Het onderzoek waarop dit proefschrift is gebaseerd werd verricht vanuit de afdelingen Interne Oncologie en Klinische Genetica, Erasmus Medisch Centrum te Rotterdam, met financiële steun van de Nederlandse Kankerbestrijding / Koningin Wilhelmina Fonds (project DDHK 95-953). De uitgave van dit proefschrift kwam tot stand met financiële steun van de Henny C. Dirven Stichting en de Nederlandse Kankerbestrijding.

De omslag en omslagfoto zijn ontworpen en gemaakt door Mieke Timmermans.

Het betreft een transversale (dwarse) doorsnede door een rode kool (**Brassica** oleracea var. rubra). Rode kool is slechts één van de gecultiveerde variëteiten van de wilde plant met een oorspronkelijk verspreidingsgebied langs de Atlantische en mediterrane kust van Europa. Andere variëteiten zijn spruitjes, koolrabi, bloemkool en broccoli. Aangenomen wordt dat het grote verspreidingsgebied van deze plant ertoe heeft bijgedragen dat er een grote genetische diversiteit bestaat waardoor selectief kweken zo veel verschillende groenten kon voortbrengen.[1]
Koolsoorten bevatten van nature een aantal bio-actieve verbindingen die (in hoge doseringen) onder andere interfereren met de jodium opname door de schildklier en het metabolisme van oestrogenen door de lever.[2] Van één van deze stoffen, indol-3-carbinol (I3C), werd aangetoond dat deze de expressie van BRCA1 in borstkanker-cellijnen doet toenemen.[3]
De auteur van dit boekje wil echter benadrukken dat van enig verband tussen rode kool en het hier beschreven onderzoek geen sprake is en dat hij zich over een mogelijke beschermende werking van koolconsumptie op borstkanker geen mening heeft gevormd.

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2 Stoewsand GS. Bioactive organosulfur phytochemicals in Brassica oleracea vegetables – a review. Food Chem Toxicol. 1995;33(6):537-43.
3 Meng Q, et al. Suppression of breast cancer invasion and migration by indole-3-carbinol: associated with up-regulation of BRCA1 and E-cadherin/catenin complexes. J Mol Med. 2000;78(3):155-65.

# Oncological and Genetic Aspects of Hereditary Breast Cancer Associated with Mutations in BRCA1 and BRCA2

*Oncologische en genetische aspecten van erfelijke borstkanker geassocieerd met mutaties in BRCA1 en BRCA2*

## PROEFSCHRIFT

ter verkrijging van de graad van doctor aan
de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. dr. ir. J.H. van Bemmel
en volgens het besluit van het College van Promoties

De openbare verdediging zal plaatsvinden op
woensdag 1 oktober 2003 om 13.45 uur

door

Leendert Cornelis Verhoog
geboren te Spijkenisse

## Promotiecommissie

**Promotoren:**

Prof.dr. J.G.M. Klijn

Prof.dr. M.F. Niermeijer

**Overige leden:**

Dr. P.M.J.J. Berns

Prof.dr. P. Devilee

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CONTENTS

# Chapter 1

## Introduction

## 1.1 General introduction

In western countries breast cancer affects approximately 1 in every 10 to 12 women. It is the leading cause of cancer death in women in these countries and the leading cause of overall mortality in women aged 35 to 55 years. Many risk factors for breast cancer have been identified including menstrual history, reproductive behavior, exogenous hormones, and a positive family history for the disease. Although most of these factors have only a small impact on breast cancer risk, the role of familial susceptibility can increase the risk for breast cancer up to a lifetime risk of close to 50% (Claus et al, 1994). Genetic susceptibility for breast cancer obtained attention and practical relevance upon the identification of the first two major breast cancer susceptibility genes, BR(east)CA(ncer)1 and BRCA2 (Miki et al, 1994; Wooster et al, 1995).

Breast cancer, like all cancers, is a 'genetic' disease, as it results from an accumulation of somatic mutations in genes or the altered expression of genes in the absence or presence of germline mutations. These genetic alterations ultimately cause the uncontrolled growth and potential for dissemination which are the hallmarks of breast cancer. It is roughly estimated that at least 5 to 10 (epi-)genetic alterations (also known as "hits") are necessary to accumulate, to promote a malignant breast tumor. Each genetic alteration arises in a single cell. It may result from spontaneous flaws in the replication of the DNA in that cell and subsequent failing of control mechanisms and DNA-repair (sometimes in the context of an increased proliferation rate). Mutations can similarly be caused by exogenous factors like radiation or mutagens. Once a cell divides, a mutation in its DNA will be passed on to the daughter-cells through the duplication of that DNA which is necessary for a cell to proliferate.

If a gene mutation is inherited from a parent this results in the presence of that genetic alteration in the DNA of each cell of the body. Carriership of these so-called germline mutations is shared with the parent that passed on the mutated gene and with siblings and other family members that similarly inherited the mutated gene. Based on the two-hit process hypothesized by Knudson, in the

case of a deleterious germline mutation in a tumor suppressor gene, only the second hit has to occur in a cell to develop a tumor (Knudson, 1971).

Up to 13% of the women diagnosed with breast cancer have one or more first-degree relatives that are also affected by the disease (Collaborative Group on Hormonal Factors in Breast Cancer, 2001). Familial aggregation of breast cancer can result from chance or shared risk factors by family members but most of the familial risk is probably genetic in origin (Peto, 2001). Genetic analysis of large families with evident clustering of breast cancer successfully led to the discovery of two breast cancer susceptibility genes, BRCA1 and BRCA2. However only one-fifth of the familial aggregation of breast cancer is attributable to the BRCA1 and BRCA2 genes (Peto et al, 1999; Anglian Breast Cancer Study Group, 2000; Antoniou et al, 2001). The remaining familial risk could be due to additional high risk susceptibility genes or to the combined effect of a number of more common susceptibility alleles with lower risks (Pharoah et al, 2002). The real situation at present is largely unknown.

## 1.2 Hereditary breast and ovarian cancer (HBOC)

In a subset of families with clustering of (early onset) breast cancer there is a clear association with ovarian cancer. The inherited tumor syndrome in these families is designated hereditary breast and ovarian cancer (HBOC). In the general population ovarian cancer is a relatively infrequent malignancy compared to breast cancer but like breast cancer its incidence is highest in western countries. In the Netherlands ovarian cancer affects approximately 1 in 70 women during their lifetime. The peak incidence of ovarian cancer is in the 7th decade. It is however the leading cause of death from gynecological cancer, also because the majority of ovarian cancers becomes diagnosed at an advanced stage when the disease is no longer confined to the ovaries. Ovarian cancer shares with breast cancer most known risk factors as hormonal and reproductive factors. Ovarian and/or breast cancer in the family history increases the risk for

these cancers, underlining the shared genetic susceptibility. The strongest risk factor is the carrier status of a germline mutation for BRCA1 giving a risk of ovarian cancer of 39% (95% confidence interval CI 18%-54%) (Antoniou et al, 2003).

Ovarian cancer is the collective name of a range of histo-pathologically distinct malignancies with different origins and natural history. Ovarian cancer can arise either from the coelomic epithelium covering the surface of the ovaries, from the ovarian stroma, or from the germcells in the ovaries. More than 90% of ovarian cancers are epithelial in origin. In its turn epithelial ovarian cancer can exhibit features of the different epithelia originating from the Mullerian ducts i.e. the epithelium lining the fallopian tubes, cervix, uterus, and part of the vagina. In this way epithelial ovarian cancers can be divided into serous, mucinous, endometrioid and clear cell cancers.

Epithelial ovarian cancer can be a fully invasive cancer or a so-called borderline malignancy. Ovarian cancer of borderline malignancy is sometimes regarded as a precursor of its invasive counterpart. The general view however is that a borderline tumor is to be considered a more or less separate neoplasm. In line with this view, borderline ovarian cancer is not clearly associated with BRCA1 or BRCA2 germline mutations (Gotlieb et al, 1998; Werness et al, 2000). BRCA1-associated ovarian neoplasms are epithelial ovarian cancers, more frequently of high grade and serous type when compared with sporadic ovarian cancer. In contrast these tumors are reported to have a better prognosis compared to age and stage matched sporadic ovarian cancers (Rubin et al, 1996; Johannsson et al, 1998).

## 1.3 Hereditary breast cancer (HBC)

### 1.3.1 BRCA1 gene

The locus of the BRCA1 gene was discovered in 1990 through genome wide linkage studies using large pedigrees with multiple women affected by breast cancer at a relatively young age at diagnosis (Hall et al, 1990). From these pedigrees with linkage to the BRCA1 gene it was clear that mutations in the BRCA1 gene conferred of a high lifetime risk for breast cancer, a substantial lifetime risk for ovarian cancer and possibly also an increased risk for colorectal cancer and prostate cancer in men (Ford et al, 1994). The gene encodes for an 1863 amino acid protein and stretches out over approximately 100 kilobases of genomic sequence at chromosome 17q21.

*Mutational spectrum of BRCA1*

Mutations may occur in all regions of the BRCA1 gene. Strong founder effects are present resulting in different distributions of mutations among populations (reviewed by Szabo and King, 1997). Germline mutations in BRCA1 are responsible for less than 3% of all breast cancer and approximately 5% of all breast cancer before the age of 40 (Ford et al, 1995; Whittemore et al, 1997) but large differences have been demonstrated to exist between populations also due to founder effects.

More than 90% of the known BRCA1- mutations are small deletions or insertions that disrupt the gene's reading frame. Pathogenic mutations and sequence variants of both BRCA1 and BRCA2 are listed in an international database (the Breast Cancer Information Core; http://research.nhgri.nih.gov/bic/) . However a number of mutations have been described that are large genomic deletions or rearrangements (Swensen et al, 1997; Puget et al, 1997; Puget et al, 1999a&b; Montagna et al, 1999; Unger et al, 2000; Rohlfs et al, 2000; Casilli et al, 2002). In the Dutch population two large genomic deletions (IVS21-36del510 and IVS12-1643del3835) are founder mutations representing ~25% of all

pathogenic BRCA1 mutations (Petrij-Bosch et al, 1997). For these recurrent mutations simple tests have been developed, but in general screening for large genomic rearrangements is laborious and time consuming, and is therefore not routinely included by most laboratories. In parallel to the Dutch situation, the fraction of mutations missed could be high in any population. Recently 5 additional large genomic rearrangements were identified in a set of 661 Dutch breast cancer families by using a novel method (Hogervorst et al, 2003). With respect to this, it is not surprising that families exist with positive evidence for linkage to the BRCA1 locus but in which (as yet) no deleterious mutation can be found (Ford et al, 1998).

*Function of BRCA1*

BRCA1 is regarded as a tumor suppressor gene. In the vast majority of breast and ovarian cancers from BRCA1 germline mutation carriers, the tumors show loss of heterozygosity of the wildtype allele at 17q21 (Smith et al, 1992). This locus was already known to be frequently involved in non-hereditary breast cancer (reviewed by Devilee and Cornelisse, 1994). Intriguingly somatic mutations of BRCA1 in breast cancer as well as ovarian cancer are a rare event in the carcinogenesis of these tumors. However in a subset of sporadic breast tumors decreased levels of BRCA1 are seen and this may be due to transcriptional inactivation of BRCA1 through aberrant methylation of its promotor (Thompson et al, 1995; Rice et al, 1998).

Many tumor suppressor genes act as "caretakers" of genomic stability by sensing and repairing different sorts of DNA damage during mitoses and meiosis. More specifically BRCA1 (and BRCA2) are implicated in DNA double strand break repair to maintain the structural integrity of chromosomes (reviewed by Welcsh et al, 2000; Venkitaraman, 2002; Starita and Parvin, 2003). BRCA1 is part of a complex (the RAD50-MRE11-NBS1$^{p95}$ complex) that mediates repair of DNA double strand breaks by recombination of homologous DNA sequences (Zhong et al, 1999). This complex in its turn is part of the BASC (BRCA1-associated genome surveillance complex) super complex that includes other

tumor suppressors and DNA repair proteins including mismatch repair proteins (Wang et al, 2000). Furthermore cells without functional BRCA1 are deficient in transcription-coupled repair (Gowen et al, 1998). Table 1 summarizes some of the multiple nuclear functions of BRCA1. Note that ultimately these processes may play a role in different phases or types of DNA repair. A more complete overview of BRCA1/2-associated proteins (>50!) is presented in a review by Sauer (Sauer, 2002).

Table 1 Some of the (putative) functions of BRCA1

| Cellular process | Related proteins and complexes |
|---|---|
| DNA damage repair | RAD50-MRE11-NBS1$^{p95}$ complex & BASC super complex |
| Ubiquitination | BRCA1/BARD1 heterodimeric complex |
| Regulation of transcription | RNA polymerase II holoenzyme |
| Chromatine remodelling | SWI/SNF*, BRG1†, histondeacetylase complex‡ |
| Homologous recombination | RAD51, BRCA2 |

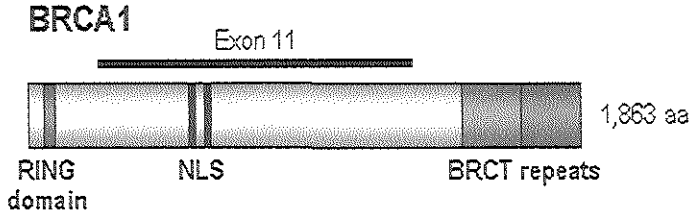
* Bochar et al, 2000; † Cantor et al, 2001; ‡ Yarden and Brody, 1999

In line with its function BRCA1 is expressed in a cell cycle dependent fashion and regulated (i.e. activated) by phosphorylation. Proteins capable of phosphorylating BRCA1 are ATM (Ataxia Telangiectasia Mutated) and CHEK2 (Checkpoint Kinase 2) (Cortez et al, 1999; Lee et al, 2000). Both proteins are involved in DNA repair processes and both are considered to be low-penetrance breast cancer susceptibility genes to breast cancer (Swift et al, 1987; Meijers-Heijboer et al, 2002).

The structure of the BRCA1 gene gives some information about its possible functions (figure 1). Exon 11 of the gene contains nuclear localization signals (NLS) thus implicating a role in the nucleus (Thakur et al, 1997). The N-terminal region of the gene is a RING finger domain that in the case of BRCA1 has been shown to interact with BARD1 (BRCA1-associated RING domain1 protein). BARD1 shows structural similarities with BRCA1 and a germline mutation in this gene was identified in a patient with breast, ovarian and endometrial cancer (Thai et al, 1998). The BRCA1/BARD1 complex functions as

a ubiquitin ligase (Hashizume et al, 2001; Ruffner et al, 2001). Other proteins that contain RING finger domains are also known to facilitate ubiquitination. The identification of substrates for the BRCA1/BARD1 complex should provide more insight in how precisely BRCA1 mediates tumor suppression.

Figure 1 Schematic representation of the functional domains in BRCA1




At the C-terminal end of the protein there are two so-called BRCT (BRCA1 C-terminal) repeats. BRCT repeats have been identified in a number of other proteins involved in DNA repair or metabolism including BRCA2. In BRCA1 these domains interact with both BRCA2 and the RAD51 protein, a protein known to be involved in these processes (see also figure 3) (Scully et al, 1997).

Other important clues about the function of the BRCA1 protein came from experiments in transgenic mice with disrupting mutations in both alleles (nullizygous or knock-out mice). These mice die early in their embryonic development and show decreased cellular proliferation instead of uncontrolled proliferation that is seen in cancer (Hakem et al, 1996). Embryonic lethality is delayed when these embryos also are nullizygous for p53 or p21 (Hakem et al, 1997). Importantly, the majority of BRCA1-associated tumours present with mutations in p53 (Crook et al, 1997; Schuyer and Berns, 1999).

*Function and tissue specific cancer susceptibility*

The processes in which BRCA1 is involved are fundamental to all cells. Therefore its (multiple) function(s) offers no simple explanation why disruption of the protein principally causes cancer in steroid hormone dependent tissues (see below). However, clues are emerging that point to an interaction of BRCA1 with estrogen receptor alpha by co-repressing both ligand-dependent (Fan et al, 2002) and ligand-independent transcriptional activity (Zheng et al, 2001) of this receptor.

Another possible explanation of organ specific cancer susceptibility could be the role of BRCA1 and BRCA2 in mammary development. In certain mouse strains, mice that are heterozygous for BRCA1 or BRCA2 mutations show inhibited mammary duct branching when treated with diethylstilbesterol (DES) (Bennet et al, 2000). Mice that are heterozygous for BRCA1 or BRCA2 mutations are not prone to developing cancer but mice carrying a germline BRCA1 mutation and a conditional BRCA1 mutation (i.e. an allele that is inactivated specifically in the mammary epithelium) also show abnormal ductal development and occasional tumor formation. Additional p53 mutations accelerate the formation of mammary tumors in these mice (Xu et al, 1999; reviewed by Moynahan, 2002).

Additional mutations in other genes are crucial for development of cancer in BRCA1/2 mutation carriers. The genes involved in the development or the progression of hereditary breast cancer may in part be different than those involved in sporadic breast cancer. This is demonstrated for instance by the increased frequency of p53 aberrations in BRCA1-associated tumors (reviewed by Schuyer and Berns, 1999) and different gene-expression profiles in hereditary breast cancer (Hedenfalk et al, 2001; Berns et al, 2001; van 't Veer et al, 2002). As a result hereditary breast cancer could also differ with respect to the outcome of the disease, in other words, BRCA1 and BRCA2 mutations may have prognostic significance.

*Cancer risk associated with BRCA1*

Estimates of the lifetime risk of breast cancer in carriers from BRCA1 mutations show wide intervals. The studies that started from pedigrees of 'high risk' families found a breast cancer risk of 59-73% by age 50 years and 82-87% by age 70 years (Easton et al, 1993; Ford et al, 1994). The same studies estimate the life-time risk for ovarian cancer in some carriers to reach 65% (Ford et al, 1998). Population based studies among Ashkenazi Jewish subjects with BRCA1/2 founder mutations thereupon found substantial lower lifetime risks for breast and ovarian cancer, ranging from 36-56% (Struewing et al, 1997; Fodor et al, 1998). The considerable variation in cancer risk is first of all accounted for by different methodologies and different populations that were investigated. However cancer risks are also influenced by the position of the mutation within the gene sequence. One report observed a correlation between the location of the gene and the ratio of breast and ovarian cancer incidence within each family (Gayther et al, 1995). A much larger study found a significantly lower breast cancer risk (relative risk of 0.71) for mutations in the central region of the gene (nucleotides 2401-4190) and a significantly lower ovarian cancer risk (0.81) for mutations 3' to that central region (Thompson and Easton, 2002).

Recently the combined analysis of 22 studies with pooled pedigree data from 500 index patients with a BRCA1/2-germline mutation, unselected for family history, was published. This study found average cumulative risks in BRCA1-mutation carriers by the age of 70 years of 65% (95% confidence interval: 44%-78%) for breast cancer and 39% (95% confidence interval: 18%-54%) for ovarian cancer (Antoniou et al, 2003). The relative risk of breast cancer declined significantly with age for BRCA1-mutation carriers. Considering the size of the study and the fact that most women are currently tested on the basis of relative weak family histories (compared to families with evidence for linkage to BRCA1), the latter risk estimates are probably the most accurate.

Regarding the risk for other cancers in BRCA1 germline mutation carriers elevated risks have been reported for colon and prostate cancer (Ford et al, 1994) but this has been disputed for both cancers. BRCA1 did not contribute to

familial prostate cancer (Gayther et al, 2000) and in (early onset) prostate cancer patients the low frequency of BRCA1 mutations did not indicate a significant risk. (Lehrer et al, 1998; Nastiuk et al, 1999; Vazina et al, 2000). Regarding colon cancer, the aforementioned large population based study showed no excess of colon cancers among BRCA1/2 carriers (Struewing et al, 1997). Another study demonstrated a large BRCA1-associated family with multiple colorectal cancers in which the tumors did not show loss of heterozygosity at the BRCA1 region (Peelen et al, 2000). This would be in conflict with a role for BRCA1 as tumor suppressor gene in colon cancer.

A recent analysis from 699 BRCA1-associated families ascertained by members of the Breast Cancer Linkage Consortium again observed an increased risk for colon cancer and prostate cancer (in male BRCA1 carriers, <65 years) as well as an increase in risks for cancer of the pancreas, cervix and uterine body (Thompson and Easton, 2002). However the marked deficit in the number of reported rectal cancers suggests that these have been misdiagnosed as colon cancers. When these two cancers were considered together the relative risk was no longer significantly elevated. Similarly ovarian cancers could have been misdiagnosed as colon cancers. Moreover the overall increased risk of cancer was only observed in women but not in men.

Male breast cancer too has occasionally been reported in BRCA1-associated families (Struewing et al, 1995, Borg et al, 2000, Ottini et al, 2003). Population based studies among male breast cancer patients failed to find evidence for an increased risk in BRCA1 mutation carriers (Friedman et al, 1997; Wolpert et al, 2000; Basham et al, 2002).

Cancer of the fallopian tubes is a rare neoplasm in the general population but has repeatedly been noted in BRCA1 families. Serous (papillary) carcinomas of uterus and fallopian tubes have been found in BRCA1 mutation carriers with loss of the wild type allele in these tumors (Lavie et al, 2000; Zweemer et al, 2000). Moreover primary papillary serous carcinoma of the peritoneum (PSCP) has been reported in BRCA1 mutation carriers (Bandera et al, 1998; Schorge et al, 1998). PSCP is microscopically reminiscent to serous epithelial ovarian

carcinoma and cases have been reported to arise even following oophorectomy (Kemp et al, 1992; Weber et al, 1992; Piver et al, 1993).

*Pathobiological characteristics of BRCA1-associated breast cancers*

Shortly after the cloning of the BRCA1 gene it became clear that breast cancers in mutation carriers had phenotypic characteristics different from non-BRCA1 related breast cancers in age matched controls. These specific pathobiological characteristics could have opposite influences on the prognosis. For example, BRCA1-tumors are more likely to be of higher grade (i.e. less differentiated) which is caused by a) a high mitotic index, reflected in a high S-phase fraction, b) more nuclear polymorphism, c) less tubule formation (BCLC, 1997). In addition these tumors are more often steroid receptor negative (Karp et al, 1997; Johannsson et al, 1997). On the other hand they are more often of the medullary or atypical medullary histologic type (BCLC, 1997, Eisinger et al, 1998). Medullary breast cancers might have a better prognosis as compared to ductal carcinomas not other wise specified (Bloom et al, 1970; Ridolfi et al, 1977; Rapin et al, 1988).

A number of other characteristics concerning possible prognostic factors do not unequivocally indicate a worse or better outcome. For instance the frequent occurrence of p53 mutations (Crook et al, 1998; Phillips et al, 1999) would suggest a worse outcome but the lack of Her2-neu (c-erbB2) over-expression (Johannsson et al, 1997; Noguchi et al, 1999) points towards a better outcome. Immuno-histochemical analyses indicated infrequent expression of the estrogen-dependent pS2 gene in BRCA1-associated tumors in line with their negative steroid receptor status (Osin et al, 1998). Others however showed a relatively high proportion positive for pS2 (Eisinger et al, 2001). The latter observation points to potential hormonal sensitivity and would be of interest for chemoprevention and response to anti-estrogen therapy. Amplification of chromosome 6q22-24, the locus of c-myb, and its subsequent over-expression was frequently detected in BRCA1-associated tumors as compared to sporadic breast cancers (29% vs 2%) (Kauraniemi et al, 2000). Expression of the

transcriptional transactivator c-myb in breast cancer cell lines is regulated by estrogens (Gudas et al, 1995) and is correlated with estrogen-receptor positive tumors in unselected breast cancer patients (Guerin et al, 1990).

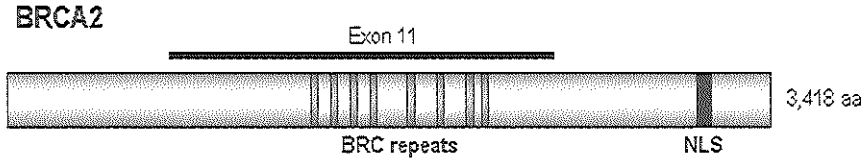
Low expression of cyclin D1 might indicate a poor prognosis in BRCA1-associated tumors (Osin et al, 1998; Armes et al, 1998). On the other hand Robson et al detected no difference in cyclin D1 expression between BRCA-associated breast tumors and sporadic controls. Similarly they also found no immuno-histochemical differences in the expression of epidermal growth factor receptor (EGFR), cathepsin D, bcl-2, p27 or p53 (Robson et al, 1998). However others found the anti-apoptotic bcl-2 gene to be down-regulated in these tumors (i.e. a possible better prognostic factor) (Freneaux et al, 2000). Finally a decreased level of angiogenesis has been reported as a feature of BRCA1-associated tumors (Lynch et al, 1998). An extensive overview of the differential role of prognostic factors in sporadic versus hereditary breast cancer is presented in paragraph 1.4 of this introduction.

### 1.3.2 BRCA2 gene

The BRCA2 gene was localized in 1994 to chromosome 13q12-13 (Wooster et al, 1994) and cloned just over one year later (Wooster et al, 1995). It has been implicated in similar risks of breast cancer compared to BRCA1, ranging from 80-84% at the age of 70 years (Easton et al, 1997; Ford et al, 1998). Similar as for BRCA1, substantially lower life-time breast cancer risks were obtained from population based studies using the kin-cohort method ranging from 37% to 56% (Struewing et al, 1997; Thorlacius et al, 1998). Compared to BRCA1, the risk of breast cancer may increase at a somewhat later age thus the age specific breast cancer risks for young women are considerably lower for BRCA2 carriers. Based on cases unselected for family history estimates of the cumulative breast cancer risk by age 70 years are 45% (95% confidence interval: 31%-56%) (Antoniou et al, 2003).

The Breast Cancer Linkage Consortium reported the risk of other cancers than breast and ovarian cancer in a large series of 173 BRCA2 families (BCLC, 1999). Significantly increased risks were found for prostate cancer, pancreatic cancer, gallbladder and bile duct cancer, stomach cancer and malignant melanoma. The role of BRCA2 in genetic susceptibility for pancreatic cancer was already suspected since mutations were relatively frequently found in sporadic pancreatic carcinomas (Goggins et al, 1996). Ovarian cancer risk in BRCA2 germline mutation carriers is estimated to be 11% (95% confidence interval 2.4%-19%), substantially less than that for BRCA1 mutation carriers. There is a genotype-phenotype correlation for the ovarian cancer risk with an increase in risk (and a decrease in breast cancer risk) for mutations in the so-called ovarian cancer cluster region (occr) stretching out over exons 10 and 11 of the gene (Gayther et al, 1997; Thompson and Easton, 2001). Germline mutations in BRCA2 are also found in cancers of the fallopian tube (Rose et al, 2000; Aziz et al, 2001). BRCA2 is associated with male breast cancer. The life-time risk for breast cancer in male carriers, linked to BRCA2, is estimated to reach 6.9% (95% confidence interval 1.2%-39%) (Thompson and Easton, 2001).

Figure 2 Functional domains of the BRCA2 protein; the BRC repeats in exon 11 are capable of binding RAD51 protein (Wong et al, 1997).

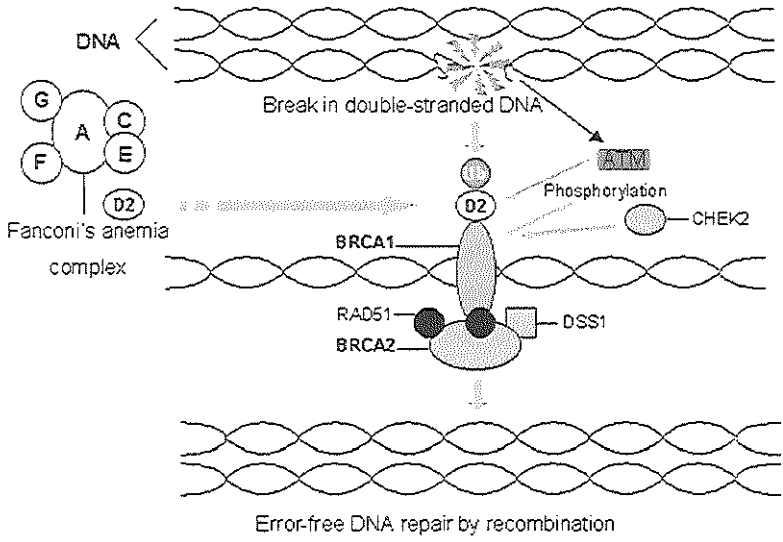


BRCA2 encodes for a 3418 amino acid protein and is believed to play a role in the same DNA damage response pathway as BRCA1 (figure 3). Among others this is based on the evidence that at a cellular level the protein co-localizes and interacts with RAD51 and BRCA1 (Chen et al, 1998). BRCA2 was recently identified as identical to the as yet unidentified Fanconi D1 (FANCD1) gene in Fanconi anemia (Howlett et al, 2002). Fanconi anemia is a rare autosomal recessive disorder caused by defects in one of at least 8 distinct genes and is characterized by bone marrow failure, cancer proneness, birth defects and hypersensitivity to DNA damage.

Five of the Fanconi anemia proteins interact in a nuclear complex that regulates the monoubiquination of FANCD2, another Fanconi anemia protein, in response to DNA damage. BRCA2 may act either upstream or downstream in this pathway. Intriguingly, following radiation induced DNA damage, BRCA1 co-localizes with FANCD2 and other proteins involved in DNA repair in nuclear foci (Garcia-Higuera et al, 2001). A simplified model on how BRCA2 and several other genes involved in breast cancer susceptibility participate in the repair of double-stranded DNA breaks is shown in figure 3.

Similar as in BRCA1, mutations in the BRCA2 gene are spread along the gene. Large genomic deletions have not been reported. Founder mutations were identified in the Ashkenazi Jewish and Icelandic population but otherwise seem to be less pronounced (Thorlacius et al, 1996; Neuhausen et al, 1996). Like BRCA1, breast cancers in BRCA2-mutation carriers are of higher histological grade although in multivariate analysis this seems predominantly due to less tubule formation in these tumors (Agnarsson et al, 1998; Lakhani et al, 1998). Similar as in BRCA1, lower levels of cyclin D1 were found in BRCA2 associated cancers (Osin et al, 1998; Armes et al, 1998). In general it is suggested that BRCA2 breast cancers have a more heterogenous phenotypic profile (reviewed by Phillips, 2000). The prognostic and pathobiological characteristics of BRCA2 associated breast cancer are more extensively discussed in chapter 6 of this thesis.

Figure 3 The proteins of several breast cancer susceptibility genes (ATM, CHEK2, BRCA1 AND BRCA2) act together in the repair of double-stranded DNA breaks by homologous recombination. Proteins from another chromosome instability disorder, Fanconi anemia, are connected to this pathway (from Venkitaraman, 2003).




Ub: mono-ubiquitin; DSS1: "deleted in split-hand/split-foot 1 region", MIM No. 601285; the BRCA2-DSS1 complex directly binds single stranded DNA (Yang et al, 2002).

### 1.3.3 Other hereditary cancer syndromes and breast cancer risk

Many hereditary cancer syndromes show clustering of one specific tumor (a so-called site-specific cancer syndrome) and in others a range of other tumors are associated with that syndrome. To establish whether a tumor occurs as part of an hereditary cancer syndrome or as a coincidental sporadic tumor in pedigrees suggestive of a cancer syndrome, the following characteristics point to a causal relation with germline susceptibility:

a) epidemiological evidence regarding the tumor spectrum of families with a syndrome;
b) in the case of a tumor suppressor gene, the tumor from an affected family member shows loss of the wild type allele in line with loss of its function;
c) sporadic tumors of that type have somatic mutations in the predisposing gene or loss of heterozygosity at its locus.

Breast cancer occurs in a number of well defined hereditary cancer syndromes e.g. Li Fraumeni syndrome and Peutz-Jeghers syndrome. For other syndromes like HNPCC, the correlation with breast cancer is less firmly established. However the total number of breast cancers from these syndromes is only a small proportion of overall breast cancer nor do they explain a significant part of the HB(O)C families without BRCA1/2 mutations.

*Li Fraumeni syndrome*

Mutations in the tumor suppressor gene p53, located at chromosome 17p13, occur in about 50% of all human tumors, making it the most frequent target for somatic genetic alterations. Germline mutations in the p53 gene, confer a risk of sarcomas, brain tumors, leukemia and adrenal tumors leading to a syndrome first described by Li and Fraumeni in 1969 (Li and Fraumeni, 1969).

Somatic mutations in p53 are an important step in the development of BRCA1-associated breast and ovarian cancer, as shown by their high frequency in BRCA1-related tumors (Crook et al, 1998; Rhei et al, 1998; Phillips et al, 1999). The model for this interaction is that loss of function of BRCA1, would in

the presence of normal functioning p53 lead to either cell cycle arrest or apoptosis. Other mutated genes in this pathway like e.g. p21 could similarly be responsible for a cell to escape these control mechanisms.

Germline mutations in p53 are present in approximately half of the families with a Li-Fraumeni phenotype (Malkin et al, 1990). Many of the p53-associated malignancies occur in childhood and the lifetime penetrance approaches 100% for female carries compared to ~70% in male carriers (Chompret et al, 2000). This difference is accounted for by the very high risk of breast cancer in female p53 germline mutation carriers, estimated to be ~90% (Garber et al, 1991). Also breast cancer in these families occurs considerably earlier than sporadic breast cancer with a peak incidence between 20 and 40 years of age. Li-Fraumeni syndrome is rare, also as a consequence of the severity of the phenotype. P53 germline mutations in breast cancer patients are equally rare (<1%) (Borresen et al, 1992) and usually are identified through the specific cancer spectrum among their relatives.

*Cowden syndrome*

The Cowden syndrome is a rare autosomal dominantly inherited genodermatosis. It is characterized by specific mucocutaneous lesions (skin adnexal tumors called trichilemmomas, acral keratosis and oral papillomas). Besides an estimated life-time risk of 25-50% of developing invasive breast cancer, there is an increased risk of (follicular) thyroid- and endometrial cancer (Starink et al, 1986). The gene for Cowden's disease is named PTEN/MMAC1 (phosphatase en tensine homolog/mutated in multiple advanced cancers 1) (Li et al, 1997; Steck et al, 1997; Liaw et al, 1997). Unselected breast cancers frequently show loss of heterozygosity at the locus of PTEN, 10q23, (Feilotter et al, 1999). Also there is an epidemiological relationship between thyroid cancer and breast cancer (Ron et al, 1984; Vassilopoulou-Sellin et al, 1999). However, germline mutations of this gene are infrequent in familial breast cancer (FitzGerald et al, 1998, Shugart et al, 1999).

*Peutz-Jeghers syndrome*

Peutz-Jeghers syndrome shows autosomal dominant inheritance and features intestinal hamartomatous polyposis and melanin pigmentation of the skin and mucous membranes. There is an increased risk for cancers in the small bowel, colorectum, stomach and pancreas but also of the breast (Riley et al, 1980; Boardman et al, 1998; Westerman et al, 1999). It is caused by germline mutations of the STK11 gene (serine/threonine kinase 11) that maps to chromosome 19p13.3 (Hemminki et al, 1998; Jenne et al, 1998). Somatic mutations in this gene do not appear to play an important role in sporadic breast cancer (Bignell et al, 1998). Despite the fact that LOH at 19p is frequently found in familial breast cancers, germline mutations in STK11 were not detected in familial breast cancer patients (Lindblom et al, 1993; Chen et al, 2000).

*FAMMM and HNPCC*

In addition to the above-mentioned syndromes breast cancer may be part of two other cancer syndromes, namely the familial atypical multiple mole-melanoma syndrome (Lynch et al, 1981; Bergman et al, 1990; Borg et al, 2000) and hereditary non-polyposis colorectal cancer (HNPCC) (Lynch et al, 1988). About half of the HNPCC families show inheritance of mutant mismatch repair genes, notably MLH1, MSH2 and MSH6 (bacterial MutL and MutS Homologs). Breast cancer appears to be over-represented in MLH1-positive HNPCC families and also in HNPCC families without evidence (as yet) of mismatch repair (MMR) gene-mutations (Scott et al, 2001). This correlation however remained unconfirmed or was disputed by other studies (Watson and Lynch, 1993; Aarnio et al, 1999; Vasen et al, 2001). Epidemiological evidence also shows that colon cancer is a relative frequent second primary malignancy following breast cancer (Ewerts et al, 1985; Agarwal et al, 1986; Mamounas et al, 1993).

Breast cancers in MMR gene-mutation carriers from HNPCC families show microsatellite instability (MSI) (Bergthorsson et al, 1995; Risinger et al, 1996) and this has also been demonstrated for the tumor of a male breast cancer patient with a MLH1 germline mutation (Boyd et al, 1999). The extent of MSI in

sporadic breast cancers varied between studies presumably dependent upon the number of loci tested and differences in stability of the markers that were used (Wooster et al, 1994; Richard et al, 2000). However, extensive MSI, similar to that in MMR deficient HNPCC-colon cancers, is infrequently noted in sporadic breast cancers (Peltomaki et al, 1993). Because germline MMR gene mutations are relatively rare they are not expected to make a large contribution to hereditary breast cancer.

Following the identification of CHEK2 (checkpoint kinase 2, the human homolog of Rad53 in yeast) as a low penetrance breast cancer susceptibility gene (see below), it was found that the CHEK2 1100delC mutation appeared related with the coincidence of colorectal cancer in non-BRCA1/2 hereditary breast cancer families. It is believed that this mutation acts in synergy with another, as yet unknown susceptibility gene(s) (Meijers-Heijboer et al, 2003). The correlation between colon and breast cancer needs further clarification but shared reproductive and hormonal risk factors may also be involved (Potter et al, 1983; Singh et al, 1985).

### 1.3.4 Additional breast cancer susceptibility genes

Approximately 95% of HBOC and approximately 85% of early onset HBC families with 6 or more breast cancer patients appear to be due to either BRCA1 or BRCA2 (Ford et al, 1998). However, as pointed out before, the contribution of BRCA1 and BRCA2 to the excess of familial risk for breast cancer is relatively small, being approximately 20% (see reviews by Nathanson et al, 2001 and Wooster and Weber, 2003). The substantial percentage of smaller families without BRCA1/2 mutations or linkage to these genes, suggests the existence of (an) other breast cancer susceptibility gene (or genes) (Antoniou et al, 2001). Others argued that families with a moderate number of breast cancer cases are most likely explained by clustering of sporadic breast cancer patients (Cui and Hopper, 2000).

Putative loci for a third high-penetrance susceptibility gene, "BRCA3", have been proposed, including chromosome 13q21 (Kainu et al, 2000) and 8p12-22 (Kerangueven et al, 1995; Seitz et al, 1997) but both have also been refuted (Rahman et al, 2000; Thompson et al, 2002). An alternative model that could account for the excess of familial risk are relative common variants (polymorphisms) in low-penetrance susceptibility alleles that may moderately increase the risk of breast cancer. Since these genetic variants are much more common in the population than BRCA1 and BRCA2, gathered they make a substantially greater contribution to breast cancer than BRCA1 and BRCA2 (reviewed by Dunning et al, 1999; de Jong et al, 2002). Also, multiple common low-penetrance genes could act in synergy (either additive or multiplicative) resulting in a polygenic model (Antoniou et al, 2001; Pharoah et al, 2002). Low-penetrance alleles will result in frequent absence of familial clustering (Cui and Hopper, 2000) and, in addition, with polygenic susceptibility there will be non-segregation of a genetic variant with the disease. This would also explain the failure to identify new major susceptibility genes (Peto, 2002).

Of special interest are additional modifier genes acting together with deleterious BRCA1/2 mutations. Their existence is likely because breast cancer risk in BRCA1/2-mutation carriers shows evidence for heterogeneity. Among others, N-acetyltransferase 2 (NAT2) and the androgen receptor have been investigated in this context but results are ambiguous (Rebbeck et al, 1999a; Spurdle et al 1999). Risk-modifying genetic factors that may explain inter-individual variability in BRCA1/2-associated breast and ovarian cancer risk have been reviewed by Rebbeck (Rebbeck, 2002).

The notion that many tumor suppressor genes play a role in DNA repair and cell cycle control prompted mutation analysis of more genes involved in these processes, as candidate breast cancer susceptibility genes. Recently the 1100delC mutation in CHEK2 was shown to contribute to approximately 5% of BRCA1/2–negative familial breast cancer (Meijers-Heijboer et al, 2002; Vahteristo et al, 2002). CHEK2 is located on chromosome 22q and is part of the p53 pathway (Chehab et al, 2000; Shieh, 2000). The penetrance of this gene

was relatively low with an estimated twofold increase of breast cancer risk in women and a tenfold increase of risk in men. Segregation of the mutation in the families was incomplete suggesting that this mutation alone may not explain the clustering of breast cancer in these families. Earlier, heterozygous germline mutations in the CHEK2 gene were identified in families with Li-Fraumeni syndrome without p53 mutations (Bell et al, 1999). The frequency of 1100delC has been estimated in healthy control populations to vary between 0.3% and 1.7% ruling out that this mutation causes the rare Li-Fraumeni syndrome (Meijers-Heijboer et al, 2002; Vahteristo et al, 2002; Offit et al, 2003)

The ATM (ataxia telangiectasia-mutated) gene can be also considered a low-penetrance breast cancer susceptibility gene. Homozygotes of deleterious mutations in this gene suffer from the rare disease AT, characterized by cerebellar ataxia, capillary telangiectases of eye and skin, immunodeficiency, susceptibility to cancer, especially lymphomas, and an increased sensitivity to ionizing radiation. Again, its function lies in a pathway that evokes the cellular response to DNA damage. ATM takes part in the activation of BRCA1 and CHEK2 by phosphorylation (Chaturvedi et al, 1999; Cortez et al, 1999). Approximately 0.5-1% of the general population is estimated to be carrier of an ATM germline mutation. Earlier studies have indicated that female carriers have a relative risk of breast cancer of up to 6.8 compared to non-carriers (Swift et al, 1987; Morrell et al, 1990). Given the population frequency of ATM mutations, these would then account for a substantial proportion of all breast cancers. Recently it was confirmed that ATM germline mutations contribute to breast cancer for a selected group of Dutch breast cancer patients diagnosed at young age and selected for longevity (Broeks et al, 2000). Controversy however remains about the magnitude of ATM-related breast cancer risk (Chenevix-Trench et al, 2002; Szabo, in press).

## 1.4 Prognostic features in sporadic versus hereditary breast cancer

Breast cancer in general is a heterogenic disease. This is apparent in the variability of the disease after it has been diagnosed. Beside numerous biochemical and genetic prognostic and predictive factors (reviewed by Dahiya and Deng 1998; Mirza et al, 2002 and Klijn, 2002) the outcome of the disease is first of all determined by the stage at time of diagnosis as expressed in the TNM classification. The stage of invasive breast cancer is established by taking into account the tumor size (T), loco-regional lymphnode involvement (N) and the presence of metastases at time of diagnosis (M) (the TNM system).

A host of single prognostic factors, in part discussed in paragraph 1.3.1, fails to hold their promise when tested in a multivariate assay system. However assessment of tumor grade and steroid receptor status became standard practice because of their additive value and therapeutic consequences. Recent developments like molecular profiling of breast tumors with micro-arrays and new designer drugs acting on specific molecular targets, show the importance of further characterizing individual tumors beyond staging and grading. Far from being comprehensive, some prognostic factors are highlighted below in view of (potential) differences between sporadic and hereditary breast cancer.

*Tumor size*

The size (pT) of a breast tumor is defined as the maximum diameter of the invasive component of a breast cancer. Tumor size is one of the strongest prognostic factors of relapse in node-negative breast cancer (Rosen et al, 1993; Quiet et al, 1995). Also there is a good correlation between tumor size and the incidence of nodal metastases and subsequent survival rate. Growth of a tumor is not just the result of the rate of cell proliferation but is strongly influenced by loss of tumor cells because of cell death and factors like angiogenesis to provide nutrients and oxygen. Size by itself however is of limited prognostic value. In 20-30% of patients with tumors up tot 2 cm (T1) and without axillary lymphnode

involvement (N0), micrometastases are present at time of diagnosis (Rosen et al, 1989).

No studies have yet conclusively shown that tumors associated with BRCA1 or BRCA2 differ in size as compared to their sporadic counterpart. With respect to hereditary or familial breast cancer it has been hypothesized that women at risk will show increased awareness for breast cancer and therefore detect tumors at a smaller size due to active breast self examination or participation in surveillance programs. In contrast it has been suggested that denial in these women might lead to patient delay.

*Nodal status*

The presence or absence of axillary lymph node metastases is one of the most important prognostic parameters. Moreover the amount of metastatic tumor and the surgical level of the axillary lymph node involvement, probably best reflected by the absolute number of affected lymph nodes, influence survival (Fisher et al, 1983). Furthermore the presence of lymph node metastases often determines the need for adjuvant therapy.

Apart from patients diagnosed by regular surveillance, no large differences appear to exist in the proportion of BRCA1/2 associated breast cancer patients with lymph node involvement. Foulkes et al showed that especially for the subgroup of node negative breast cancer patients, BRCA1 may be a strong adverse prognostic factor and suggested a differential role of nodal status in BRCA1-associated patients compared to sporadic patients (Foulkes et al, 1998a&b; Foulkes et al, 2000).

*Age at diagnosis*

A young age (<35 years) at diagnosis is considered to be an adverse prognostic feature of breast cancer (Adami et al, 1986; Nemoto et al, 1980). However relative survival of premenopausal women in general might be better than those diagnosed with breast cancer after the age of 50 years (Host and Lund, 1986).

The worse prognosis of young age appears to be independent of clinical stage, tumor grade and therapy (de la Rochefordiere et al, 1993).

Since the majority of BRCA1- and BRCA2-associated breast cancer patients will be diagnosed before the age of 50, age at onset in hereditary breast cancer does not unequivocally points towards a worse prognosis. In our experience age at onset in BRCA1-associated breast cancer patients appeared to be of little prognostic value with respect to overall survival (chapter 5).

*Tumor grade*

Microscopic grading of breast carcinomas uses the Nottingham modification of the Bloom-Richardson system and includes the degree of tubule formation, the degree of nuclear pleomorphism and the mitotic count (Elston et al, 1991). This grading has a robust correlation with prognosis (Fisher et al, 1990; Garne et al, 1994), as have its single components, especially the mitotic count. The mitotic count is a measure for cell proliferation such as is the S-phase fraction determined by flow cytometry or immunostaining with proliferation markers (Biesterfeld et al, 1995). As mentioned above both BRCA1- and BRCA2-breast cancers are often of high grade with an especially high mitotic count in BRCA1, suggesting a worse clinical outcome.

*Estrogen (ER) and progesterone receptors (PgR)*

Steroid hormone receptors became extensively studied as prognostic factors (reviewed by Osborne, 1998). Positive steroid receptor status correlates with a lower tumor grade and it is accordingly associated with a longer disease-free interval and overall survival in the first years following diagnosis. The long-term prognosis is less clearly related to receptor status. The overall prognostic significance is modest, but a positive steroid receptor status is a strong predictor of response to endocrine treatment. Possibly other prognostic interactions are relevant, like between ER and PgR, the nodal status and length of the disease free interval (Alanko et al, 1985; Mason et al, 1983; Aamdal et al, 1984). With

respect to BRCA1/2-associated breast cancer, receptor status is extensively discussed in chapters 4-6.

*Her2-neu over-expression*

Over-expression of Her2-neu (c-erbB2) protein is proven to be of prognostic importance and is used in the clinical management of breast cancer. Her2-neu is a member of the epidermal growth factor receptor family. Its over-expression results from amplification of the gene and occurs in approximately 20% of invasive ductal carcinomas. This feature is associated with poor prognosis, especially in node positive patients and closely correlates with tumor grade. A humanized antibody (herceptin), directed against this receptor, has become available for the treatment of metastasized disease (reviewed by Spigel and Burnstein, 2002). Interestingly Her2-neu is not frequently over-expressed in BRCA1 tumors (Johannsson et al, 1997; Armes et al, 1999; Noguchi et al, 1999). BRCA2-related tumors do not show a lower frequency of Her2-neu over-expression as compared with sporadic breast tumors (Lakhani et al, 2002).

*Cytoarchitectural type*

The two major histological types in which breast cancer is routinely classified, i.e. invasive ductal carcinoma and invasive lobular carcinoma, give a similar prognosis in breast cancer. Some (sub-)types correlated with a better prognosis are tubulo-lobular (du Toit et al, 1989), tubular (Carstens et al, 1985), mucinous (Fentiman et al, 1997) and medullary carcinomas (Ridolfi et al, 1977). Most of these types correspond to either low grade malignancies (e.g. tubular carcinoma) or are associated with absence of lymph node metastases (e.g. mucinous carcinoma) and therefore have little practical significance next to routine staging and grading of breast cancer.

BRCA1 tumors are more frequently of the medullary type, and in an unexpectedly high number of unselected medullary carcinomas nonsense BRCA1 mutations were detected (Eisinger et al, 1998). One of the characteristics of a medullary carcinoma is that the borders of the tumor are of the "pushing"

type i.e. rounded and sharply separated from the surrounding stroma. Also in breast carcinomas other than typical medullary carcinomas, tumors with this type of pushing margins have a more favorable prognosis (Carter et al, 1978). Pushing margins are a feature of BRCA2 tumors as well (Lakhani et al, 1998). Some other characteristics of medullary carcinoma, like prominent nuclear pleomorphism and high mitotic count are also shared by BRCA1 tumors but out of the context of a medullary carcinoma, suggest a worse outcome.

*Bilateral breast cancer*

Bilateral breast cancer would appear to be an adverse prognostic feature of breast cancer. In general there is absence of a link between prognostic factors of the first and the second breast tumor which supports the notion that contralateral breast cancer is in fact a second primary breast cancer (Broët et al, 1995). However the relationship between metachronous bilateral breast cancer and survival is difficult to investigate because of a strong bias that only women who will survive from their first tumor can be diagnosed with a second contralateral breast cancer (discussed in chapter 5). Factors that influence the outcome of breast cancer patients with bilateral disease are stage of the second breast cancer, age at diagnosis of the second breast cancer and the interval between the diagnosis of the first and second cancer (Heron et al, 2000). These aspects of bilateral breast cancer have not been separately looked into for BRCA1/2.

Bilateral disease is a dominant feature of BRCA1/2-associated breast cancer (this thesis). Epidemiological evidence shows that especially in young women with their second breast cancer before the age of 50 years, survival rates after diagnosis of the second tumor are worse (Brenner et al, 1993). In contrast an interval of more than five years between the first and second tumor is associated with a better distant recurrence-free survival rate measured from the time of treatment for the second cancer (Gustafsson et al, 1994). Both scenarios seem applicable to BRCA1/2 mutation carriers. With regard to synchronous bilateral breast cancer, also more frequent in BRCA1/2, patients are at greater

risk for distant metastasis than women with unilateral or metachronous breast cancer (Heron et al, 2000).

## 1.5 Genetic testing and cancer risk management

Once a woman has been identified as a carrier of a BRCA1/2 germline mutation she faces the before mentioned risks for breast and ovarian cancer. She can then opt for a number of choices that is highly depending on her personal situation, psychological and cultural aspects. Irrespective of these latter aspects, a female carrier has a number of options in trying to reduce her risk of succumbing of breast or ovarian cancer i.e. regular surveillance, prophylactic surgery and chemoprevention.

Hypothetically a small proportion of risk reduction for BRCA1/2 mutation carriers could also be achieved from life style changes. Firstly these factors like e.g. parity, obesity and consumption of alcohol only have a small impact on breast cancer risk in general as compared to the magnitude of the genetic cancer risk of a BRCA1/2 mutation. Moreover the environmental effects in hereditary breast cancer may not act in a similar fashion as compared to non-hereditary breast cancer. For instance the risk of parity in BRCA1/2 carriers has been reported to be the opposite of what is observed in the general population (Jernstrom et al, 1999). Finally some life style factors may act beneficially with regard to breast cancer risk but are otherwise harmful to a woman's health. In this context it has been suggested that smoking lowers breast cancer risk in BRCA1 carriers (Brunet et al, 1998). External modifying factors of the genetic risk for breast and ovarian cancer have recently been reviewed by Narod (Narod, 2002).

### 1.5.1 Breast surveillance

In many western countries women who are at risk for developing breast cancer because of a strong family history for the disease are offered to participate in a breast surveillance program (Vasen et al, 1998). Besides physical examination and breast self-examination these surveillance programs rely mainly on mammography as a method for early detection. The (additional) value of magnetic resonance imaging is currently under investigation, with promising preliminary results (Warner et al, 2001; Stoutjesdijk et al, 2001).

Regular mammography has been shown to reduce mortality in women in the ages 50-70 years and additional evidence exists with regard to younger age groups (40-50 years) (Bjurstam et al, 1997; Falun Meeting Report, 1996), although the debate concerning the evidence has recently flared up (Olson and Gotzsche, 2001; Nyström et al, 2002). Since breast cancer risk in BRCA1/2 carriers is evident at ages far younger, mammography is performed from the age of +/-25 years. The value of surveillance in young women in general could be less because of increased density of the breast tissue, masking a malignant lesion in a mammography.

Tumors in BRCA1/2-mutation carriers potentially are fast growing tumors as indicated by the high proliferation indices of BRCA1 breast cancer. In that case intervals between rounds of surveillance will be too long to detect a tumor while it is still small. Characteristics of the tumor itself could make it hard to detect like the absence of high grade DCIS with calcifications or the presence of pushing margins, thus mimicking a benign lesion (Tilanus-Linthorst et al, 2002). Finally it appears that breast screening in women age 40 to 49 years does not reduce mortality in women with grade 3 ductal carcinomas (Tabar et al, 1997) but in fact the large majority of BRCA1/2-associated tumors is of high grade. For all these reasons it might be that, especially in this group of women, mammography is insufficiently effective and might not prevent death of breast cancer.

Experiences from the Rotterdam Family Cancer Clinic indeed showed less favorable results of screening by mammography in BRCA1/2 mutation carriers (Brekelmans et al, 2001). However, preliminary screening results with MRI appear to be much more promising. No matter how effective breast surveillance will be, some women with small node-negative invasive breast tumors, detected on mammography or MRI, will eventually die from breast cancer.

### 1.5.2 Ovarian surveillance

The most applied surveillance strategy for ovarian cancer is the use of vaginal ultrasonography with blood measurement of the tumor marker, CA-125. Additional value could be derived from other tumor markers or the use of color Doppler ultrasound (Crump et al, 2000; Schelling et al, 2000). Neither vaginal ultrasound nor CA-125 measurement has been shown to reduce mortality from ovarian cancer (Rosenthal and Jacobs, 1998, Einhorn et al, 2000), but this could be different for ovarian cancer screening in a group of high-risk women. A major problem is the low positive predictive value of ovarian cancer screening or in other words the large number of benign conditions that is detected requiring further investigation and thus leading to unnecessary surgery (van Nagell et al, 2000).

### 1.5.3 Prophylactic mastectomy

Prophylactic mastectomy is considered a mutilating procedure and requires careful discussion with a patient. Acceptance of this measure by women who have undergone prophylactic surgery is good (Stefanek et al, 1995a&b; Klijn et al, 1997; Lodder et al, 2002) provided that the discussion leading to a decision for prophylactic mastectomy should primarily be a patient-initiated one, instead of a physician-initiated discussion (Payne et al, 2000). Aim of the procedure should be to remove all breast tissue, in order to optimally reduce the risk for breast cancer. This cannot be technically achieved in all cases. Prophylactic

mastectomy is usually followed by immediate breast reconstruction. Investigating the efficacy of prophylactic mastectomy in prospective randomized trials is impossible because it is considered unethical to randomize.

Large retrospective series of prophylactic mastectomy in high-risk women show a risk reduction below the level of breast cancer risk in the general population (Hartmann et al, 1999). The remaining risk for breast cancer after bilateral prophylactic mastectomy is estimated to be less than 10% and relates to the amount of breast tissue not surgically removed. Short-term results from the Rotterdam Family Cancer Clinic show that prophylactic bilateral mastectomy significantly reduces the incidence of breast cancer. No breast cancers were observed in 76 women, 3 years after prophylactic mastectomy whereas in the same period 8 breast cancers occurred in 63 women who remained under regular surveillance (Meijers-Heijboer et al, 2001). However reports of breast cancer following prophylactic mastectomy are not unique (reviewed by Hughes et al, 1999).

### 1.5.4 Prophylactic oophorectomy

Because of the uncertainties regarding the efficacy of ovarian cancer screening women who are carrier of BRCA1/2-germline mutations are often advised to undergo prophylactic oophorectomy, that is when childbearing is considered to be complete. Because the fallopian tubes are also at risk for developing cancer in BRCA1/2 carriers a salpingo-oophorectomy is undertaken. This is a relatively small surgical procedure that can be performed using laparoscopy. The first short-term results of prophylactic oophorectomy are now emerging, showing a decrease in both breast cancer and BRCA1/2 related gynecologic cancer (Kauff et al, 2002).

Of some concern is the remaining risk of subsequent papillary serous carcinoma of the peritoneum (PSCP), also called 'extra-ovarian ovarian cancer'. PSCP is reported to occur following prophylactic oophorectomy and since it is one of the tumors belonging to the BRCA1-cancer spectrum it is clear that

prophylactic oophorectomy can not completely prevent the development of peritoneal 'ovarian' cancer. The additional benefit of prophylactic oophorectomy in the reduction of breast cancer risk in BRCA1/2 carriers has recently been confirmed (Kauff et al, 2002; Rebbeck et al, 2002b). In one study prophylactic oophorectomy was shown to cause a 50-70% risk reduction for breast cancer in BRCA1-mutation carriers (Rebbeck et al, 1999b). However a surgically induced menopause at a young age may cause other serious health problems such as its psychological aspects and an increased risk of cardiovascular disease and osteoporosis. Moreover it is conceivable that hormonal replacement therapy to overcome some of these problems could increase the breast cancer risk in BRCA1/2-mutation carriers.

### 1.5.5 Chemoprevention.

The role for chemoprevention with respect to breast cancer is currently under investigation. In a group of women with a positive family history or who were otherwise at increased risk for developing breast cancer, tamoxifen reduced (the short-term) breast cancer risk (Fisher et al, 1998). Although this was first disputed by other studies (Veronesi et al, 1998; Powles et al, 1998), a recent overview combining the results of four tamoxifen prevention trials showed a 38% reduction in breast-cancer incidence (Cuzick et al, 2003). The decrease was only seen in ER-positive tumors. Tamoxifen causes an increased risk for endometrial cancer and women from these trials also had an increased risk of thrombo-embolic complications.

The potential of tamoxifen in BRCA1 mutation carriers, who more frequently are diagnosed with ER-negative tumors, is less clear. In a subgroup analysis of 19 BRCA1/2 carriers participating in the aforementioned NSABP-trial, no reduction of frequency of breast cancer was observed in BRCA1 carriers, whereas there was a suggestion of a benefit in BRCA2 carriers (King et al, 2001). Other estrogen-antagonists are being tested which may be more

specifically directed against estrogen receptors in the breast tissue in that way evading other side effects (reviewed by Cuzick et al, 2003).

Ovarian cancer risk in BRCA1-mutation carriers has been shown to be decreased by using oral contraceptives (Narod et al, 1998). The role of oral contraceptives with regard to breast cancer risk in BRCA1/2 carriers has been controversial (Ursin et al, 1997). The largest study addressing this subject, performed in 1311 BRCA1 and BRCA2 carriers, showed a modestly increased risk of breast cancer from the use oral contraceptives for BRCA1 mutation carriers (Narod et al, 2002).

## 1.6 Aims and outline of this thesis

The aims of this thesis are:

1 To gain insight into the contribution germline mutations in BRCA1 and BRCA2 make to clinically ascertained breast cancer families.

2 To investigate the demand of genetic testing and analyze the choices of women with a mutation with respect to cancer risk management.

3 To study the prognostic significance of BRCA1/2-germline mutations in breast cancer patients with respect to disease-free and overall survival, contralateral breast cancer risk, and the risk of ipsilateral recurrence following breast conserving therapy.

The first two objectives address some of the clinical genetic aspects of hereditary breast (and ovarian) cancer (chapters 2 and 3). The latter focuses on the outcome and characteristics of BRCA1/2-associated breast cancer (chapters 4-8).

Germline mutations in BRCA1 and BRCA2 account for 15-20% of breast cancer that clusters in families. A large number of different mutations in these genes have been identified. Presymptomatic genetic testing of individuals at risk can therefore only be offered after a BRCA1/2 mutation is identified in the family concerned. Chapter 2 describes HB(O)C family characteristics in correlation with

the proportion of mutations found. Because of apparent large regional differences in the frequency of distinct BRCA1/2 mutations, the contribution of 2 specific founder mutations in a small population based series of breast cancer patients is determined.

The uptake of pre-symptomatic testing and subsequent choices of female mutation carriers for breast and ovarian cancer prevention are reported in chapter 3. The demand for BRCA1/2 testing by unaffected individuals at risk and the subsequent option for prophylactic surgery by female mutation carriers were correlated with age, presence of children and pre-test genetic risk.

Data on outcome and tumor characteristics of hereditary breast cancer are of great importance for the clinical decision making on choice of therapy, surveillance and prophylactic surgery. The prognostic significance of germline BRCA1 and BRCA2 mutations in breast cancer patients with regard to disease-free interval, overall survival, contralateral breast cancer occurrence and ipsilateral breast cancer recurrence is investigated.

In chapters 4 and 5 the disease free interval and overall survival of breast cancer patients from families with a proven BRCA1 or BRCA2 mutation were compared to patients with non-BRCA1/2 breast cancer patients matched for age and year of diagnosis. The role of BRCA2 as a prognostic factor is further discussed in chapter 6. Breast cancer caused by BRCA2 mutations has features distinct from BRCA1-associated breast cancer especially with regard to steroid receptor status.

Chapter 7 shows that the contralateral breast cancer risk in BRCA1-associated patients is related to the age at onset of the first tumor. In chapter 8 the outcome of breast conserving therapy in hereditary and sporadic breast cancer patients is investigated. A higher frequency of (late) ipsilateral breast cancer recurrences was seen in hereditary patients as a result from the occurrence of new primary tumors in the treated breast as well as recurrence of the first tumor. This is in line with the notion that in these patients all remaining breast tissue is genetically predisposed for developing breast cancer. Both findings have implications for clinical decision making.

Finally chapter 9 discusses the results of the studies described in this thesis and gives new perspectives. A number of crucial questions, remaining to be answered, are posed.

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# Chapter 2

## Large regional differences in the frequency of distinct BRCA1/BRCA2 mutations in 517 Dutch breast and/or ovarian cancer families.

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Verhoog LC, van den Ouweland AM, Berns E, van Veghel-Plandsoen MM, van Staveren IL, Wagner A, Bartels CC, Tilanus-Linthorst MM, Devilee P, Seynaeve C, Halley DJ, Niermeijer MF, Klijn JG, Meijers-Heijboer H.

# Large regional differences in the frequency of distinct *BRCA1/BRCA2* mutations in 517 Dutch breast and/or ovarian cancer families

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**Abstract**

In 517 Dutch families at a family cancer clinic, we screened for *BRCA1/2* alterations using the Protein Truncation Test (PTT) covering approximately 60% of the coding sequences of both genes and direct testing for a number of previously identified Dutch recurrent mutations. In 119 (23%) of the 517 families, we detected a mutation in *BRCA1* ($n=98$; 19%) or *BRCA2* ($n=21$; 4%). *BRCA1/2* mutations were found in 72 (52%) of 138 families with breast and ovarian cancer (HBOC), in 43 (13%) of the 339 families with breast cancer only (HBC), in 4 (36%) of 11 families with ovarian cancer only (HOC), and in none of 29 families with one single young case (<40 years) of breast cancer. Between the different subgroups of families (subdivided by the number of patients, cancer phenotype and age of onset) the proportion of *BRCA1/2* mutations detected, varied between 6 and 82%. Eight different mutations, each encountered in at least six distinct families, represented as much as 61% (73/119 families) of all mutations found. The original birthplaces of the ancestors of carriers of these eight recurrent mutations were traced. To estimate the relative contribution of two important regional recurrent mutations (*BRCA1* founder mutation IVS12-1643del3835 and *BRCA2* founder mutation 5579insA) to the overall occurrence of breast cancer, we performed a population-based study in two specific small regions. The two region-specific *BRCA1* and *BRCA2* founder mutations were detected in 2.8% (3/106) and 3.2% (3/93) of the unselected breast tumours, respectively. Of tumours diagnosed before the age of 50 years, 6.9% (3/43) and 6.6% (2/30) carried the region-specific founder mutation. Thus, large regional differences exist in the prevalence of certain specific *BRCA1/BRCA2* founder mutations, even in very small areas concerning populations of approximately 200 000 inhabitants. 

*Keywords:* Breast cancer; *BRCA1*; *BRCA2*; Mutation detection rate; Founder mutations; Population-based studies; Family cancer clinic

## 1. Introduction

Since the identification of the breast cancer susceptibility genes *BRCA1* [1] and *BRCA2* [2] in 1994 and 1995, respectively, a growing number of members from families with clustering of breast and/or ovarian cancer have sought genetic counselling [3]. In general, genetic testing of individuals at risk can only be offered when a specific mutation that segregates with the disease has been identified within the family. Both *BRCA1* and *BRCA2* are large genes and germline mutations in these genes are scattered throughout the coding sequences.

Both for practical and cost-effectiveness reasons, the probability that an individual with breast or ovarian cancer may have a mutation in *BRCA1/BRCA2* is an important consideration in genetic testing. Therefore models have been developed, based on characteristics such as age at diagnosis of breast cancer and the number of breast and/or ovarian cancer patients in a family, to predict mutation carrier status before testing [4–7].

The ethnic background of a patient can strongly influence these probability models. For example, Ash-

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kenazi Jewish breast cancer patients have significantly higher probabilities for carrying a *BRCA1* mutation [4]. This is explained by the fact that 3 *BRCA1/2* founder mutations (*BRCA1* 185delAG, 5382insC and *BRCA2* 6174delT) are encountered at frequencies of 1, 0.1 and 1.5%, respectively, in the Ashkenazi Jewish population [8,9]. Similar effects were observed in breast cancer patients from the Icelandic population, in which the *BRCA2* 999del5 founder mutation is prevalent (population frequency of 0.6%) [10,11].

In other countries, including The Netherlands, several recurrent mutations in the *BRCA1* and *BRCA2* genes have been described [12–21]. Thus far, haplotype analysis of Dutch recurrent mutations was consistent with a single origin of these mutations, indicating that they are founder mutations. In particular, the recurrent mutations IVS12-1643del3835 in *BRCA1* and 5579insA in *BRCA2*, highlighted in the present study, were also shown to be founder mutations [15,18].

By the end of 1998, 517 families with either clustering of breast and/or ovarian cancer or a single case of early onset breast cancer were registered at the Family Cancer Clinic of the Daniel den Hoed Cancer Center and/or the Department of Clinical Genetics of the Erasmus University Rotterdam. We determined family characteristics in terms of the age at onset of breast cancer, the presence of ovarian cancer and the number of affected individuals in the pedigree in relation to the percentage of mutations identified with a routinely applied set of mutation-detection methods.

Frequencies of *BRCA1* and *BRCA2* founder mutations detected in the Southwestern part of The Netherlands differed from those reported elsewhere in The Netherlands [16] (see the BIC database). Therefore, we looked more closely into the geographical origin of the families with an identified mutation and investigated the prevalence of certain founder mutations in population-based series of breast cancer patients from specific regions within the South-western part of The Netherlands.

## 2. Patients and methods

### *2.1. Families and geographical distribution of families with a mutation*

A series of families with clustering of breast and/or ovarian cancer was referred for onco-genetic and medical counselling to our departments between 1 January 1994 and 1 January 1999; this closing date was chosen because the routinely applied mutation-detection methods at that time took 6 to 12 months. Eligible for the present study were all families out of these series in which *BRCA1/BRCA2* mutation analysis was performed ($n = 517$), according to a protocol approved by the Medical Ethical Committees of our institutes. In general, a family was eligible for screening for mutations in *BRCA1/BRCA2* when it met one of the criteria listed in Table 1. The number of first and second-degree relatives with breast and/or ovarian cancer was determined by the relationship of an affected relative to the nearest affected individual in the pedigree. Considering the high penetrance of *BRCA1/BRCA2* mutations in women, as well as the heterogenetic origin of breast cancer, we excluded second-degree affected relatives who were daughters of unaffected women, whereas second-degree affected relatives who were daughters of men were included.

For each family, a detailed pedigree encompassing at least four generations was constructed. Whenever possible, hospital records and pathology reports were collected from individuals with malignancies to confirm the diagnosis. Age at onset of breast cancer was registered in three categories: the number of relatives diagnosed before the age of 40 years, the number of relatives diagnosed from 40 to 49 years and the number of relatives diagnosed with breast cancer from the age of 50 years and over. Pedigree data were used to identify the ancestors most likely to have transmitted the genetic predisposition in each of the families. On average, such an ancestor was born around 1890. The place of birth of that ancestor was taken as the place of origin of a family. Occasionally, it was possible to link separate families of which the probands were not aware they were related: these families were then considered as one family.

### *2.2. Population-based breast cancer patients*

In view of the results with respect to the geographical origin of the two founder mutations IVS12-1643del3835 (*BRCA1*) and 5579insA (*BRCA2*), we performed a population-based study for the prevalence of these two mutations (Fig. 1).

From previously isolated DNA of 1052 stored breast tumour samples which were sent to our regional central laboratory for routine steroid receptor assays [22], two

Table 1

Minimal criteria for *BRCA1/BRCA2* mutation analysis
- A single woman affected by breast cancer before the age of 40
- A single woman affected by both breast and ovarian cancer
- Two first- or second-degree[a] relatives affected by breast cancer, one of them diagnosed before the age of 45 years
- Two first- or second-degree[a] relatives, one of them affected by ovarian cancer and the other affected by breast cancer before the age of 50 years
- Two first- or second-degree[a] relatives affected by ovarian cancer
- Three first- or second-degree[a] relatives affected by either breast cancer or ovarian cancer

[a] Only second-degree relatives who were paternally related to another affected relative, and not maternally, were taken into account.

groups of breast cancer patients were selected on the basis of their region of residence: (a) patients ($n=106$) who at time of diagnosis were living in the region (West-Brabant) of clustering of the IVS12-1643del3835 *BRCA1* founder mutation; and (b) patients ($n=93$) from the region (Zuid-Beveland) of clustering of the 5579insA *BRCA2* founder mutation (see also Fig. 1). In both groups, no selection was made for age at diagnosis or family history.

These 199 DNA samples were irreversibly made anonymous, with only the geographical region of where the patient lived and the age at diagnosis recorded. The region was defined and registered as the zip code area. The Netherlands (population of approximately 16 million inhabitants) is divided into 90 zip code areas. All samples were tested for both mutations, and for 2804delAA which is one of the most frequently detected *BRCA1* mutation throughout the whole Dutch population.

### 2.3. *DNA analysis*

In 517 separate families, DNA analysis was performed using genomic DNA, preferably of all living affected relatives with breast and/or ovarian cancer. On average, 1.9 patients per family were tested. As screening of the entire coding sequences of both genes is costly and was not accessible in our clinical setting on a routine basis, we applied in all these families a set of mutation-detection assays covering at least 60% of the coding sequences of both genes. This set consisted of a Protein Truncation Test (PTT) of exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2* that was performed as previously described [23] with minor modifications. In addition, single strand conformation polymorphism (SSCP) analysis of exon 2 of *BRCA1* (which included detection of the mutations 185insA and 185delAG); allele specific oligonucleotide hybridisation (ASO) analysis of the founder mutations 5382insC and IVS20+1G>A was performed. Finally, the founder mutations IVS12-1643del3835 and IVS21-36del510 were tested by a polymerase chain reaction (PCR) analysis specific for these large genomic deletions [15].

In a subset of 106 families, SSCP analysis of the remaining coding exons of the *BRCA1* gene was performed and in subset of 23 families, PTT analysis of the complete *BRCA2* gene from reverse transcriptase (RT)-PCR obtained products was undertaken. The *BRCA1/2* mutations identified by these additional analyses were not taken into account with regard to the proportion of mutations identified in relation to family characteristics (Table 2) to make comparisons between the subgroups possible. For the population-based study, DNA from tumour samples of breast cancer patients was tested with an ASO analysis for the *BRCA1* mutation 2804delAA and the *BRCA2* mutation 5579insA. Deletion-specific PCR analysis was used to detect the *BRCA1* mutation IVS12-1643del3835.

| | | | | | |
|---|---|---|---|---|---|
| | *BRCA1* | | ◆ | IVS20+1G>A | n=10 |
| ★ | 185insA | n=6 | ◆ | IVS21-36del510 | n=8 |
| ✤ | 1411insT | n=7 | | *BRCA2* | |
| ▼ | 2804delAA | n=8 | ⊙ | 5579insA | n=6 |
| ⊗ | IVS12-1643del3835 | n=17 | + | 6503delTT | n=7 |

○ Other BRCA1/2 mutations found <6 times each; n=41

Fig. 1. Geographical distribution of the places from which each family with one of the eight recurrent BRCA1/BRCA2 mutations originate; the arrow indicates Rotterdam.

Table 2
Frequency of *BRCA1* and *BRCA2* mutations, depending on the number, age at diagnosis and site of origin of the cancers in the family[a]

| | 0 BC below 40 years | 1 BC below 40 years | ≥2 BC below 40 years | Total |
|---|---|---|---|---|
| HBC | | | | |
| Total | 172 | 118 | 49 | 339 |
| Mutations | | | | |
| *BRCA1* | 8 | 14 | 9 | 31 |
| *BRCA2* | 3 | 4 | 4 | 11 |
| Either gene | 11 (6%) | 18 (15%) | 13 (27%) | 42 (12%) |
| HBOC with 1 OC | | | | |
| Total | 47 | 30 | 17[b] | 94 |
| Mutations | | | | |
| BRCA1 | 7 | 12 | 12 | 31 |
| BRCA2 | 1 | 3 | 0 | 4 |
| Either gene | 8 (17%) | 15 (50%) | 12 (71%) | 35 (37%) |
| H(B)OC with ≥2 OC | | | | |
| Total | 27 | 12[b] | 16[b] | 55 |
| Mutations | | | | |
| *BRCA1* | 12 | 7 | 8 | 27 |
| *BRCA2* | 2 | 1 | 1 | 4 |
| Either gene | 14 (52%) | 8 (67%) | 9 (56%) | 31 (56%) |

BC, breast cancer; OC, ovarian cancer; HBC, hereditary breast cancer; HBOC, hereditary breast and ovarian cancer; HOC, hereditary ovarian cancer.
[a] Only mutations detected with the routinely applied set of mutation-detection methods are shown.
[b] Taking these 3 subgroups together, the proportion of identified *BRCA1*/*BRCA2* mutations rose from 64% (29/45) to 78% (35/45) when results of the more extensive mutation analyses were included.

### 2.4. Statistical analysis

*P* values were calculated using the two-sided Fisher's Exact test. All analyses were performed using STATA 6.0 software.

## 3. Results

### 3.1. Family characteristics and mutation spectrum

Overall, in the 517 families 119 (23%) mutations in total were detected in *BRCA1* ($n=98$; 19%) and in *BRCA2* ($n=21$; 4%). Table 3 lists the general clinical characteristics of the families in which genetic analysis was performed and the number of mutations found per gene. In 52% ($n=72$) of 138 families with both breast and ovarian cancer (HBOC), a mutation was identified: in *BRCA1* in 46% ($n=64$) and in *BRCA2* in 6% ($n=8$). In families with breast cancer only (HBC), in 13% ($n=43$) of the 339 families a mutation was detected: in *BRCA1* in 9% ($n=31$) and in *BRCA2* in 4% ($n=12$); and in families with ovarian cancer only (HOC) in 36% ($n=4$) of 11 families a mutation was detected: in *BRCA1* in 27% ($n=3$) and in *BRCA2* in 9% ($n=1$).

Table 3
Frequency of *BRCA1* and *BRCA2* mutations in relation to the presence of breast cancer and ovarian cancer

| Family characteristics | No of families | BRCA1 (%) | BRCA2 (%) | Either gene (%) |
|---|---|---|---|---|
| HBC | | | | |
| 1 patient with BC below 40 years | 29 | 0 | 0 | 0 |
| 2 patients with BC | 131 | 11 (8) | 4 (3) | 15 (11) |
| ≥3 patients with BC | 208 | 20 (10) | 8 (4) | 28 (13) |
| HOC | | | | |
| ≥2 patients with OC | 11 | 3 (27) | 1 (9) | 4 (36) |
| HBOC | | | | |
| 1 patient with both BC and OC and ≥1 patients with BC | 27 | 14 (52) | 2 (7) | 16 (59) |
| 1 patient with OC and ≥1 patients with BC | 67 | 22 (33) | 3 (4) | 25 (37) |
| 2 patients with OC and ≥1 patients with BC | 27 | 15 (56) | 2 (7) | 17 (63) |
| ≥3 patients with OC and ≥1 patients with BC | 17 | 13 (76) | 1 (6) | 14 (82) |
| Total | 517 | 98 (19) | 21 (4) | 119 (23) |

BC, breast cancer; OC, ovarian cancer; HBC, hereditary breast cancer; HOC, hereditary ovarian cancer; HBOC, hereditary breast and ovarian cancer.

The majority (68%: 67 out of 98 families) of *BRCA1* mutations were found in the HBOC/HOC families, whereas less than half of *BRCA2* mutations were detected in the HBOC/HOC families (43%: 9 out of 21 families); this difference was statistically significant ($P = 0.04$).

Table 4 lists all 38 distinct mutations identified and the number of families in which each mutation was found. In addition, the total number of breast and ovarian cancer cases and relative percentages per mutation are shown. Fig. 2a and b show for each family the position of the mutation in the gene and the relative contribution of the number of breast cancer cases and ovarian cancer cases to the clinical phenotype. By far the most frequent mutation was the large 3.8 kb genomic deletion IVS12-1643del3835 encompassing exon 13 in *BRCA1*, which was found in 20 families with a total of 109 breast and/or ovarian cancer cases. Six of the *BRCA1* and two of the *BRCA2* recurrent mutations were encountered six times or more, together being responsible for 61% (73/119) of the families with a detected mutation.

Table 2 shows the probability of finding a *BRCA1* and *BRCA2* mutation with the routinely applied mutation screen, in relation to the cancer phenotype in the family. Initially, the number of affected relatives diag-

Table 4
Number of families for each mutation, and frequency of cases of breast cancer (BC) and ovarian cancer (OC) per mutation

| BRCA1 | Exon | No. of families | No. of BC/OC[a] | No. of BC (%) | No. of OC (%) |
|---|---|---|---|---|---|
| 185insA | 2 | 6 | 26 | 21 (78) | 6 (22) |
| 185delAG | 2 | 3 | 9 | 8 (89) | 1 (11) |
| W372X | 11 | 1 | 2 | 1 (50) | 1 (50) |
| 1411insT | 11 | 7 | 35 | 30 (86) | 5 (14) |
| S510X | 11 | 1 | 3 | 2 (67) | 1 (33) |
| 2312del5 | 11 | 3 | 8 | 7 (78) | 2 (22) |
| Q780X | 11 | 3 | 17 | 14 (82) | 3 (18) |
| 2524delTG | 11 | 1 | 3 | 1 (33) | 2 (67) |
| 2765delTGC | 11 | 1 | 6 | 4 (67) | 2 (33) |
| 2804delAA | 11 | 8 | 27 | 17 (63) | 10 (37) |
| E908X | 11 | 4 | 19 | 12 (57) | 9 (43) |
| 2846del4 | 11 | 1 | 7 | 5 (71) | 2 (29) |
| 3604delA | 11 | 1 | 2 | 2 (100) | 0 |
| 3668delAGinsT | 11 | 1 | 5 | 2 (40) | 3 (60) |
| E1214X | 11 | 1 | 5 | 5 (100) | 0 |
| 3875del4 | 11 | 1 | 3 | 3 (100) | 0 |
| 3889delAG | 11 | 2 | 8 | 4 (44) | 5 (56) |
| 4284delAG | 12 | 2 | 12 | 11 (79) | 3 (21) |
| IVS12-1643del3835 | 13 | 20 | 109 | 82 (71) | 33 (29) |
| R1443X | 13 | 2 | 7 | 6 (75) | 2 (25) |
| 5149del4 | 17 | 1 | 3 | 3 (75) | 1 (25) |
| 5256delG | 18 | 1 | 4 | 4 (80) | 1 (20) |
| IVS18-1G > A | 19 | 1 | 8 | 6 (67) | 3 (33) |
| 5382insC | 20 | 5 | 19 | 16 (80) | 4 (20) |
| IVS20 + 1G > A | 20 | 10 | 30 | 26 (81) | 6 (19) |
| 5448insC | 22 | 1 | 5 | 4 (67) | 2 (33) |
| IVS21-36del510 | 22 | 9 | 35 | 30 (75) | 10 (25) |
| IVS22 + 5G > A | 22 | 1 | 14 | 11 (79) | 3 (21) |
| Total | | 98 | 431 | 337 | 120 |
| BRCA2 | Exon | No of families | No of BC/OC[a] | No of BC (%) | No of OC (%) |
| 862delAG | 8 | 1 | 4 | 3 (75) | 1 (25) |
| 4682del4 | 11 | 1 | 3 | 3 (100) | 0 |
| 4708insA | 11 | 1 | 8 | 7 (64) | 4 (36) |
| 5578delAA | 11 | 1 | 2 | 2 (67) | 1 (33) |
| 5579insA | 11 | 6 | 22 | 13 (59) | 9 (41) |
| S1882X | 11 | 1 | 3 | 3 (100) | 0 |
| Y1894X | 11 | 1 | 4 | 4 (100) | 0 |
| 6503delTT | 11 | 7 | 25 | 25 (93) | 2 (7) |
| 6872del4 | 11 | 1 | 4 | 3 (60) | 2 (40) |
| 9900insA | 27 | 1 | 3 | 3 (100) | 0 |
| total | | 21 | 78 | 66 | 19 |

[a] Figures of breast and ovarian cancer do not add up because of cases with both breast and ovarian cancer.

nosed with breast cancer at ages 40–49 years and at ages ≥ 50 years were also taken into account for each of the subgroups of Table 2. However, these 2 parameters did not play a role in the probability of detecting *BRCA1/BRCA2* mutations once the classification according to the number of early onset breast cancer and number of ovarian cancers was made. The only exception was a minor influence of the number of breast cancer patients diagnosed between 40 and 49 years in families with HBC (data not shown).

The proportion of *BRCA1/BRCA2* mutations that was detected varied from 6% (11/172) in the HBC families without breast cancer patients diagnosed before the age of 40 years (Table 2), to 82% (14/17) in the HBOC families with 3 or more ovarian cancer patients and one or more breast cancer patients (Table 3). In our series, we recorded in total 13 male breast cancer patients in 12 different families. In 5 (42%) out of these 12 families, a mutation was detected: four *BRCA1* mutations (in three HBOC and one HBC families) and one *BRCA2* mutation (in a HBOC family).

### *3.2. Geographical distribution of families with a mutation*

The geographical origin of the families with the eight most frequently occurring recurrent *BRCA1* and *BRCA2* mutations is shown in Fig. 1. Five pairs of

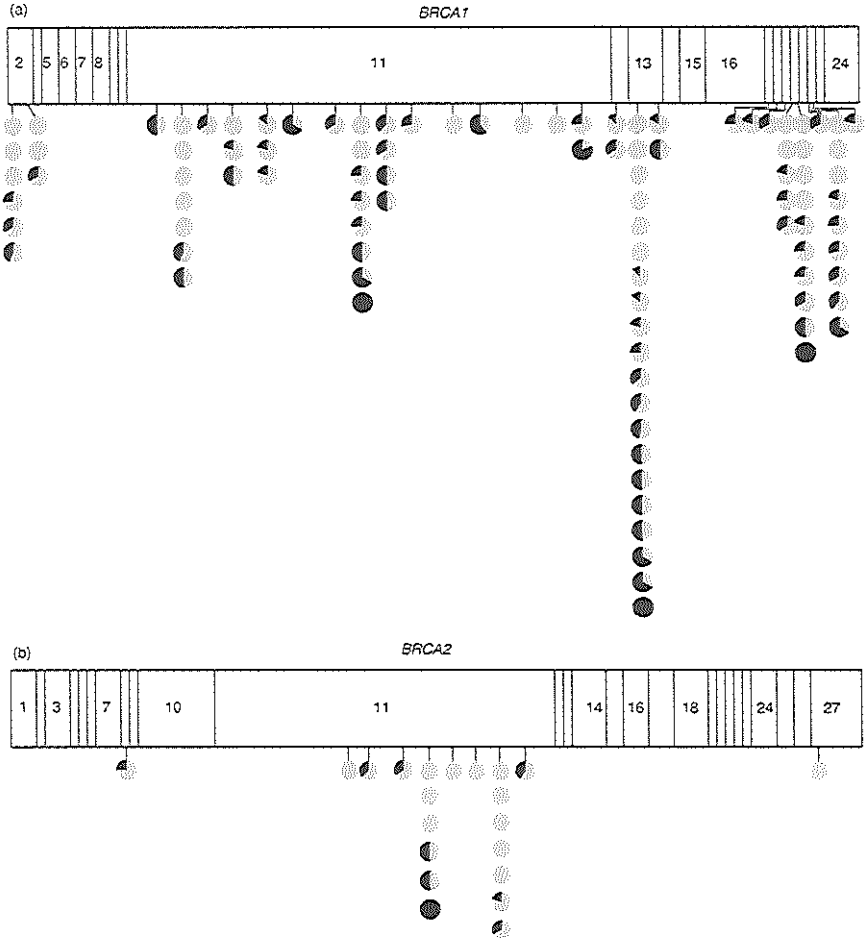


Fig. 2. (a) Position of germline mutations in *BRCA1* in 98 families; the ratio of cases of ovarian cancer (black) to cases of breast cancer in each separate family is represented within one circle. (b) Position of germline mutations in *BRCA2* in 21 families; the ratio of cases of ovarian cancer (black) to cases of breast cancer in each separate family is represented within one circle.

families, of which the probands were not aware that they were related, appeared to be linked; each of those pairs was mapped out as a single family.

The arrow in Fig. 1 refers to the urban area of Rotterdam, in which at present approximately 750 000 inhabitants are living. Geographical clustering was seen for a number of recurrent mutations, particularly the *BRCA1* mutations 185insA, 1411insT, IVS12-1643del3835, and the *BRCA2* mutation 5579insA. Most striking is the situation for the *BRCA1* IVS12-1643del3835 mutation and for the *BRCA2* 5579insA mutation: these cluster in two distinct, geographically adjacent regions of a number of small towns and villages that until now were independent rural districts with current populations of approximately 250 000 and 150 000 inhabitants, respectively.

### *3.3. Mutation analysis in population-based tumour samples*

Of the 199 tumours selected for testing for either the *BRCA1* mutation IVS12-1643del3835 ($n=106$) or the *BRCA2* 5579insA mutation ($n=93$), the mean age at diagnosis was 57 years (range 24–85 years). In both regions, the 'region-specific' mutation was found in 3/106 (2.8%; breast cancers diagnosed at ages 34, 43 and 48 years) and 3/93 (3.2%; diagnosed at ages 42, 47 and 53 years), respectively, of the unselected breast tumours. In the '*BRCA1*-founder' region, the *BRCA2* founder mutation 5579insA and the other Dutch founder *BRCA1* mutation (2804delAA), were detected once (both 1/106; 0.9% age at diagnosis, 42 and 39 years, respectively). In the '*BRCA2*-founder region' none of the other two founder mutations were detected. Of the eight tumours with one of the three germline mutations, seven were diagnosed before the age of 50 years. If only breast tumours diagnosed below the age of 50 years were considered, the prevalence of these founder mutations in the regions of clustering was 6.9% (3/43) for the *BRCA1* mutation and 6.6% (2/30) for the *BRCA2* mutation. Regarding all tumours from both regions diagnosed before the age of 50 years, in 10% (7/73) one of the three *BRCA1/BRCA2* founder mutations was detected.

## 4. Discussion

In this report, we describe the results of a *BRCA1/BRCA2* germline mutation analysis in a large series of 517 families visiting our Family Cancer Clinic. Overall, we detected a *BRCA1* mutation in 19% of the families, while in 4% a *BRCA2* mutation was identified. In accordance with others, we found that the presence of ovarian cancer, early onset of breast cancer (<40 years), and increasing numbers of young affected women in a family, greatly enhanced the probability of finding a mutation [4–7,24]. In addition, our data confirm that apart from *BRCA2*, BRCA1 mutations are also involved in male breast cancer [2,24] and that both *BRCA1* and *BRCA2* analysis is warranted in HBC/HBOC families with a case of male breast cancer.

We detected no *BRCA1/BRCA2* mutations in 29 families with a single case of breast cancer before the age of 40 years. This seems to be in contrast with breast cancer population studies, where *BRCA1* or *BRCA2* mutations were identified in 5.9–9.4% of the patients diagnosed at ages below 35/36 years [25,26]. However, our 29 patients in fact were strongly selected for not having a positive family history for the disease since for each family an extended pedigree encompassing at least 4 generations was constructed. In contrast, in population-based studies cases with a positive family history for the disease will inevitably be included. Therefore, detailed pedigree analysis is an important tool in determining the probability of finding a mutation in *BRCA1/BRCA2*.

Currently, only a few studies describe a complete analysis of the coding sequences of the *BRCA1* and *BRCA2* genes in a series of families visiting a family cancer clinic [7,16,27]. With the set of mutation-detection methods completed in all 517 families, we analysed approximately 60% of the coding sequences of the *BRCA1* and *BRCA2* genes, and therefore will have missed an unknown number of mutations. Despite this limitation, our overall *BRCA1/BRCA2* mutation-detection rate in 138 HBOC families (52%) was similar to the *BRCA1/BRCA2* mutation-detection rate (50%) found by Frank and colleagues [7] in another large series of clinically ascertained HBOC families ($n=117$). This could indicate that we have detected the majority of identifiable mutations in these families. Moreover, our results appear to be nearly identical to those of two recently presented smaller studies involving 100 HBOC [27] and 268 HB(O)C families [28], respectively, analysing the complete coding sequence of *BRCA1* and/or *BRCA2*.

The two Dutch founder mutations in *BRCA1* (IVS12-1643del3835) and *BRCA2* (5579insA) were mainly detected in families originating from small, confined regions in the South-western part of The Netherlands. The cause of the geographical differences in the prevalence of founder mutations on such a small map-scale may be specific demographic or geographical conditions. In the 16th century, the region of clustering of the *BRCA1* founder mutation (West-Brabant) was nearly de-populated due to a religious war (Roman-Catholics against protestants); afterwards the region was re-populated by large scale reproduction of a limited number of people. Our findings may be explained by a founder mutation carried by one of these ancestors. Interestingly, one village in the *BRCA1* founder-region has already been shown to be a genetic isolate for other

inherited diseases [29]. In the past, religious preferences contributed also significantly to the isolation of communities in our country. We found that all ancestors of the families with the *BRCA1* founder mutations were Roman Catholics, while all ancestors of the families with the *BRCA2* founder mutation were protestant. Furthermore, the region of clustering of the *BRCA2* founder mutation (Zuid-Beveland) was a rather isolated island until the nineteenth century. Apart from migration-characteristics of a population, the time period of origin of the mutation is an important factor with respect to geographical clustering of founder mutations. In this respect, it is interesting to note that families with the Dutch *BRCA1* founder mutation 2804delAA, which was estimated to have originated about 32 generations ago, have places of origin more scattered across The Netherlands [14] (Fig. 1).

In order to estimate the clinical impact of these two specific founder mutations on breast cancer incidence in the two geographical regions, we performed a population-based study of breast tumours from these regions. First of all, it is noteworthy that there are no significant regional differences in the age-adjusted mortality rates from either breast or ovarian cancer in The Netherlands [30]. As much as 7% of breast tumours selected for age at diagnosis below 50 years, but unselected for family history, were due to the region-specific founder mutations only. In a British population-based study, 6.1% of patients with breast cancer at ages below 50 years were estimated to be carriers of any *BRCA1* or *BRCA2* mutation [25]. Since we tested for only three *BRCA1/2* mutations in the population study, all other mutations remained undetected. Thus, already a relatively large proportion of breast cancer below the age of 50 years from these two regions was due to the *BRCA1/BRCA2* founder mutations (10%; 7/73).

By further comparison, at least one of the three founder mutations in the Ashkenazi Jewish population and the single founder mutation in the Icelandic population are found in 14 and 7.7%, respectively, of women with breast cancer below the age of 50 years that were unselected for family history [11,31]. Finally, the percentage of mutations we detected in our population-based study was comparable to the prevalence of the total of *BRCA1* mutations identified in a hospital-based study of 642 breast cancer patients from the Western part of The Netherlands [32].

Mapping out the origin of the ancestors of the HBC/HBOC/HOC families may facilitate the search for as yet undetectable *BRCA1/BRCA2* mutations in families from the same geographical region by reconstructing haplotypes [19]. In well-defined populations, it may even be possible to map unknown breast cancer susceptibility genes using haplotype-sharing.

In conclusion, even in a small and densely populated industrial country such as The Netherlands, large regional differences may exist in the prevalence of a *BRCA1* and a *BRCA2* founder mutation. In addition to the familial cancer history (early onset breast cancer as well as ovarian cancer), knowledge about the presence and prevalence of founder mutations in specific populations is of importance for selecting families eligible for *BRCA1/BRCA2* analysis and will greatly facilitate the detection of mutations.

## 5. Electronic-Database Information

Accession numbers and URLs for data in this article are as follows: BIC: http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/ (for *BRCA1* and *BRCA2* mutations); Dutch database: http://ruly70.-medfac.leidenuniv.nl/~devilee/Lab/b1nl5.htm; http://ruly70.medfac.leidenuniv.nl/~devilee/Lab/b2nl5.htm; (for *BRCA1* and *BRCA2* mutations in The Netherlands); Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov./Omim/ (for *BRCA1* [MIM 113705] and *BRCA2* [MIM 600185]).


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# Chapter 3

## Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation.

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Meijers-Heijboer EJ, Verhoog LC, Brekelmans CT, Seynaeve C, Tilanus-Linthorst MM, Wagner A, Dukel L, Devilee P, van den Ouweland AM, van Geel AN, Klijn JG.

# Presymptomatic DNA testing and prophylactic surgery in families with a *BRCA1* or *BRCA2* mutation

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## Summary

**Background** Germline mutations in the *BRCA1* and *BRCA2* genes highly predispose to breast and ovarian cancer. In families with *BRCA1* or *BRCA2* mutations, identification of mutation carriers is clinically relevant in view of the options for surveillance and prevention.

**Methods** We assessed presymptomatic DNA testing and prophylactic surgery in 53 consecutive families presenting to the Rotterdam Family Cancer Clinic with a known *BRCA1* or *BRCA2* mutation. We identified predictors for DNA testing and prophylactic surgery with univariate and multivariate analysis.

**Findings** 682 unaffected individuals with a 50% risk (275 women and 271 men) or with a 25% risk (136 women) for carrying a mutation were identified and offered a DNA test. Presymptomatic DNA testing was requested by 48% (198 of 411) of women and 22% (59 of 271) of men (odds ratio for difference between sexes 3·21 [95% CI 2·27–4·51]; p<0·001). In women, DNA testing was significantly more frequent at young age, in the presence of children, and at high pre-test genetic risk for a mutation. Of the unaffected women with an identified mutation who were eligible for prophylactic surgery, 51% (35 of 68) opted for bilateral mastectomy and 64% (29 of 45) for oophorectomy. Parenthood was a predictor for prophylactic mastectomy but not for prophylactic oophorectomy. Age was significantly associated with prophylactic oophorectomy, but not with prophylactic mastectomy, although there was a tendency towards mastectomy at younger ages.

**Interpretation** In a clinical setting, we show a high demand for *BRCA1* and *BRCA2* testing by unaffected women at risk, and of prophylactic surgery by unaffected women with the mutation. Young women with children especially opt for DNA testing and prophylactic mastectomy.

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## Introduction

Apart from age, genetic predisposition is the strongest risk factor for breast cancer. *BRCA1* and *BRCA2* gene mutations are involved in most families with the autosomal dominant inherited breast-ovarian cancer syndrome, and in about 60% of families with four or more cases of just breast cancer before the age of 60 years.[1] Women with a *BRCA1* or *BRCA2* mutation have a cumulative lifetime risk of invasive breast cancer of about 55–85%, and of invasive epithelial ovarian cancer of 15–65%.[1–5] By contrast, men with mainly *BRCA2* mutations have only a 6% lifetime risk of breast cancer, whereas risks for some other types of cancer are only slightly increased.[4–6] A large variety of mutations in the *BRCA1* and *BRCA2* genes are associated with inherited breast and/or ovarian cancer, so mutation identification is necessary in every family.[7] In the context of a known mutation in the family, identification of individuals with or without the mutation is possible by presymptomatic DNA testing. Clearly the absence or presence of a mutation will have considerable medical and psychological significance. In women who are carriers of mutations, regular surveillance, prophylactic mastectomy and oophorectomy, and chemoprevention are options that are currently considered.

Results of several attitudinal studies have shown that many (81–91%) of healthy first-degree female relatives of patients with breast or ovarian cancer are potentially interested in *BRCA1/BRCA2* testing.[8,9] By contrast, use of the DNA test was significantly lower in a series of affected and unaffected women (66%) from families with an identified *BRCA1* mutation.[10] No research has focused on the actual use of presymptomatic *BRCA1/BRCA2* testing in clinical settings (eg, family cancer clinics). There are few reports highlighting requests from patients for prophylactic surgery before genetic testing[11,12] and scarcely any following genetic testing.[10,11] No data on the actual choices made with regard to prophylactic surgery by carriers of *BRCA1/BRCA2* mutations are available.

The main aims of our study were to assess in a Family Cancer Clinic whether unaffected individuals from families with *BRCA1* and *BRCA2* mutations use the opportunity to find out their mutation carrier-status, and to assess the decisions taken by women who are identified as mutation carriers. In addition, we looked for major predictive factors for use of the DNA test and prophylactic surgery. To assess what proportion of individuals eligible for DNA testing and prophylactic surgery might be interested in the long term, we studied the time-dependent rates for the various decisions.

## Methods

### Patients

The eligible families had had a mutation in the *BRCA1* or *BRCA2* genes identified at our Family Cancer Clinic and Department of Clinical Genetics between Jan 1, 1994, and Jan 1, 1998 (in three families presymptomatic DNA testing was initially based on DNA linkage analysis). The families were part of a series of about 350 families who were at that time undergoing DNA analysis for familial breast and/or ovarian cancer; families had been referred to us by general practitioners and medical specialists since 1991. DNA analysis was done according to standard procedures.[13,14]

Use of presymptomatic DNA testing was investigated in all individuals aged 20 years and older with a genetic risk of 50% for the mutation. Women affected by breast or ovarian cancer were not included in the study. The choice between regular surveillance and prophylactic bilateral mastectomy and/or oophorectomy was offered to carriers of the mutation by a shared decision-making process. We only included unaffected female carriers at ages eligible for mastectomy and oophorectomy. None of them were lost to follow-up as most stayed under surveillance at our Family Cancer Clinic; from all others we received medical reports on the findings during follow-up.

### Procedures

Ethical approval for the study was obtained from the medical ethic's committee of our Cancer Centre in 1991 (protocol DDHK 91-17, updated in 1995). Informed consent, comprising items from the American Society of Medical Oncology,[15] was given by the individuals involved in the study.

In view of the genetic heterogeneity of breast and ovarian cancer, the search for a causative *BRCA1/BRCA2* mutation in a family was preferably performed on all living family members affected with breast and/or ovarian cancer. After identification of the family-specific mutation, the initial counsellees (index individuals) were asked to inform all adult first-degree and second-degree relatives of the patients with breast or ovarian cancer about the hereditary nature of the cancer in their family. Written information was available for the index individuals to distribute among their relatives that included facts on the inheritance of cancer in their family, the possibility of presymptomatic DNA testing, the risks of breast and ovarian cancer for female mutation carriers, and the options for regular breast and ovarian surveillance or prophylactic surgery. Relatives were invited to contact the clinic if they needed further information or wanted DNA testing.

DNA testing was done after one or more individual counselling sessions with the clinical geneticist, depending on knowledge about the issue beforehand and the need to reconsider DNA testing. During pre-test interviews, *BRCA1* and *BRCA2* related cancer risks and the efficacy of regular surveillance and prophylactic surgery were discussed.[16] Possible psychosocial sequelae of DNA testing were extensively addressed and psychological support was offered to all individuals. Disclosure of test results followed 6–12 weeks after blood sampling.

A breast and ovarian surveillance programme was offered to women with a mutation, and to women with a genetic risk of 50% or 25% (solely daughters of untested men) for the mutation. Breast surveillance started at the age of 25 years consisting of physical examination by a specialist every 3–6 months and annual mammography. Ovarian surveillance started at the age of 30 years and consisted of physical examination by a gynaecologist, vaginal ultrasonography of the ovaries, and assessment of serum CA125 concentrations twice a year. Prophylactic mastectomy was offered at age 25 years and older, and oophorectomy was offered at 35 years and older to all unaffected women with a mutation. A psychologist supported all women who considered prophylactic mastectomy. Standard bilateral simple mastectomy (including the nipple) was done on request with a simultaneous breast reconstruction by subpectoral implantation of silicone prostheses. To monitor postmastectomy breast-cancer risk and morbidity, follow-up was offered twice a year. Prophylactic oophorectomy was preferentially done by laparoscopy. Because of the residual peritoneal cancer risk, annual gynaecological follow-up was advised. Hormone-replacement therapy was prescribed in premenopausal unaffected mutation carriers who underwent both prophylactic oophorectomy and mastectomy. At the time of the study, chemoprevention was not an option offered to unaffected women who were mutation carriers.

### Statistical analysis

Descriptive statistics were used to find out the rates of DNA testing and of prophylactic mastectomy and oophorectomy. All individuals were classified according to whether they had or had not been tested or whether they had or had not had a prophylactic operation.

The predictive value of the variables of age, parenthood, and pre-test genetic risk (risk of 25% or 50% for a mutation, only in women) for the utilisation of DNA testing was first assessed separately for men and women by univariate analysis. Odds ratios and 95% CIs were calculated. Second, to assess the effect of the variables simultaneously, multivariate logistic regression was used. Odds ratios and 95% CIs were adjusted for the factors that were significant in the univariate model. The presence of interaction was tested by including product terms in the model. In the same way, the predictive value of the variables of age and parenthood for prophylactic mastectomy and oophorectomy was tested. Participants were categorised into three age-groups: less than 40 years old; 40–54 years; and 55 years and older.

We calculated Kaplan-Meier survival probabilities to assess the time-dependent rate of tested individuals. This was done for individuals with a first-degree relative affected by breast or ovarian cancer—ie, individuals with a 50% risk of carrying the mutation, based on the pedigree. Individuals with a pedigree-based risk of 25% may have awaited the test result of a parent and were therefore not included in this analysis. The time when the causative mutation was identified in the family was recorded as the date of disclosure of the family test result to the family.

Kaplan-Meier survival analysis was used to analyse the time between personal DNA-test disclosure and the decision for prophylactic surgery. The date of prophylactic surgery was used as the definite date of decision.

## Results

53 consecutive families were identified in whom a mutation in the *BRCA1* or *BRCA2* genes had been identified. These included 682 unaffected individuals with a 50% risk of carrying the mutation (275 women, 271 men) or with a 25% risk (136 women). All were offered a DNA test.

The median follow-up after identification of the family-specific mutation was 26 months (range 16–62). Table 1 lists the cases of breast and ovarian cancer in the families studied and the mean age at diagnosis per

| Gene | Number of families | Breast cancer | | Ovarian cancer | |
|---|---|---|---|---|---|
| | | Number of patients (number per family) | Mean age at diagnosis (SD) | Number of patients (number per family) | Mean age at diagnosis (SD) |
| *BRCA1* | 43 | 161 (3-7) | 42-7 (12-7) | 61 (1-4)* | 52-6 (7-2) |
| *BRCA2* | 10 | 43 (4-3) | 48-7 (12-7) | 14 (1-4)† | 62-8 (13-2) |
| Total | 53 | 204 (3-8) | 43-1‡ (12-8) | 75 (1-4) | 55-2 (9-6)† |

*Eight patients with both breast and ovarian cancer. †Two patients with both breast and ovarian cancer. ‡Mean age based on 151 breast cancer and 64 ovarian cancer patients with confirmation of the diagnosis through hospital record or pathology report.
SD=standard deviation.

Table 1: **Characteristics of studied families**

| Variable | Number tested (%) | Univariate odds ratio (95% CI) | p | Multivariate odds ratio (95% CI) | p |
|---|---|---|---|---|---|
| **Women** | | | | | |
| Age | | | | | |
| <50 years | 162/292 (55) | 1·00 | .. | 1·00 | .. |
| ≥50 years | 36/119 (30) | 0·54 (0·37–0·79) | 0·002 | 0·32 (0·20–0·50) | <0·001 |
| Parenthood* | | | | | |
| No children | 47/106 (44) | 1·00 | .. | 1·00 | .. |
| Children | 151/235 (64) | 2·25 (1·46–3·48) | <0·001 | 3·45 (2·12–5·62) | <0·001 |
| Genetic risk | | | | | |
| 25% | 40/136 (29) | 1·00 | .. | 1·00 | .. |
| 50% | 158/275 (57) | 3·47 (2·22–5·40) | <0·001 | 2·45 (1·42–4·20) | 0·001 |
| **Men** | | | | | |
| Age | | | | | |
| <50 years | 29/151 (19) | 1·00 | .. | 1·00 | .. |
| ≥50 years | 30/120 (25) | 1·32 (0·78–2·24) | 0·29 | 1·01 (0·58–1·77) | 0·97 |
| Parenthood† | | | | | |
| No children | 6/70 (9) | 1·00 | .. | 1·00 | .. |
| Children | 53/186 (28) | 5·17 (2·13–12·57) | <0·001 | 5·16 (2·09–12·72) | <0·001 |

*Not all data add up to total because of missing data on parenthood in 85 individuals.

Table 2: ***BRCA1* and *BRCA2* test use in relation to sex, age, parenthood, and genetic risk**

gene. At the time of the study 78 patients with breast and/or ovarian cancer were still alive; 63 with breast cancer, ten with ovarian cancer, and five with both. Affected women were not included.

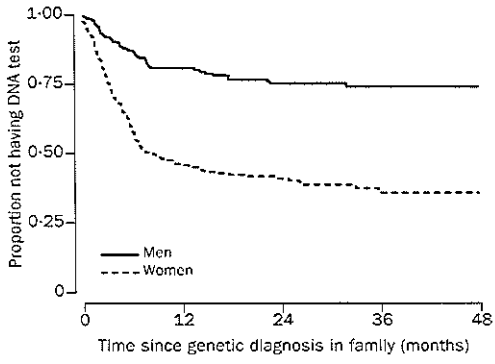
DNA testing was utilised by 38% (257 of 682) of eligible unaffected risk carriers. The median follow-up after the disclosure of the individual DNA-test result was 21 months (range 10–61) in 68 women eligible for prophylactic mastectomy and 24 months (11–61) in 45 women eligible for oophorectomy. 48% (198 of 411) women opted for testing and 22% (59 of 271) men (odds ratio for difference between sexes 3·21 [95% CI 2·27–4·51]; p<0·001; table 2). In women, signficant preditors for utilisation of DNA testing in the univariate analysis were age (odds ratio for ≥50 years *vs* <50 years 0·54 [0·37–0·79]), parenthood (children *vs* no children 2·25 [1·46–3·48]), and genetic risk for a mutation (pre-test risk of 50% *vs* 25% 3·47 [2·22–5·40]). In men, DNA testing was associated with parenthood (children *vs* no children 5·17 [2·13–12·57]) but not with age. Results did not alter after multivariate modelling (table 2). With inclusion of only individuals with children and at a pre-test risk of 50% for a mutation, the DNA test rate in women below the age of 50 years was significantly higher than for men at the same ages (83% [90 of 108] *vs* 27% [23 of 86]; odds ratio 12·74 [95% CI 6·40–25·35], whereas at the age of 50 years and older the test rates in women and men were almost the same (40% [31 of 78] *vs* 30% [30 of 100]; odds ratio 1·57 [95% CI 0·85–2·89]).

Of unaffected women with a pre-test genetic risk of 50% and 25% for a mutation, who had a DNA test, 44% (69 of 158) and 15% (6 of 40), respectively, had a mutation, as did 44% (26 of 59) of tested men with a 50% risk.

Unaffected women and men at a pedigree-based 50% risk for a mutation took about the same time to decide whether or not to have DNA testing (figure 1). Most of them decided before and only a few after a follow-up of 9 months. At a follow-up of 9 months, 1 year, and 2 years after identification of the family-specific *BRCA1/BRCA2* mutation 19%, 19%, and 24% of men and 51%, 54%, and 58% of women, respectively, were tested.

68 women aged 25 years and older were eligible for mastectomy and 45 women aged 35 years and older were eligible for oophorectomy.

Prophylactic mastectomy was done in 35 (51%) of the 68 eligible unaffected mutation carriers and the others opted for regular surveillance (figure 2, table 3). The following choose prophylactic mastectomy: women aged below 40 years, 55% (21 of 38); women aged




**Number at follow-up**

| | | | | |
|---|---|---|---|---|
| Men | 271 | 220 | 120 | 31 |
| Women | 275 | 127 | 63 | 23 |

Figure 1: **Proportion not having a DNA test**
Unaffected men and women with pedigree-based 50% risk for mutation opting for DNA testing.

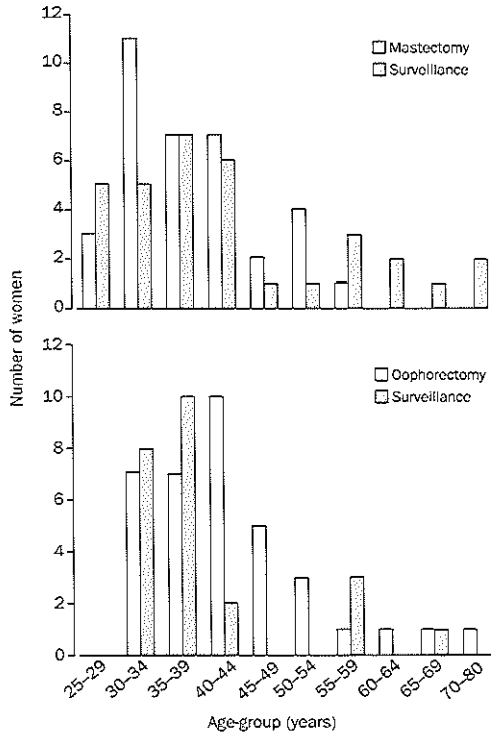



Figure 2: **Unaffected carriers opting for mastectomy, oophorectomy, or regular surveillance by age-group**
Top: Unaffected carriers (n=68) opting for prophylactic mastectomy or regular surveillance. Bottom: Unaffected carriers (n=60) opting for prophylactic oophorectomy or regular surveillance.

| | Mastectomy* | | p | Oophorectomy† | | p |
|---|---|---|---|---|---|---|
| | Number with mastectomy (%) | Univariate odds ratio (95% CI) | | Number with oophorectomy (%) | Univariate odds ratio (95% CI) | |
| **Age** | | | | | | |
| <40 years | 21/38 (55) | 1·00 | .. | 7/17 (41) | 1·00 | .. |
| 40–54 years | 13/21 (62) | 1·07 (0·29–2·82) | 0·87 | 18/20 (90) | 12·8 (2·23–74·1) | 0·004 |
| ≥55 years | 1/9 (11) | 0·24 (0·06–1·04) | 0·06 | 4/8 (50) | 1·43 (0·26–7·73) | 0·68 |
| **Parenthood** | | | | | | |
| No children | 2/14 (14) | 1·00 | .. | 2/5 (40) | 1·00 | .. |
| Children | 33/54 (61) | 9·43 (1·92–46·4) | 0·006 | 27/40 (68) | 3·12 (0·46–20·9) | 0·24 |
| **Total** | 35/68 (51) | .. | .. | 29/45 (64) | .. | .. |

*Only individuals aged ≥25 years included. †Only individuals aged ≥35 years included.

Table 3: **Choices of unaffected carriers**

40–54 years, 62% (13 of 21); and women aged ≥55 years and older) 11% (one of nine). There was a tendency towards mastectomy in younger (<55 years) women. In the 30–35 year age-group, 69% (11 of 16) opted for prophylactic mastectomy (figure 2). The oldest woman to choose prophylactic mastectomy was 55 years of age at time of surgery.

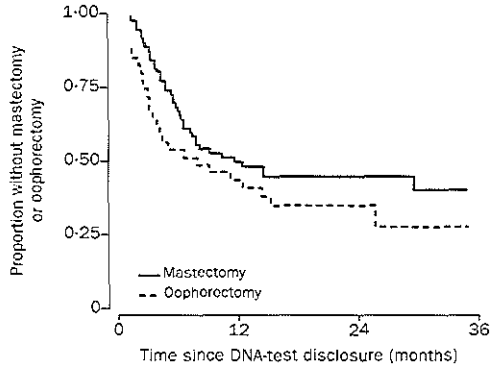
Having children was a significant predictor towards the choice for prophylactic mastectomy: 61% (33 of 54) of unaffected women with children chose prophylactic mastectomy versus 14% (two of 14) childless women (odds ratio 9·43 [95% CI 1·92–46·4]; p=0·006; table 3). Combining both predictors of age and parenthood 70% (28 of 40) of women aged below 50 years with children opted for prophylactic mastectomy.

At a follow-up of 9 months, 1 year, and 2 years after DNA-test disclosure, 46%, 51%, and 55%, respectively, of unaffected women carrying the mutation had a prophylactic mastectomy (figure 3). Of all 35 unaffected mutation carriers who opted for prophylactic mastectomy, 31 (89%) underwent this surgical intervention within 9 months; only four women took more than 9 months to decide to have prophylactic mastectomy (11, 13, 28, and 33 months, respectively). Breast reconstruction was simultaneously done in all but one woman.

The choice of surveillance or prophylactic oophorectomy in 60 unaffected carriers of the mutation (aged ≥30 years) are shown according to different age-groups in figure 2. Five women had prophylactic oophorectomy before the genetic diagnosis in the family. Although prophylactic oophorectomy is advised in women older than age 35 years, seven of 15 women younger than 35 preferred to have this surgical intervention simultaneously done with their prophylactic mastectomy (figure 2). Overall 36 (60%) of these 60 unaffected carriers had prophylactic oophorectomy.

Of unaffected eligible carriers of the mutation (≥35 years of age) 64% (29 of 45) had prophylactic oophorectomy, whereas 36% opted for regular surveillance (table 3). According to univariate analysis only age was a significant predictor towards prophylactic oophorectomy. Women aged 40–54 years were more likely to opt for this intervention than women at younger ages (odds ratio 12·8 [95% CI 2·23–74·1]; table 3).

At a follow-up of 9 months, 1 year, and 2 years after DNA-test disclosure, 47%, 53%, and 59%, respectively, of the unaffected carriers had a prophylactic oophorectomy (figure 3). Of all 29 unaffected carriers who had prophylactic oophorectomy, 24 (83%) underwent this surgical intervention within 9 months. Only five women took longer than 9 months to decide to have prophylactic oophorectomy (10–25 months).




**Number at follow-up**

| | | | | |
|---|---|---|---|---|
| Prophylactic mastectomy | 68 | 33 | 13 | 5 |
| Prophylactic oophorectomy | 45 | 16 | 6 | 3 |

Figure 3: **Proportion without mastectomy or oophorectomy**
Unaffected female carriers opting for prophylactic mastectomy and prophylactic oophorectomy after presymptomatic DNA-test disclosure

## Discussion

The identification of the *BRCA1* and *BRCA2* genes in 1994 and 1995[17,18] has had increasing clinical impact. In our clinical setting we found that 57% of unaffected women and 22% of men with a genetic risk of 50% for a mutation opted for a DNA test. Our data fall below the previously reported rates of DNA testing of 66% in women and of 48% in men.[10] However, dissimilarities in the mode of enrolment of the families, characteristics of the studied groups, and the counselling process might have contributed to these differences. Important factors influencing the decision for *BRCA1/BRCA2* testing were whether the individual had children for both women and men and whether they wanted surveillance or prophylaxis (mainly in women).[8,12] We show that parenthood is a strong predictor towards DNA testing in both men and women. Interestingly, age influenced the rate of DNA testing mainly in women and men with children. Younger women were more likely to be tested than men, but uptake was similar at older ages. This suggests that older women wanted DNA testing because of its impact on children and less so for any personal medical benefit. However, there is no a-priori reason to expect that the same decision-making process pertains to both sexes.

Currently, unaffected women with a *BRCA1* or *BRCA2* mutation face the choice of regular surveillance, prophylactic surgery, or chemoprevention. Studies on the possible interest in prophylactic mastectomy in untested high-risk women showed a wide range of outcomes: 32% (10 of 31)[11] and 62% (59 of 95)[12] of women said they would consider prophylactic mastectomy in case they carried a mutation. Of proven *BRCA1* mutation carriers, 35% (11 of 31) and 17% (2 of 12) expressed interest in prophylactic mastectomy and 73% (27 of 37) and 33% (4 of 12) in prophylactic oophorectomy shortly after disclosure of DNA-test results.[10,11] At our family cancer clinic 51% of unaffected women with a proven mutation choose prophylactic mastectomy, and 64% prophylactic oophorectomy. The results of the univariate analysis of predictors for mastectomy and oophorectomy have to be interpreted with some caution, as the numbers in some subgroups were small and 95% CIs were wide. However, parenthood is likely to be a significant predictor towards prophylactic mastectomy in unaffected carriers of the mutation. No women older than age 55 years opted for prophylactic mastectomy, which is less advisable in view of the significantly declining estimated gains in life expectancy with increasing age by this surgical intervention.[19]

Most women choose to undergo prophylactic surgery shortly after disclosure of DNA-test results. However, we stress that many of the family members awaited for several years, the results of DNA testing giving ample time to consider prophylactic surgery while under regular surveillance. The time-dependent rates suggest that most individuals interested in both DNA testing and prophylactic surgery had already come forward during the period of our study. Therefore, it is unlikely that uptake of DNA testing and prophylactic surgery will significantly increase over time.

The utilisation of both prophylactic surgery and DNA testing in our centre may differ from those in other countries for several reasons. In the Netherlands, cancer susceptibility is no ground for exclusion by the health-insurance system, or in access to employment. Costs for genetic testing, surveillance, and prophylactic surgery are covered by both public and private health insurances. Accordingly, families and risk carriers are free from social or financial constraints, something that may be different in other countries. The risk for social and financial discrimination has been noted as an important reason to refrain from *BRCA1/BRCA2* testing.[10,12] Furthermore, cultural differences in views on health and disease, risks and prevention, paternalism versus autonomy, and femininity might greatly influence interests in presymptomatic DNA testing and prophylactic surgery.[16,20]

The efficacy of the various medical options and the durability of its effects are of major concern to female *BRCA1/BRCA2* mutation carriers, and will influence their choices. Based on reported stage of incident breast cancers in young high-risk women under regular surveillance, it is likely that at least a quarter of these breast-cancer patients will ultimately die of distant metastasis despite a relatively early diagnosis.[16] In 1998, in one large American study, the chemopreventive agent tamoxifen was shown to reduce the risk of invasive breast cancer by 49% during a median follow-up of 55 months.[21] However, it is uncertain whether tamoxifen will be equally effective in *BRCA1/BRCA2* mutation carriers, and whether it will affect overall survival.[21,22] In particular, the option of prophylactic mastectomy has been a matter of debate.[16,23] However, Hartmann and colleagues[24] reported a reduction of about 90% in the incidence of invasive breast cancer by prophylactic (mainly subcutaneous, thus incomplete) mastectomy in high-risk women on the basis of family history during a median follow-up of 14 years. Breast cancers occurred in seven of 575 subcutaneous mastectomy cases and none of 64 total mastectomy cases (p=0·38 in comparison for type of surgery). Because information on the *BRCA1/BRCA2* mutation status was not known, it is likely that in their study at least 50% of the women who underwent prophylactic mastectomy were in fact not at increased risk of breast cancer, but could not be discriminated for at the time of surgery. With respect to ovarian cancer, the overall 5-year survival is about 30%. No screening strategy has been shown conclusively to decrease mortality[25] and after prophylactic oophorectomy women at familial risk for ovarian cancer still have a risk of about 2% of peritoneal cancer.[26]

Prophylactic mastectomy is a mutilating and irreversible intervention, affecting body image and sexual relations. There is much concern about the potential psychological harm of DNA testing for *BRCA1* and *BRCA2* and prophylactic surgery, in particular mastectomy. However, in our experience and that of others, women who had mastectomy after adequate counselling rarely express regret, instead they are relieved from fear of cancer.[16,27,28] In one study, risk of cancer for those refraining from DNA testing was attributed to higher depression rates in proven carriers of the mutation, because they experienced unresolved uncertainty and fear.[29]

Studies are underway on the efficacy and morbidity of regular surveillance and prophylactic strategies for *BRCA1/BRCA2* mutation carriers and on the long-term psychological effect of DNA testing, regular surveillance, and prophylactic surgery. In our clinical practice, women increasingly base their decision for prophylactic surgery on proven susceptibility. Overall, since 1998, about 90% of high-risk women based their choice for prophylactic mastectomy on a proven *BRCA1/BRCA2* mutation in contrast to less than 20% before 1996.

*Contributors*

E J Meijers-Heijboer and L C Verhoog were the principal investigators of the study and J G M Klijn was the co-ordinating investigator. C T M Brekelmans did the statistical analysis. E J Meijers-Heijboer and A Wagner were involved in the genetic counselling. A N van Geel, L Dukel, J G M Klijn, C Seynaeve, and M M A Tilanus-Linthorst did the prophylactic mastectomies and oophorectomies, oncological counselling, and the regular surveillance. A M W van den Ouweland and P Devilee did the mutation analysis. E J Meijers-Heijboer and J G M Klijn wrote the paper and the other investigators contributed comments.

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# Chapter 4

## Survival and tumour characteristics of breast-cancer patients with germline mutations of BRCA1.

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Verhoog LC, Brekelmans CT, Seynaeve C, van den Bosch LM, Dahmen G, van Geel AN, Tilanus-Linthorst MM, Bartels CC, Wagner A, van den Ouweland A, Devilee P, Meijers-Heijboer EJ, Klijn JG.

# Survival and tumour characteristics of breast-cancer patients with germline mutations of *BRCA1*

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## Summary

**Background** Hereditary breast cancer has been associated with mutations in the *BRCA1* and *BRCA2* genes and has a natural history different from sporadic breast cancer. We investigated disease-free and overall survival for patients with a proven *BRCA1* alteration.

**Methods** We estimated disease-free and overall survival for 49 Dutch patients from 19 consecutive families with a proven specific *BRCA1* mutation and one family with strong evidence for linkage to the *BRCA1* gene. We compared clinical outcome and data on tumour size, histology, axillary nodal status, contralateral breast cancer, and oestrogen-receptor and progesterone-receptor status with those of 196 patients with sporadic breast cancer, matched for age and year of diagnosis.

**Findings** Disease-free survival for *BRCA1* and sporadic patients at 5 years was 49% (95% CI 33–64) and 51% (43–59), respectively (p=0·98). Overall survival at 5 years was 63% (47–76) and 69% (62–76), respectively (p=0·88). Recurrence and death rates did not differ significantly between groups. Hazard ratios for recurrence and death among *BRCA1* patients were 1·00 (0·65–1·55) and 1·04 (0·63–1·71) relative to sporadic patients (p=0·88), and these did not differ significantly after adjustment for prognostic factors. Patients with *BRCA1*-associated breast cancer had twice as many progesterone-receptor-negative tumours (p<0·005) and development of contralateral breast cancer was four to five times as frequent as in the sporadic group (p<0·001).

**Interpretation** We showed that disease-free and overall survival were similar for sporadic and hereditary breast cancer in the presence of different tumour characteristics, which has implications for screening prophylactic therapy, and different treatments of hereditary breast cancer.

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## Introduction

In western countries, up to 20% of women diagnosed with breast cancer have at least one affected relative. 5% of all breast cancers are estimated to be attributable to highly penetrant dominant genes, and about 40–50% of so-called site-specific hereditary breast cancers and most cases of hereditary breast-ovarian cancer syndrome are thought to be due to mutations in the *BRCA1* gene.[1] Since the mapping[2] at chromosome 17q21 and subsequent cloning[3] of the *BRCA1* gene, several characteristics of *BRCA1*-associated breast cancer have been reported. These characteristics include younger age of onset,[2,4] frequent bilateral occurrence,[1,2,5] and worse histoprognostic features, such as more aneuploidy, higher grade, and higher proliferation indices.[6–13] Although these characteristics are generally associated with a poor outlook for unselected breast-cancer patients,[14,15] Porter and colleagues[16] found a longer survival for patients from probable *BRCA1*-linked families with breast cancer than in patients with breast cancer with similar age and date of diagnosis identified from the breast cancer registry. However, stage of disease was not provided. Lynch and colleagues[7] and Marcus and colleagues[8] found a general trend toward lower specific relapse and death rates, but found similar rates after adjustment for age and stage.

For familial breast cancer, however, studies that have investigated survival are not conclusive and results are not consistent.[8,17–20] Differences in the definition of familial breast cancer and the populations investigated could result in a diverse contribution of genetic factors, which

| Mutation | Number of families | Number of breast-cancer patients | Number of ovarian-cancer patients | Number of breast-cancer patients with pathology and follow-up |
|---|---|---|---|---|
| 185delAG | 1 | 3 | 0 | 1 |
| 1411insT | 4 | 13 | 3 | 9 |
| 2312del5 | 1 | 4 | 1 | 2 |
| C2457T | 1 | 3 | 1 | 3 |
| 2765delTGC | 1 | 4 | 2 | 3 |
| 2804delAA | 4 | 9 | 7 | 5 |
| G2841T | 2 | 7 | 7‡ | 6 |
| 3668delA+G3669T | 1 | 2 | 3 | 1 |
| G3759T | 1 | 5 | 0 | 3 |
| 5382insC | 1 | 3 | 0 | 3 |
| IVS22+5G>A* | 1 | 10 | 2 | 5 |
| 3·8 Kb deletion*† | 1 | 5 | 1 | 4 |
| Linkage | 1 | 7 | 2‡ | 4 |
| Total | 20 | 75 | 29 | 49 |

*Initially linkage to *BRCA1*. †Large genomic deletion comprising exon 13. ‡One of seven and two out of two patients, respectively, had breast and ovarian cancer.

Table 1: **Characteristics of *BRCA1* families**

might be one explanation for these conflicting results, apart from differences between control groups.

Survival in *BRCA1*-associated breast cancer might depend on the position of mutations in the gene. For example, histoprognostic grade segregates as a genetic trait in families and is thought to correlate with *BRCA1* mutations in conserved regions of the gene.[10,11] Specific mutations in the *BRCA1* gene could, therefore, affect the clinical outcome of *BRCA1*-associated breast cancer. However, whether heritability imparts a favourable or unfavourable prognosis is not clear.[21] No results on disease-free and overall survival of proven *BRCA1*-mutation carriers with breast cancer have been published. There are a few reports on breast-cancer survival in linkage-based *BRCA1* families,[9,16] but in these studies control groups matched for important confounding factors, such as age, were not used. Furthermore, linkage analysis in families with a high number of patients alive may have been in the past more readily conclusive, which might have led to a bias towards longevity.

For these reasons, we investigated disease-free and overall survival in relation to several clinical and pathological characteristics in patients with breast cancer from the first 20 consecutive non-selected families who presented at our family cancer clinic with a proven *BRCA1* alteration and who were counselled.

## Patients and methods

We identified families through the family cancer clinic of the Dr Daniel den Hoed Cancer Centre and the department of clinical genetics, Erasmus University, Rotterdam, according to a protocol approved by the medical ethical committees of the cancer centre and the academic hospital. Of the families that were eligible for this study, 19 had an identified germline mutation and one had strong evidence (>98% posterior probability) for being linked to *BRCA1*. The families were not selected by size or number of affected individuals, although each family had to have one or more cases of histologically confirmed breast cancer, with follow-up hospital records. The presence of a *BRCA1* mutation was detected by protein truncation test[22] followed by mutation analysis,[23] allele-specific oligonucleotide hybridisation for distinct mutations by Southern-blot analysis to detect gross genomic rearrangements,[24] and linkage analysis. 13 different mutations were found (table 1). 49 patients had hereditary breast cancer with follow-up, of whom 30 were treated in the Dr Daniel den Hoed Cancer Centre and 19 were treated elsewhere. For every *BRCA1* case four patients with sporadic breast cancer were matched for age and date of diagnosis (within 1 year); these patients were also selected from the Dr Daniel den Hoed cancer registry. We were able to exclude patients with a positive family history from the sporadic group since the registry contains information on tumours of identical type occurring in the family.

| | *BRCA1* (n=49) | Sporadic (n=196) | p |
|---|---|---|---|
| **Menopausal state at time of diagnosis** | | | 0·81 |
| Premenopausal | 40 (87·0%) | 161 (88·0%) | |
| Postmenopausal | 6 (13·0%) | 22 (12·0%) | |
| Unknown | 3 | 13 | |
| **Operative procedure** | | | 0·60 |
| Breast-conserving therapy | 18 (37·5%) | 90 (47·4%) | |
| Mastectomy | 29 (60·4%) | 96 (50·5%) | |
| Other | 1 (2·1%) | 4 (2·1%) | |
| Unknown | 1 | 6 | |
| **Tumour size** | | | 0·47 |
| ≤2 cm | 20 (45·5%) | 82 (46·3%) | |
| 2–5 cm | 20 (45·5%) | 64 (36·2%) | |
| >5 cm | 2 (4·5%) | 19 (10·7%) | |
| Any size with extension to chest wall | 2 (4·5%) | 12 (6·8%) | |
| Unknown | 5 | 19 | |
| **Axillary nodal status** | | | 0·15 |
| Negative | 28 (58·3%) | 83 (46·1%) | |
| Positive | 20 (41·7%) | 97 (53·9%) | |
| Unknown | 1 | 16 | |
| **Distant metastases** | | | 0·99 |
| Absent | 44 (95·7%) | 177 (95·7%) | |
| Present | 2 (4·3%) | 8 (4·3%) | |
| Unknown | 3 | 11 | |
| **Tumour stage** | | | 0·89 |
| I | 15 (34·9%) | 50 (28·7%) | |
| II | 21 (48·8%) | 93 (53·4%) | |
| III | 5 (11·6%) | 23 (13·2%) | |
| IV | 2 (4·7%) | 8 (4·6%) | |
| Unknown | 6 | 22 | |
| **Histological tumour type** | | | 0·36 |
| No special type | 39 (84·8%) | 173 (88·7%) | |
| Lobular | 3 (6·5%) | 8 (4·1%) | |
| Medullary | 4 (8·7%) | 7 (3·6%) | |
| Mucinous | 0 | 3 (1·5%) | |
| Other | 0 | 4 (2·1%) | |
| Unknown | 3 | 1 | |
| **Contralateral breast cancer** | | | .. |
| No | 37 (75·5%) | 184 (93·9%) | |
| Yes, synchronous | 2 (4·1%) | 1 (0·5%) | |
| Yes, metachronous | 10 (20·4%) | 11 (5·6%) | |
| **Oestrogen-receptor status** | | | 0·005 |
| Negative (<10 fmol/mg) | 16 (64·0%) | 52 (34·7%) | |
| Positive (>10 fmol/mg) | 9 (36·0%) | 98 (65·3%) | |
| Unknown | 24 | 46 | |
| **Progesterone-receptor status** | | | 0·002 |
| Negative | 14 (70·0%) | 45 (33·8%) | |
| Positive | 6 (30·0%) | 88 (66·2%) | |
| Unknown | 29 | 63 | |
| **Number of recurrences** | 24 (44·9%) | 101 (51·5%) | .. |
| **Number of deaths** | 19 (38·8%) | 80 (40·8%) | .. |

Table 2: **Distribution of selected characteristics in patients with *BRCA1*-related and sporadic breast cancer**

We gathered data on age at onset, menopausal status, surgical procedure, tumour-node-metastasis status, histology, contralateral breast cancer, and oestrogen-receptor and progesterone-receptor status from hospital records and pathology reports. In the two groups, breast cancer was diagnosed between 1969 and 1995 (median for each, 1987). The median age at diagnosis was 40 years (range 27–76). Our main endpoints were date of death (due to breast cancer or other disease), date of first local or distant recurrence, and the occurrence of a second primary breast tumour. We excluded two patients with distant metastases at diagnosis from all analyses of recurrence rates, but not analysis of death rates.

In most cases, tumour oestrogen-receptor and progesterone-receptor concentrations were routinely measured with radioligand binding assays, as recommended by the European

Organization for Research and Treatment Center, or with EIAs.[25] Values of less than 10 fmol/mg protein were taken as negative.

Because *BRCA1* patients and controls were treated in the same time period and had similar median year of diagnosis, median age of diagnosis, and menopausal status, they were treated according to the same protocols for primary surgery, adjuvant therapy, and systemic treatment for metastatic disease.

We compared characteristics of patients and tumours between groups by $\chi^2$ tests. We calculated Kaplan-Meier survival probabilities and differences were tested by the logrank rest. We investigated the simultaneous effect of several prognostic factors on disease-free and overall survival by Cox's proportional hazards method. The adequacy of the proportional hazards assumption was tested by logminlogplots.[26]

To exclude possible bias based on potential differences in survival and prognostic factors between treatment centres, we analysed separately patients treated in the Dr Daniel den Hoed Cancer Centre. In addition, *BRCA1* patients were analysed with and without probands because these patients might be selected for longevity. We did all statistical analyses with SPSS-PC (version 5) and STATA (version 5) including and excluding alternately missing variables as a separate group.

## Results

Characteristics of patients and tumours of *BRCA1*-mutation-associated and sporadic breast cancers are summarised in table 2. We found no significant differences in menopausal status, operative procedure and tumour stage. We found a possible trend towards more node-negative tumours (58·3 *vs* 46·1%, p=0·15) and tumours of the medullary histological type (8·7 *vs* 3·6%, p=0·23) among *BRCA1* patients than among patients with sporadic breast cancer, but the differences were not significant. By contrast, contralateral breast cancer was significantly more frequent among *BRCA1* patients than among controls (synchronous, 4·1 *vs* 0·5%; metachronous, 20·4 *vs* 5·6%, p=0·001). More *BRCA1* tumours with known steroid-receptor status were oestrogen-receptor or progesterone-receptor negative, (64·0 *vs* 34·7%, p=0·005; 70·0 *vs* 33·8%, p=0·002, respectively).

Because of many missing data (no clear indication of grade in the pathology report), we did not assess differentiation grade, but included some families with proven *BRCA1* mutations in the study of the Breast Cancer Linkage Consortium,[12] which showed a significantly higher rate of undifferentiated tumours in *BRCA1*-mutation carriers than in age-matched controls.

Disease-free survival at 2 and 5 years was 68% (95% CI 52–80) and 49% (33–64) for *BRCA1* patients and 73% (66–79) and 51% (43–59) for sporadic cases (p=0·98, figure 1). Overall survival at 2 and 5 years was 78% (63–87) and 63% (47–76) for *BRCA1* patients and 88% (83–92) and 69% (62–76) for patients with sporadic breast cancer (p=0·88, figure 1).

Recurrence and death rates did not differ significantly between groups at 2 and 5 years after diagnosis (p=0·98 and 0·88, respectively), nor did local recurrence rate after breast-conserving therapy (table 3). By contrast, the occurrence of metachronous contralateral breast cancer at 5-year follow-up was nearly four times higher in the *BRCA1*-mutation group than in the sporadic breast-cancer group (p=0·02, table 3). The unadjusted hazard ratios for recurrence and all-cause mortality among *BRCA1*-mutation-associated cases were 1·00 (0·65–1·55) and 1·04 (0·63–1·71), respectively, relative to sporadic patients. Adjustment for prognostic factors, including

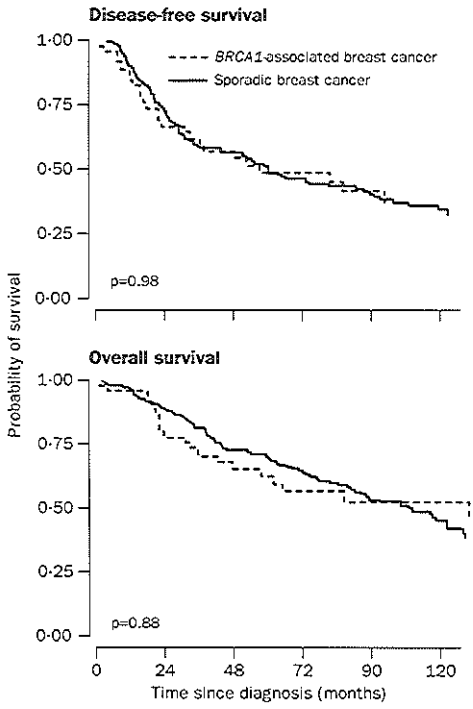


Figure 1: **Disease-free and overall survival for patients with *BRCA1*-associated or sporadic breast cancer since time of diagnosis**
*BRCA1* group n=49, sporadic group n=196.

tumour size, nodal status, oestrogen-receptor and progesterone-receptor status, and the occurrence of contralateral breast cancer, did not substantially alter the results. After correction for tumour stage, which is generally the most important prognostic factor, among patients with *BRCA1*-mutation-associated tumours, the hazard ratios for recurrence and all-cause mortality were 1·09 (0·70–1·70) and 1·21 (0·72–2·04), respectively (p=0·46).

A few patients who had died may have had sporadic tumours. However, restriction of the analysis to 30 affected proven carriers of gene mutations and their matched controls did not alter the results (difference between survival curves, p=0·88). Our matched control group consisted entirely of cases treated in the Dr Daniel den Hoed Cancer Centre, whereas some *BRCA1*-related cases were treated elsewhere; therefore, we further

| Outcome | BRCA1 | | Sporadic | | p |
|---|---|---|---|---|---|
| | 2 year | 5 year | 2 year | 5 year | |
| Overall death rate | 0·22 | 0·37 | 0·12 | 0·31 | 0·88 |
| Death rate by breast cancer | 0·20 | 0·36 | 0·11 | 0·29 | 0·81 |
| Recurrence rate* | 0·32 | 0·51 | 0·26 | 0·49 | 0·96 |
| Local recurrence rate after Breast conservation | 0·10 | 0·14 | 0·09 | 0·16 | 0·84 |
| Contralateral breast-cancer rate† | 0·09 | 0·19 | 0·03 | 0·05 | 0·02 |

*Stage IV tumours excluded. †Synchronous tumours excluded.

Table 3: **2 and 5 year actuarial outcomes for patients with *BRCA1*-mutation-related and sporadic breast cancer**

| | Recurrence | | Death of all causes | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | p | Hazard ratio (95% CI) | p |
| Unadjusted | 1·00 (0·65–1·55) | 0·98 | 1·04 (0·63–1·71) | 0·88 |
| Adjusted for tumour stage | 1·09 (0·70–1·70) | 0·69 | 1·21 (0·72–2·04) | 0·46 |

Table 4: **Crude and adjusted hazard ratios for patients with *BRCA1*-related versus sporadic breast cancer**

restricted our analyses to the 30 cases treated in the Dr Daniel den Hoed Cancer Centre. The findings did not differ significantly (difference between curves p=0·77). To look further into the representative value of our hospital control group we compared our data with cancer-registry data of the southeast Netherlands: in this area, 5-year survival for patients aged 15–59 years during 1980–92 was 72%,[27] which was similar to that of our control group (69%).

If many *BRCA1* families were identified by inclusion of an affected proband with breast cancer, inclusion of these probands might result in a selection for longevity and, therefore, positively affect the survival curves of the *BRCA1*-related group of patients. Exclusion of 13 probands with breast cancer (without a non-affected index patient at the same time) negatively affected survival curves: 2-year and 5-year overall survival of the remaining 36 hereditary breast-cancer cases was 73% and 56%, which was worse than that of their matched controls (89% and 71%), although not significantly

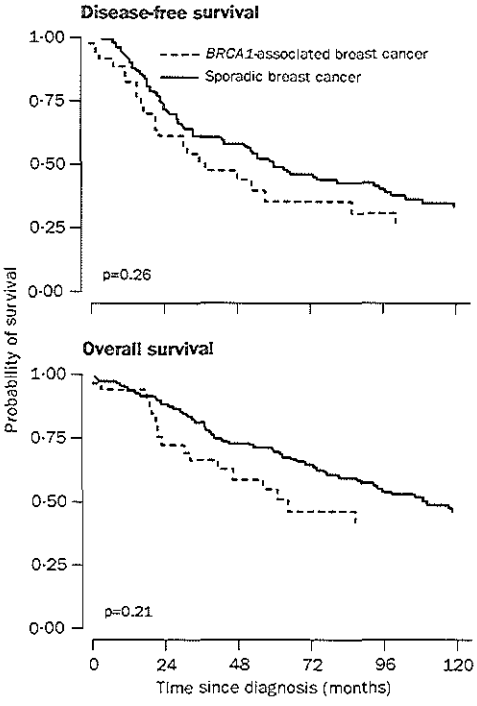


Figure 2: **Disease-free and overall survival for patients with *BRCA1*-associated breast cancer and matched controls since time of diagnosis**
13 affected probands excluded from *BRCA1* group. *BRCA1* group n=36, sporadic group n=144.

(figure 2). We found a non-significant trend to a worse disease-free (p=0·26) and overall survival (p=0·21).

## Discussion

Disease-free and overall survival among patients with breast cancer associated with *BRCA1* mutation did not differ significantly from patients with sporadic breast cancer matched for age and date of diagnosis and adjusted for tumour stage. Our findings, although perhaps relevant only to the Dutch population, partly support those of Marcus and colleagues,[9] who described a similar crude overall survival for patients with hereditary breast-ovarian cancer syndrome, with or without evidence for linkage to *BRCA1* (but without exclusion for *BRCA2* mutations) and unmatched controls of patients without hereditary breast cancer. Further, preliminary unpublished results from southern Sweden suggest an equal or shorter survival for *BRCA1* patients than that for breast cancer in general in this area.[13] However, Porter and colleagues[10] described a significantly longer survival in patients with breast cancer linked to *BRCA1* than in an age-matched Scottish population. Their median year of first diagnosis was 1974 (compared with 1987 in our study), without adjustment for stage, and their probable *BRCA1* carriers were identified by genetic-linkage analysis with markers located some genetic distance from the gene; in five of their eight pedigrees, the maximum odds of linkage was less than 95%. This finding contrasts with our first study, which included a known spectrum of distinct germline mutations. Nevertheless, it cannot be excluded that in populations with other mutation spectrums, survival curves might differ.

A general difficulty with studies of hereditary cancer is the prevention of bias, which is seldom completely possible. To prevent bias as much as possible, we analysed survival by taking into account various important factors. First, we matched for age (an important prognostic factor[15]) and year of diagnosis, because young age of onset is one of the main characteristics in carriers of a *BRCA1* mutation, and because the time period of diagnosis is strongly associated with distribution of stage (higher stages are more frequent in early time periods). Second, we did not select families, but included them consecutively. We analysed the data with and without exclusion of subgroups of patients and by multivariate analysis. After exclusion of affected probands we found a non-significant tendency towards worse disease-free and overall survival for *BRCA1*-mutation carriers, compared with matched controls. However, exclusion of affected probands might lead to the same amount of bias (negative selection) than their inclusion (positive selection for longevity). Such negative selection of patients may have increased when the reason for seeking counselling by some non-affected index-patients was the death of a close relative. Therefore, we preferred to include all patients in the main analysis who had no clear difference for survival. Nevertheless, for sufficient power to show smaller differences, hundreds to thousands of patients with a *BRCA1*-mutation would have been needed depending on the difference looked for. This size of sample should be included in future multicentre international studies. However, these studies will have their specific risks of bias by differences in mutation spectrum, in treatment policy between centres, and so on.

Our finding of a (non-significant) trend towards more node-negative tumours in *BRCA1*-related tumours might suggest an increased awareness or selective screening of family members at risk. Since distribution of tumour size in the group of *BRCA1*-mutation carriers was not different from sporadic cases, this explanation is not likely. Furthermore, only one case from a *BRCA1* family had been regularly screened before diagnosis. Marcus and colleagues[4] and Johansson and colleagues[13] also found a trend towards a higher percentage of node-negative *BRCA1*-positive patients, but their control and study groups were not easily comparable. Results on node-negativity could be of interest in the interpretation of results of mammographic screening in high-risk women. The percentage of 41% node positivity in our study is higher than the range of 8–28% seen in screened high-risk women according to five retrospective and prospective studies.[28] In line with the results of the studies of Marcus and colleagues[4] and the Breast Cancer Linkage Consortium,[12] we found a trend towards a higher frequency of the medullary tumour type in *BRCA1*-related breast cancers (8·7 *vs* 3·6%), which differs from that of Johansson and colleagues[13] (0 *vs* 2%). The frequently reported bilateral occurrence of hereditary breast cancer[1,2,5] was confirmed in our study. We also found that within 5 years after primary surgery, contralateral breast cancer developed in 25% of patients with *BRCA1*-mutation-associated breast cancer, which might support the application of prophylactic contralateral mastectomy.

Very few data have been reported on the relation between *BRCA1* positivity and steroid-receptor status. In agreement with our data Johansson and colleagues,[13] mainly through use of immunostaining, found that *BRCA1*-related tumours were significantly more often oestrogen-receptor and progesterone-receptor negative. This negative correlation between *BRCA1* and oestrogen and progesterone-receptor status may limit the application and efficacy of endocrine therapy, especially antioestrogens, in all stages of breast cancer and may also limit use of chemoprevention in families with hereditary breast cancer. Therefore, subgroup analysis in the continuing chemoprevention trials might be useful.

Intraductal component is a risk factor for local failure after breast-conservation therapy. However, this risk seems to be less common in *BRCA1*-associated breast cancers[29] and, therefore, a lower local recurrence rate can be expected. Local failure rate after breast-conservation therapy might be higher because of a higher proliferation rate, young age at onset, and a potentially greater chance of ipsilateral second primaries in *BRCA1*-mutation carriers. We found that the local relapse rate after breast-conservation therapy did not differ between affected *BRCA1*-mutation carriers and controls matched for age and year of diagnosis. This finding is in agreement with an observation about breast-conservation therapy in patients with possible hereditary breast cancer, which shows that a family history of breast cancer was not associated with a higher local recurrence rate after breast-conservation therapy.[30] However, because we do not know how many of the local recurrences in our patients who received breast-conservation therapy were new primary, ipsilateral tumours, further analysis to address this important clinical question is needed in larger series of patients with hereditary breast cancer. Because of the high number of contralateral breast cancers, some of the local recurrences might have been second primary tumours.

Our results show that breast-cancer cases from 20 Dutch families with proven specific germ-line *BRCA1* mutations differed from a control set of sporadic tumours in various clinical variables. The most important findings were the significantly increased occurrence of oestrogen-receptor-negative and progesterone-receptor-negative and bilateral tumours. Despite these differences and other reported tumour characteristics, disease-free and overall survival, and local failure rate, did not significantly differ from those in patients with sporadic breast cancer. However, larger studies are needed to detect small differences with sufficient statistical power.

*Contributors*

L C Verhoog and C T M Brekelmans were the principal investigators of the overall study and J G M Klijn was the coordinating investigator. C T M Brekelmans and L M C van den Bosch did the statistical analysis. G Dahmen gathered the matched controls. C Seynaeve, A N van Geel, M M A Tilanus-Linthorst, and C C M Bartels participated in the medical care (diagnosis and treatment) of the patients. A Wagner and E J Meijers-Heijboer did the genetic counselling, and A van den Ouweland and P Devilee did the DNA analysis. L C Verhoog, C T M Brekelmans, and J G M Klijn wrote the paper and the other investigators contributed comments.

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# Chapter 5

## Contralateral breast cancer risk is influenced by the age at onset in BRCA1-associated breast cancer.

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***Summary:*** BRCA1/2 mutation carriers diagnosed with breast cancer have a strongly elevated life-time risk of developing a contralateral tumour. We studied the contralateral breast cancer risk in 164 patients from 83 families with a proven BRCA1 mutation in relation to the age at diagnosis of the first primary breast cancer. In the actuarial outcomes after 10 years' follow-up, 40% of the 124 BRCA1-patients diagnosed with breast cancer < 50 years had developed contralateral breast cancer, vs 12% of the 40 patients > 50 years at first diagnosis ($P_{logrank}$ = 0.03). These data suggest that age at diagnosis of the first tumour should be taken into account when prophylactic mastectomy in BRCA1-patients is considered.

It has long been recognised that familial breast cancer is associated with increased bilateral occurrence of this disease (Anderson, 1971). The identification of the BRCA1 and BRCA2 genes has made it possible to more accurately determine hereditary breast cancer risk for women who carry a germline mutation in one of these genes. In BRCA1 mutation carriers there is a strongly elevated life-time risk of breast cancer while the risk of contralateral breast cancer has been estimated to be as high as 64% (Ford et al, 1994). We previously reported that the rate of metachronous contralateral disease in a group of BRCA1-associated breast cancer patients (n=49) was 19% after 5 years follow-up, while synchronous presentation occurred in 4% of the patients (Verhoog et al, 1998). More recently, Robson and colleagues found that in a group of 30 BRCA1/2 mutation carriers (diagnosed with breast cancer before the age of 42) 31% developed contralateral breast cancer within 5 years (Robson et al, 1998).

Because of the high risk of a second primary breast cancer, affected BRCA1 mutation carriers and their physicians may have to face the decision about more radical surgery, including contralateral prophylactic mastectomy at the time of, or in the years following diagnosis of the first tumour. Therefore, it is of interest to identify any subgroup of patients with hereditary breast cancer which is particularly at risk of developing contralateral breast cancer. With respect to the general population, the risk of contralateral disease is clearly correlated with a younger age at onset of the disease (Prior and Waterhouse, 1978; Hislop et al, 1984; Adami et al; 1985; Broet et al, 1995). This, however,

could primarily result from the fact that a larger proportion of breast cancer occurring at a younger age is attributable to genetic susceptibility. We investigated the risk of developing contralateral breast cancer in relationship to age at diagnosis of the first breast cancer, in women from families with an identified BRCA1 mutation.

## Patients and Methods

Families were identified through the Family Cancer Clinic of the Daniel den Hoed Cancer Centre and the Department of Clinical Genetics of the Erasmus University Rotterdam. The 83 families that were eligible for this study consisted of consecutive unselected families with an identified germline mutation in the BRCA1 gene. In total 24 different BRCA1 mutations were identified. The presence of a BRCA1 mutation was detected by protein truncation test, by allele-specific oligonucleotide hybridisation for distinct mutations or by PCR-analysis specific for two large genomic deletions (Hogervorst et al, 1995; Petrij-Bosch et al, 1997). Whenever possible, histological and clinical data from breast cancer patients were collected through hospital records and pathology reports.

Follow-up data of 179 women with histologically confirmed breast cancer from these families were available. In 129 of these 179 patients, a BRCA1 mutation was proven by direct DNA-testing in blood cells or tumour material or because of their position in the pedigree. Six breast cancer patient who were identified as not carrying the familial BRCA1 mutation were excluded. Of the remaining 173 breast cancer patients, nine patients were excluded either because they were diagnosed with synchronous bilateral breast cancer (n = 4; 2%) or because they underwent bilateral mastectomy at the time of diagnosis of the first primary (n = 5); finally 164 patients were evaluated.

The date of a second contralateral primary breast tumour, date of bilateral mastectomy and date of death were selected as endpoints. Occurrence of a second contralateral breast cancer was studied in relation to

the age of onset of the first primary breast cancer (Tables 1 and 2). Incidence rates and 95% confidence intervals were calculated assuming a Poisson distribution. Finally the cut-off age at first diagnosis of 50 years was chosen because this age is frequently used to make the distinction between premenopausal vs postmenopausal breast cancer (Early Breast Cancer Trialists' Collaborative group, 1998). Kaplan-Meier probabilities were computed with respect to the contralateral breast cancer-free and overall survival and differences were tested by the logrank test.

**Table 1** Actuarial contralateral breast cancer risk for 164 BRCA1 associated breast cancer patients in relation to the age at diagnosis of the first primary breast cancer (BC)

| Age at first BC | N (%) of patients | Follow-up | | |
|---|---|---|---|---|
| | | 2-year | 5-year | 10-year |
| ≤ 40 | 74 (45) | 0.08 | 0.21 | 0.27 |
| 41-50 | 50 (30) | 0.09 | 0.33 | 0.52 |
| 51-60 | 29 (18) | 0.04 | 0.04 | 0.15 |
| > 60 | 11 (7) | 0.00 | 0.00 | 0.00 |

## Results

Median age at diagnosis of the 164 patients with unilateral breast cancer and follow up was 41 years (range 22-80). The four women (2%) with synchronous bilateral breast cancer that were excluded from the analyses presented with breast cancer at the ages of 33, 34, 34 and 51 years. Table 1 shows the number of patients diagnosed with breast cancer < 40 years, at 41-50 years, 51-60 years and > 60 years, with the 2-, 5- and 10-year contralateral breast cancer probabilities for the different age-groups. The annual incidence of contralateral breast cancer in relation to age of diagnosis of the first primary is listed in Table 2 as well as the number of contralateral cancers that

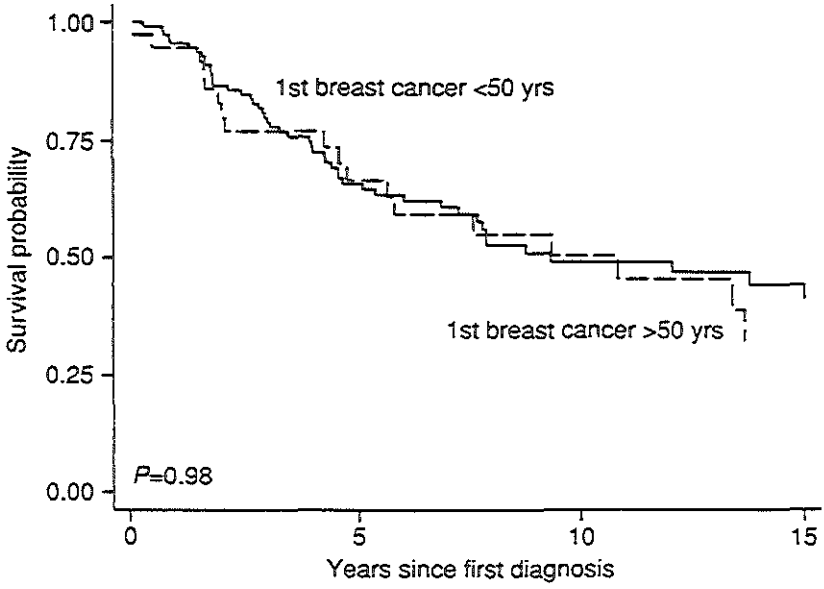
occurred and the number of women years at risk. No contralateral cancers were observed in the 11 patients that were older than 60 years at time of diagnosis of the first breast cancer.

**Table 2** Annual incidence of contralateral breast cancer (CBC) in BRCA1-associated breast cancer patients in relation to the age at diagnosis of the first primary breast cancer (BC)

| Age at first BC | Woman-years at risk | CBC (n) | Incidence per year of CBC (95% CI) | |
|---|---|---|---|---|
| ≤ 40 | 419 | 19 | 4.5% | (2.9-7.1) |
| 41-50 | 268 | 17 | 6.3% | (3.7-10.6) |
| 51-60 | 184 | 3 | 1.6% | (0.4-4.8) |
| >60 | 56 | 9 | 0 | (0-6.6) |

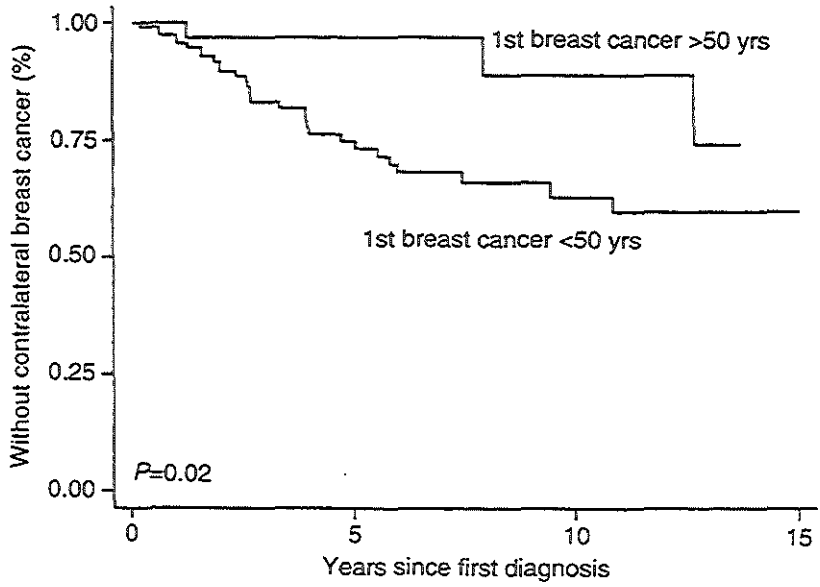
Forty (24%) of the 164 patients were diagnosed with their first breast cancer after the age of 50. Thirty-six (29%) of the remaining 124 patients diagnosed before the age of 50 years developed metachronous contralateral breast cancer vs three (7.5%) of the 40 patients over the age of 50 on diagnosis (P = 0.005). Median follow-up of all 164 patients was 47 months and the overall survival at 5 and 10 years was 66% and 50%, respectively. Between the two age groups there was no significant difference in overall survival ($P_{logrank}$ = 0.98, Figure 1).

**Figure 1** Overall survival for BRCA1-associated breast cancer patients (< 50 years at first diagnosis, n = 124, continuous line; > 50 years at first diagnosis, n = 40, dashed line).




In the actuarial outcomes after 5 and 10 years follow-up, metachronous contralateral breast cancer was found in 24% and 34%, respectively, of all BRCA1-associated breast cancer patients. In the group of patients diagnosed with breast cancer before the age of 50, contralateral breast cancer after 5 and 10 years follow up was seen in 30% and 40%, respectively, vs 4% and 12% respectively in BRCA1-associated breast cancer patients older than 50 years at first diagnosis ($P_{logrank}$ = 0.02, Figure 2).

**Figure 2** Proportion of BRCA1-associated breast cancer patients without contralateral breast cancer.




## Discussion

In the general population, young age at onset of first breast cancer has been shown to be a risk factor for the occurrence of contralateral breast cancer. However the proportion of breast cancer due to genetic susceptibility will also correlate with a younger age at onset of the disease. With regard to the contribution of BRCA1 mutations to breast cancer in the general population, it has previously been estimated that 5.3% of the women diagnosed before the age of 40 carry this mutation, compared to only 1.1% of those aged 50-70 at diagnosis (Ford et al, 1995). Population-based studies in early-onset breast cancer patients confirm these results (Newman et al, 1998; Peto et al, 1999). Therefore, young age at onset as a risk-factor for contralateral breast cancer could at least partly be due to the fact that early-onset breast cancer is more

frequently the result of genetic susceptibility, whereas a woman with breast cancer resulting from genetic susceptibility could be at an increased risk of developing contralateral breast cancer regardless of the age at onset of her disease. In the present study we show that with respect to BRCA1-associated breast cancer this risk is also correlated with the age at onset of the first breast cancer.

We are not sure of the relevance of these results in the management of contralateral breast cancer risk in BRCA2-associated breast cancer patients, or patients with a strong family history for the disease in general. Earlier we reported that the 5-year rate of contralateral breast cancer in BRCA2 mutation carriers was 12% (Verhoog et al, 1999). In the present study the rate in BRCA1 mutation-carriers is approximately twice that. Our present results are well in line with studies showing that the breast cancer risk in BRCA1 carriers declines with age (Easton et al, 1995). Although the penetrance at ages < 50 years is less for BRCA2 mutations, the life-time penetrance for breast cancer due to BRCA2 mutations may be equal to that of BRCA1 mutation carriers (Ford et al, 1998). This could indicate that, in BRCA2 mutation carriers, the risk for developing contralateral breast cancer does not diminish after the menopause.

Because it was not possible to test all the breast cancer patients from families with an identified BRCA1 mutation for the presence of such a specific mutation, it cannot be ruled out that a few of the patients in our series are in fact sporadic cases. However, restricting to proven BRCA1 carriers did not essentially alter the observed trend although numbers were to small to reach statistical significance.

Since the 1980's breast cancer in postmenopausal women with axillary lymph node involvement is generally treated with adjuvant hormonal therapy, i.e. tamoxifen for 2-5 years while 4-6 courses of adjuvant chemotherapy are preferentially used in premenopausal patients. Long-term adjuvant treatment with tamoxifen resulted in a 45% reduction of the risk of contralateral breast cancer (Early Breast Cancer Trialists' Collaborative Group, 1998). This might partly explain the lower rate of contralateral breast cancer in women diagnosed after the age of 50. In our series of 40 postmenopausal patients, information on the use of antiestrogens was available for 36 patients; only six

received adjuvant tamoxifen therapy. Exclusion of these six women did not essentially alter the observed trend, which makes this therapy an unlikely explanation of the observed difference in the rate of contralateral breast cancer between the two age groups in our study.

In conclusion, these results suggest that apart from the stage of the primary tumour, and time that has elapsed since the onset of disease, age at diagnosis of the first breast cancer in BRCA1 gene-mutation carriers should be taken into account in the decision-making process with respect to contralateral prophylactic mastectomy.

## Acknowledgement

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# Chapter 6

## Survival in hereditary breast cancer associated with germline mutations of BRCA2.

*J Clin Oncol. 1999 Nov;17(11):3396-402.*

Verhoog LC, Brekelmans CT, Seynaeve C, Dahmen G, van Geel AN, Bartels CC, Tilanus-Linthorst MM, Wagner A, Devilee P, Halley DJ, van den Ouweland AM, Meijers-Heijboer EJ, Klijn JG.

# Survival in Hereditary Breast Cancer Associated With Germline Mutations of *BRCA2*

By L.C. Verhoog, C.T.M. Brekelmans, C. Seynaeve, G. Dahmen, A.N. van Geel, C.C.M. Bartels, M.M.A. Tilanus-Linthorst, A. Wagner, P. Devilee, D.J.J. Halley, A.M.W. van den Ouweland, E.J. Meijers-Heijboer, and J.G.M. Klijn

**Purpose:** Breast cancer in *BRCA1* and *BRCA2* gene-mutation carriers may differ from so-called sporadic breast cancer in clinical features and behavior. These potential differences may be of importance for the prevention, screening, and, ultimately, treatment of breast cancer in women with such germline mutations. Thus far, there have been very few studies on the survival of *BRCA2*-associated breast cancer patients.

**Patients and Methods:** We determined the disease-free and overall survival of 28 breast cancer patients from 14 consecutive families with eight different *BRCA2* germline mutations. These patients' survival and tumor characteristics were compared with those of a control group of 112 sporadic breast cancer patients matched to them by age and year of diagnosis.

**Results:** The 5-year disease-free survival was 52% for each group ($P = .91$); the overall survival was 74% for *BRCA2* carriers and 75% for sporadic cases ($P = .50$). At the time of diagnosis, tumors from the *BRCA2* carriers were borderline significantly larger in comparison to the tumors in sporadic cases ($P = .05$), but axillary nodal status was not significantly different in the two groups (node-negativity, 63% v 52.8%, respectively; $P = .34$). With respect to steroid receptor status, *BRCA2*-associated tumors were more likely to be steroid receptor-positive, especially regarding progesterone receptor status (100% v 76.7% positive, respectively; $P = .06$). Stage-adjusted recurrence and death rates were nonsignificantly better for *BRCA2* cases (hazard ratios of 0.84 and 0.59 [$P = .61$ and $P = .19$], respectively). In contrast, after 5 years, the rate of metachronous contralateral breast cancer in *BRCA2* patients was 12% (v 2% in controls; $P = .02$).

**Conclusion:** Patients with hereditary breast cancer due to *BRCA2* have a similar prognosis when compared with age-matched sporadic breast cancer patients. Contrary to our previous observation regarding *BRCA1*-associated breast cancer, *BRCA2* tumors tended to be steroid receptor-positive, instead of steroid receptor-negative.

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IN WESTERN COUNTRIES, up to 20% of women diagnosed with breast cancer have at least one relative who is also affected by the disease.[1] Part of this familial clustering shows autosomal dominant inheritance with high penetrance and is due to mutations in the *BRCA1* and *BRCA2* breast cancer genes.[2,3] Germline mutations in the *BRCA1* and *BRCA2* genes confer a strongly elevated risk of breast cancer. Women who inherit a mutated copy of the *BRCA1* gene have an estimated lifetime risk of breast cancer ranging from 56% to 87%, which is comparable to the lifetime risk for *BRCA2* mutation carriers.[4-7] Both genes are known to be involved in the hereditary breast and ovarian cancer syndrome,[8] although the majority of cases of this syndrome are thought to occur as a result of a mutation in the *BRCA1* gene.[9]

A number of other similarities exist in breast cancer associated with either one of these genes.[7] Familial and proven *BRCA1*- and *BRCA2*-associated breast cancers are both characterized by a younger age of onset of the disease, frequent bilateral occurrence,[10-12] and worse histoprognostic features.[13-16] However, it is possible that the higher grade of malignancy of *BRCA2*-associated tumors is merely determined by reduced tubule formation.[16]

A positive family history for breast cancer has been correlated with better, similar, and worse prognoses relative to nonfamilial breast cancer.[17-21] Differences in the definitions of a positive family history for breast cancer and in the ethnic backgrounds of the populations investigated may have resulted in a varied contribution of genetic susceptibility, and this, apart from disparate control groups, might explain the conflicting results regarding prognosis of familial breast cancer.

We and other groups showed in earlier reports that despite the adverse pathobiologic features of *BRCA1*-related tumors, the prognosis for patients with *BRCA1* hereditary breast cancer is not significantly different from that of sporadic controls.[21-27] However, others have found that the presence of a *BRCA1* germline mutation is an adverse prognostic factor in breast cancer patients.[28,29]

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Because little is known about the natural history of *BRCA2*-associated breast cancer, we investigated disease-free and overall survival in relation to a number of clinical and pathologic characteristics in breast cancer patients from 14 consecutive families with a proven *BRCA2* mutation who were counseled at our family cancer clinic. Identifying the potential differences in survival and tumor characteristics could benefit the management of breast cancer in women with a *BRCA2* germline mutation and might have implications for screening, chemoprevention, prophylactic surgery, and treatment.

## PATIENTS AND METHODS

### *Families and Patients*

Families were identified through the Family Cancer Clinic of the Daniel den Hoed cancer center and the Department of Clinical Genetics of Erasmus University Rotterdam. The 14 families eligible for this study (Table 1) consisted of consecutive unselected families with an identified germline mutation in the *BRCA2* gene. The presence of a *BRCA2* mutation was detected by a protein truncation test,[12] followed by sequence analysis to characterize the precise alteration and by allele-specific oligonucleotide hybridization for distinct mutations as described.[22] Each family had to include one or more patients with histologically confirmed breast cancer for whom follow-up data were available through hospital records. This criterion was fulfilled for 28 breast cancer patients from 14 families; 17 of these patients were treated in the Daniel den Hoed Cancer Center. Six of the total group of 28 patients were probands; that is, they were the initial persons counseled in their families.

An overview of the mutation spectrum and number of cases of breast and ovarian cancer per family is listed in Table 1. Seventeen of the total of 45 breast cancer patients from the *BRCA2* families were not eligible because of lack of histologic confirmation of the original diagnosis or because no consent was obtained for the extraction of their data from hospital records. Twenty-two of the 28 *BRCA2* cases were proved to be carriers of a *BRCA2* mutation by direct tests of their blood cells or tumor material or because of their position in the pedigree; for the remaining six *BRCA2* patients, it was not possible to confirm carrier status because no blood or tissue samples were available.

**Table 1. Characteristics of *BRCA2* Families**

| Mutation | No. of Families | No. of Breast Cancer Patients | No. of Ovarian Cancer Patients | No. of Breast Cancer Patients With Pathology and Follow-Up Data |
|---|---|---|---|---|
| 862delAG | 1 | 3 | 1 | 2 |
| 4684del4 | 1 | 3 | 0 | 1 |
| 4708insA | 1 | 7 | 4 | 4 |
| 5579insA | 5 | 13 | 3 | 10 |
| S1882X | 1 | 3 | 0 | 1 |
| Y1894X | 1 | 4 | 0 | 2 |
| 6503delTT | 3 | 9 | 3 | 6 |
| 6872del4 | 1 | 3 | 2 | 2 |
| Total | 14 | 45 | 13* | 28† |

*Five patients had both breast and ovarian cancer.

†Three of the five patients with both breast and ovarian cancer had pathology and follow-up data.

The hereditary breast cancer patients were matched for age and date of diagnosis in a 1:4 ratio to a sporadic group of patients selected from the Daniel den Hoed cancer registry. This registry includes data from more than 10,000 breast cancer patients treated in the hospital since 1980. For the majority of cases, matching was performed within 1 year of the age and diagnosis date of the corresponding *BRCA2* patient. This was not possible for seven *BRCA2*-related cases diagnosed before 1980. Patients with a positive family history could generally be excluded from the sporadic group because the registry contains information regarding tumors of the identical type that had occurred in other family members.

We extracted data regarding age at onset of the disease, menopausal status, surgical procedure performed, tumor-node-metastasis system status, histology, contralateral breast cancer, and estrogen/progesterone receptor (ER/PgR) status from hospital records and pathology reports. In the majority of cases, tumor ER and PgR levels were routinely determined with radioligand binding assays as recommended by the European Organization for Research and Treatment of Cancer or with enzyme immunoassays as previously described by our group.[30] Values below 10 fmol/mg protein were considered negative.

Breast cancer in the *BRCA2*-associated patients had been diagnosed between 1960 and 1996; the median year of diagnosis for both groups was 1985 and the mean year of diagnosis was 1983 for the *BRCA2* cases and 1985 for the sporadic cases. The mean age at diagnosis was 46 years for both groups (median, 42 years; range, 32 to 85 years). We selected the date of first local or distant recurrence, the occurrence of a second primary breast tumor, and date of death (due to breast cancer or other cause) as the end points of interest. The three eligible *BRCA2*-associated breast cancer patients with ovarian cancer tumors as second primary tumors were not likely to influence survival because only one woman died as a result of ovarian cancer (33 years after the first diagnosis). From the 17 *BRCA2* patients without pathology and/or follow-up data, information about the age at diagnosis and date of death were collected as reported by their family members. This was done to check for possible bias toward longevity of the eligible *BRCA2* cases.

### *Statistical Methods*

The comparability of patient and tumor characteristics in the *BRCA2*-associated and sporadic breast cancer patients was tested by chi-square tests. Kaplan-Meier survival probabilities were computed, and differences were tested using the log-rank test. The simultaneous effect of several prognostic factors on disease-free and overall survival was investigated by use of the Cox proportional hazards regression method. The adequacy of the proportional hazards assumption was tested graphically by examination of logminlogplots.[31]

To exclude any possible biases related to potential differences in treatment, separate analyses were performed for the *BRCA2*-associated patients diagnosed after 1980. In addition, we performed a separate analysis of the *BRCA2* cases that excluded the six probands because these cases might be selected for longevity. All statistical analyses were performed using SPSS-PC version 5 (SPSS Inc, Chicago, IL) and STATA version 5 software (STATA Corporation, College Station, TX), alternately including and excluding the missing variables as a separate group.

## RESULTS

### *Patient and Tumor Characteristics*

The characteristics of patients and of the tumors in cases of *BRCA2*-associated and sporadic breast cancers are listed in Table 2. By matching for age at onset, we found no

Table 2. Distribution of Characteristics of *BRCA2*-Related and Sporadic Breast Cancer Patients at the Time of Diagnosis

| Characteristic | *BRCA2* (n = 28) No. | % | Sporadic (n = 112) No. | % | P |
|---|---|---|---|---|---|
| Menopausal state at the time of diagnosis | | | | | .75 |
| Premenopausal | 20 | 76.9 | 75 | 79.8 | |
| Postmenopausal | 6 | 23.1 | 19 | 20.2 | |
| Unknown | 2 | | 18 | | |
| Operative procedure | | | | | .11 |
| Breast-conserving therapy | 10 | 35.7 | 56 | 50.0 | |
| Mastectomy | 18 | 64.3 | 46 | 41.1 | |
| Other | 0 | | 7 | 6.3 | |
| Unknown | 0 | | 3 | | |
| Tumor size | | | | | .05 |
| 2 Cm or less | 10 | 37.0 | 53 | 54.6 | |
| 2 to 5 cm | 13 | 48.1 | 34 | 35.1 | |
| More than 5 cm | 4 | 14.8 | 4 | 4.1 | |
| Any size, with extension to chest wall | 0 | | 6 | 6.2 | |
| Unknown | 1 | | 15 | | |
| Axillary nodal status | | | | | .34 |
| Negative | 17 | 63.0 | 56 | 52.8 | |
| Positive | 10 | 37.0 | 50 | 47.2 | |
| Unknown | 1 | | 6 | | |
| Distant metastases | | | | | .48 |
| Absent | 28 | 100.0 | 110 | 98.2 | |
| Present | 0 | | 2 | 1.8 | |
| Unknown | 0 | | 0 | | |
| Tumor stage | | | | | .76 |
| I | 9 | 36.0 | 35 | 44.3 | |
| II | 13 | 52.0 | 37 | 46.8 | |
| III | 3 | 12.0 | 6 | 7.6 | |
| IV | 0 | | 1 | 1.3 | |
| Unknown | 3 | | 33 | | |
| Histologic tumor type | | | | | .46 |
| NST | 23 | 82.1 | 98 | 89.1 | |
| Lobular | 1 | 3.6 | 7 | 6.4 | |
| Mixed NST/lobular | 2 | 7.1 | 0 | | |
| Medullary | 2 | 7.1 | 2 | 1.8 | |
| Mucinous | 0 | | 1 | 0.9 | |
| Other | 0 | | 2 | 1.8 | |
| Unknown | 0 | | 2 | | |
| Contralateral breast cancer | | | | | .002 |
| No | 20 | 71.4 | 103 | 92.8 | |
| Yes, synchronous | 1 | 3.6 | 3 | 2.7 | |
| Yes, metachronous | 7 | 25.0 | 5 | 4.5 | |
| Estrogen receptor status | | | | | .37 |
| Negative (< 10 fmol/mg) | 1 | 6.7 | 10 | 15.6 | |
| Positive (> 10 fmol/mg) | 14 | 93.3 | 54 | 84.4 | |
| Unknown | 13 | | 48 | | |
| Progesterone receptor status | | | | | .06 |
| Negative | 0 | | 14 | 23.3 | |
| Positive | 12 | 100.0 | 46 | 76.7 | |
| Unknown | 16 | | 52 | | |

Abbreviation: NST, no special type.

significant difference in menopausal status at time of diagnosis. *BRCA2*-associated tumors were borderline significantly larger in size ($P = 0.05$) than the sporadic group of tumors, which is possibly related to the lower occurrence of breast-conserving therapy in the former group. Despite their larger size, *BRCA2* tumors were slightly more often node-negative (63.0% *v* 52.8%; $P = .34$). The histologic type of the *BRCA2* tumors did not differ significantly from that of the sporadic tumors ($P = .46$).

Metachronous contralateral breast cancer occurred in 25% of the *BRCA2*-associated patients. This was approximately five times more frequent than the occurrence of contralateral breast cancer in the group of sporadic controls (4.5%; $P = .002$). Of the *BRCA2* cases with known receptor status, 14 of 15 tumors were $ER^+$ (93.3%) and 12 of 12 tumors (100%) were $PgR^+$ (sporadic breast cancer patients: $ER^+$, 84.4%, $P = .37$; $PgR^+$, 76.7%, $P = .06$). In addition, the receptor statuses of three contralateral *BRCA2*-associated breast tumors was available, and all three tumors were steroid receptor-positive.

*Survival Analysis and Multivariate Analysis*

Figures 1 and 2 show Kaplan-Meier curves for disease-free and overall survival, respectively, in *BRCA2*-associated and sporadic breast cancer patients. Disease-free survival at 2 and 5 years (Table 3) was 82% and 52%, respectively, for *BRCA2* patients versus 78% and 52%, respectively, for sporadic patients ($P_{logrank} = .91$). Overall survival at 2 and 5 years was 93% and 74%, respectively, for the *BRCA2* patients versus 92% and 75% for the sporadic patients ($P_{logrank} = .50$). The overall clinical outcome of the patients, including the breast cancer–specific death rate, is summarized in Table 3. No significant differences between the *BRCA2* and sporadic cases were observed, with the exception of the rate of contralateral breast cancer. After 2 and 5

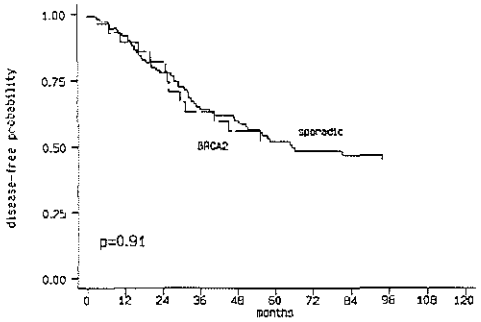


Fig 1. Disease-free survival for patients with *BRCA2*-associated (n = 28; – – –) or sporadic breast cancer (n = 112; ——) since the time of diagnosis.

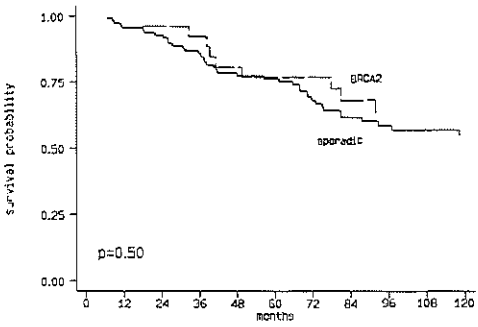


Fig 2. Overall survival for patients with *BRCA2*-associated (n = 28; - - - -) or sporadic breast cancer (n = 112; ——) since the time of diagnosis

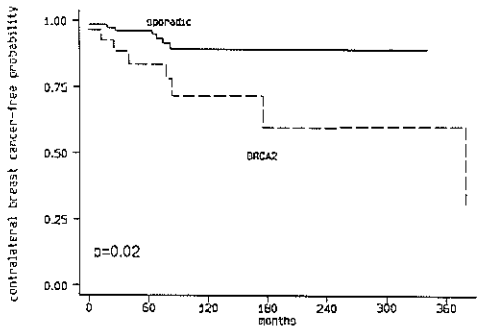


Fig 3. Contralateral breast cancer–free survival for patients with *BRCA2*-associated (n = 27; - - - -) or sporadic breast cancer (n = 109; ——) since the time of diagnosis (patients with synchronous bilateral breast cancer excluded).

years of follow-up, metachronous contralateral breast cancer was diagnosed in 4% and 12%, respectively, of the *BRCA2*-associated breast cancer patients versus 1% and 2%, respectively, in the sporadic breast cancer patients ($P_{logrank}$ = .02). Kaplan-Meier curves for the occurrence of contralateral breast cancer in *BRCA2*-associated patients and the matched sporadic controls are shown in Fig 3.

In Table 4, uni- and multivariate hazard ratios and 95% confidence intervals (CIs) of recurrence and all-causes mortality are listed. With sporadic cases as the reference group, the unadjusted hazard ratios for *BRCA2*-associated cases regarding recurrence and all-causes mortality were 0.92 (95% CI, 0.52 to 1.62) and 0.75 (95% CI, 0.37 to 1.50), respectively. Adjustment for a number of major prognostic factors, including tumor size, nodal status, steroid receptor status, and the occurrence of contralateral breast cancer, did not substantially alter the results. After correction for tumor stage, which is, in general, the most important prognostic factor, the hazard ratios for recurrence and all-causes mortality in the group of *BRCA2*-associated breast cancer patients were 0.84 (95% CI, 0.44 to 1.63) and 0.59 (95% CI, 0.27 to 1.29) ($P$ = .19).

Table 3. Two- and 5-Year Actuarial Outcomes for 28 *BRCA2*-Related and 112 Sporadic Breast Cancer Patients

| Outcome | *BRCA2* 2 Years | *BRCA2* 5 Years | Sporadic 2 Years | Sporadic 5 Years | *P* |
|---|---|---|---|---|---|
| Death rate | | | | | |
| Overall | 0.07 | 0.26 | 0.08 | 0.25 | .50 |
| By breast cancer | 0.04 | 0.23 | 0.07 | 0.24 | .40 |
| Recurrence rate* | 0.18 | 0.48 | 0.22 | 0.48 | .91 |
| Contralateral breast cancer rate† | 0.04 | 0.12 | 0.01 | 0.02 | .02 |

*Stage IV tumors excluded.
†Synchronous tumors excluded.

To further investigate the validity of the control group from the Daniel den Hoed Cancer Registry, we compared our data with the cancer registry data of the southeast Netherlands. In the Southeast, the 5-year survival for patients 15 to 59 years of age who had been diagnosed between 1980 and 1992 was 72%, which is similar to the 5-year survival of our control group (75%).[32]

*Survival Analysis After Exclusion of Affected Probands*

As we discussed previously, ascertainment of *BRCA2* families by affected probands might lead to a selection bias of patients who are characterized by longevity.[22] Therefore we performed separate analyses that excluded the six probands who were affected by breast cancer and also excluded their matched controls. This did not essentially alter the results: disease-free survival at 2 and 5 years was 77% and 45%, respectively, for *BRCA2*-associated patients versus 76% and 50%, respectively, for sporadic cases ($P_{logrank}$ = .78). Overall survival at 2 and 5 years was 91% and 68%, respectively, for *BRCA2* cases versus 92% and 72%, respectively, for sporadic breast cancer patients ($P_{logrank}$ = .79).

Table 4. Crude and Adjusted Hazard Ratios for *BRCA2*-Related Versus Sporadic Breast Cancer Patients

| | Recurrence HR | Recurrence 95% CI | Recurrence *P* | All-Causes Mortality HR | All-Causes Mortality 95% CI | All-Causes Mortality *P* |
|---|---|---|---|---|---|---|
| Unadjusted | 0.92 | 0.52-1.62 | .78 | 0.75 | 0.37-1.50 | .42 |
| Adjusted for tumor stage | 0.84 | 0.44-1.63 | .61 | 0.59 | 0.27-1.29 | .19 |

Abbreviations: HR, hazard ratio; CI, confidence interval.

We also performed separate analyses for cases diagnosed after 1980 because we were not able to fully match seven *BRCA2* cases diagnosed before 1980 with respect to year of diagnosis.

Again, there were no significant differences: disease-free survival at 2 and 5 years was 86% and 45%, respectively, for the *BRCA2* group versus 76% and 56%, respectively, for sporadic cases ($P_{logrank} = .72$). Overall survival at 2 and 5 years was 95% and 74%, respectively, for *BRCA2* patients and 90% and 76% for sporadic patients ($P_{logrank} = .99$).

Finally, we estimated the crude overall survival for the entire group of 45 *BRCA2*-associated breast cancer patients, including the 17 patients without pathology and/or sufficient follow-up. When these patients were included, 2- and 5-year overall survival was 91% and 75%, respectively.

## DISCUSSION

Disease-free and overall survival of 28 breast cancer patients from Dutch families with an identified *BRCA2* germline mutation did not significantly differ from the disease-free and overall survival of 112 sporadic patients matched for age of onset of disease and year of diagnosis. Despite some reports on the more adverse histoprognostic characteristics of *BRCA2*-related breast cancer,[13-16] the clinical outcome for patients with *BRCA2*-related breast cancer did not appear to be worse. Overall survival of the *BRCA2*-associated breast cancer patients was even nonsignificantly better when we adjusted for tumor stage. There are currently only a very few studies with which we can compare our findings. Furthermore, the data on the survival of breast cancer patients with a *BRCA2* germline mutation are primarily limited to a few mutations that are characteristic for specific populations.[27,33] An overview of those studies that have addressed the issue of survival of *BRCA1*- and/or *BRCA2*-associated breast cancer patients is given in Table 5. It must be noted that there are essential differences in the methods used in these studies and therefore also in the way that comparison groups were selected. In part this is reflected by the wide range of values for survival in control patients in the different studies.

One study described the overall survival of 20 breast cancer patients from five *BRCA2* families.[25] These families were all large families that had previously been used in linkage studies, and a germline mutation had only been identified in three of them. It has been pointed out previously that linkage analysis may have been more conclusive, considering the high number of breast cancer patients still alive at the time of study; therefore selection of these families might have led to a bias toward longevity. Nevertheless, overall survival was similar for *BRCA2*-associated breast cancer patients and breast cancer patients from a cancer registry who had been matched for age, date of diagnosis, and tumor size.

In the study by Marcus et al.[21] a poorer prognosis was demonstrated for hereditary breast cancer patients whose disease was not related to the *BRCA1* gene. It was suggested

**Table 5. Disease-Free and Overall Survival in *BRCA1*- and *BRCA2*-Associated Breast Cancer**

| | First Author | Year | No. of Carriers | 5-Year DFS (%) | | P | 5-Year OS (%) | | P | Possible Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Carriers | Controls | | Carriers | Controls | | |
| *BRCA1* | Porter[34] | 1994 | 35 | NR | NR | — | 83 | 61 | < .05 | Better |
| | Marcus[21] | 1996 | 72 | NR | NR | — | 67 | 59 | NS | Equal or better* |
| | Foulkes[28] | 1997 | 12 | 68† | 89† | .0188 | 64 | 96 | .0023 | Worse |
| | Verhoog[22] | 1998 | 49 | 49 | 51 | .96 | 63 | 69 | .88 | Equal |
| | Johannsson[24] | 1998 | 40 | NR | NR | — | 65‡ | 80‡ | NS | Equal or worse |
| | Gaffney[25] | 1998 | 30 | NR | NR | — | 75 | 70 | NS | Equal |
| | Ansquer[29] | 1998 | 15 | NR | NR | — | 70‡ | 90‡ | .04 | Worse |
| | Lee[27] | 1999 | 35§ | NR | NR | — | 79 | 78 | NS | Equal |
| Combined *BRCA1* and *BRCA2* | Robson[35] | 1998 | 28 | 65 | 69 | .51 | NR | NR | NS | Equal |
| | Lee[27] | 1999 | 82‖ | NR | NR | — | 70-87¶ | 75-93¶ | NS | Equal |
| *BRCA2* | Sigurdsson[33] | 1996 | 42 | NR# | NR# | .009 | NR | NR | — | Worse (or better?)** |
| | Gaffney[25] | 1998 | 20 | NR | NR | — | 73 | 70 | NS | Equal |
| | Lee[27] | 1999 | 23§ | NR | NR | — | 60 | 78 | NS | Equal |
| | Present study | | 28 | 52 | 52 | .91 | 74 | 75 | .50 | Equal |

Abbreviations: DFS, disease-free survival; OS, overall survival; NR, not reported; NS, not significant.

*Trend toward worse outcome after adjustment for age and disease stage.

†Survival until occurrence of distant disease.

‡Figures estimated from Kaplan-Meier curves.

§First-degree relatives of carriers affected by breast cancer.

‖Inferred mutation carrier status.

¶Range of maximum likelihood estimates for different groups according to age and year of diagnosis.

#Only DFS after 10 years of follow-up reported; DFS was 40% and 55% for carriers and controls, respectively.

**Allegedly better outcome when matched for year of diagnosis.[13]

that the group of 66 patients in the Marcus et al study predominantly consisted of *BRCA2*-related cases. However, only seven cases were included from families with evidence for linkage to the *BRCA2* gene. At present, it seems unlikely that all of the cases from families not linked to the *BRCA1* gene consist of *BRCA2* mutation carriers. A worse outcome was also found by Sigurdsson et al.[33] who studied Icelandic breast cancer patients from families with evidence for linkage to the *BRCA2* gene. However, in that study, controls were not matched for year of diagnosis, which most likely led to large differences in the treatment of these patients as well as differences in the distribution of the stage of the disease. When controls were matched for year of diagnosis in their study, there was a trend toward a more favorable outcome.[13] Moreover, as a result of endogamy and the geographic isolation of the Icelandic population, only one single specific mutation is present in the Icelandic breast cancer families linked to the *BRCA2* gene.[36] The position of this mutation in the gene, as well as its genetic and environmental background, may very well influence tumor and patient characteristics and subsequent survival.[37]

Robson et al[35] described a similar clinical outcome in a combined group of Ashkenazi Jewish breast cancer patients with *BRCA1* or *BRCA2* germline mutations, compared with cases not associated with *BRCA* mutations. This study, as well as some other studies, failed to discriminate between *BRCA1* and *BRCA2* mutations.[27,38–40] In the study presented here, we demonstrated that it may be of interest to differentiate between *BRCA1*- and *BRCA2*-associated breast cancers, especially with regard to the endocrine aspects of these tumors.

Earlier, we reported that *BRCA1*-associated breast cancers are clearly more often steroid receptor-negative, compared with sporadic controls.[22] In the study presented here, *BRCA2* tumors instead showed a trend toward a higher incidence of steroid receptor positivity, especially with respect to PgRs. At present, few data exist regarding the relationship between steroid receptor status and the presence of a *BRCA2* germline mutation. Loman et al[41] found that *BRCA2*-associated breast cancer was more likely to be $ER^+$, compared with *BRCA1* cases. In line with our observation, a study of *BRCA2*-associated tumors with the Icelandic founder mutation 999del5 showed significantly higher ER and PgR levels, compared with a group of tumors from age-matched controls.[13] Other studies however, that have used immunohistochemical analysis to assess steroid receptor status have not made this observation.[15,42] If confirmed, the higher percentage of steroid receptor-positive tumors in *BRCA2* carriers is of special interest in chemoprevention of breast cancer with antiestrogens.[43]

It has been known for a long time that familial breast cancer is associated with bilateral disease.[10,44] The lifetime risk for contralateral breast cancer in *BRCA1* carriers was estimated to be as high as 64%.[5] We reported the rate of metachronous contralateral disease in *BRCA1*-associated breast cancer to be 19% after 5 years of follow-up.[22] In the study presented here, we found a contralateral breast cancer rate of 12% after 5 years for *BRCA2* carriers. In total, bilateral disease was seen in 28.6% of the patients included in our study. Although the high rate of contralateral disease in *BRCA2* carriers might support prophylactic contralateral mastectomy as a treatment method, patients with *BRCA2* mutations might benefit from adjuvant hormonal therapy with respect to the possible prevention of contralateral disease, considering the high percentage of steroid receptor-positive tumors.

Studying survival in self-ascertained families has been said to lead to selection for longevity of cases because families with more living affected women are more likely to be (self-) referred to a family cancer clinic. In our study, only six probands were women affected with breast cancer. To exclude any possible bias, we performed separate analyses that excluded these six probands; however, this did not essentially alter the results. In two families, which contributed six *BRCA2*-associated cases, none of the affected family members were alive and a mutation was detected by testing first-degree relatives and then confirming the presence of the mutation in tumor material. In two other families, the diagnosis of breast cancer in the proband was the direct motive to refer a family to the family cancer clinic; therefore, these cases were included in a prospective manner. Finally, estimates for overall survival, including the *BRCA2*-cases without pathology or follow-up, did not yield significantly different results. For these reasons, we believe that bias is not a major problem in our study.

In conclusion, in this study, we observed that despite the differences in histologic and biochemical characteristics, the disease-free and overall survival of breast cancer patients with germline mutations in the *BRCA2* gene are similar to those of sporadic breast cancer patients. One important finding is the significantly increased rate of contralateral breast cancer in our unselected families. In contrast to those in *BRCA1*-related breast cancer, *BRCA2* tumors are not more often $ER^-$ and $PgR^-$ but rather tended to be receptor-positive. For the detection of potential small differences, larger studies are needed.


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# Chapter 7

## Prognostic significance of germline BRCA2 mutations in hereditary breast cancer patients.

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# Prognostic Significance of Germline *BRCA2* Mutations in Hereditary Breast Cancer Patients

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**Purpose:** Breast cancer in *BRCA2* gene mutation carriers differs from *BRCA1*-associated breast cancer or so-called sporadic breast cancer in clinical features and behavior. These differences may be of importance for the prevention, screening, and ultimately treatment of breast cancer in women with such germline mutations.

**Methods:** We reviewed the few studies that have reported on survival in patients with *BRCA2*-associated breast cancer. In this article we discuss why family history is no substitute for hereditary breast cancer with regard to studying survival and possible reasons why studies using family history yield contradictory results, why *BRCA2*-associated breast cancer should be considered a unique entity, and what methodological problems may exist, especially with regard to family-based studies.

**Results:** Five studies have reported on survival in *BRCA2*-associated breast cancer. Two studies showed a statistically significant worse survival for *BRCA2* patients, but the patients from one of these studies were later claimed to have a trend toward better prognosis when controls were matched for age and year of diagnosis. The other study found that the unfavorable prognosis of *BRCA2* patients was, to a great extent, due to a worse stage of the disease at time of diagnosis. The remaining three studies showed no significant effect of germline *BRCA2* mutations on survival. The numbers of *BRCA2* patients investigated in these studies were 42, 20, 23, 28, and 54 patients. Five-year overall survival in these patients varied from 65% to 74%.

**Conclusion:** No definite conclusion can be made with regard to the prognosis of *BRCA2*-associated breast cancer, but large differences in comparison with sporadic breast cancer are not likely to exist. Breast cancer caused by *BRCA2* mutations is also a distinct entity with its own features when compared with *BRCA1*-associated breast cancer. In contrast with *BRCA1*-associated breast cancer, *BRCA2* tumors tend to be more often steroid receptor–positive.

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THE DISEASE-FREE AND overall survival rate for invasive breast cancer is modified by a large variety of clinical, pathologic, and cell biologic factors. For breast cancer to occur, normal cells have to undergo growth transformation, which is the result of a multistep process as a consequence of an accumulation of several genetic alterations. Of special interest are inherited genetic alterations (ie, germline mutations) in so-called cancer susceptibility genes. Inheritance of mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* is thought to be responsible for 5% to 10% of all breast cancers.[1-3]

Over recent decades, an increasing number of cell biologic parameters, such as oncogenes, tumor suppressor genes, hormone receptors, growth factors, and secretory proteins, have been discovered that seem to influence strongly the behavior of a tumor with respect to growth rate, apoptosis, extent of cellular differentiation, metastatic pattern, and development of resistance to therapy. The possibility that a hereditary aspect of breast cancer might modify these aspects and thus influence prognosis was already investigated before the identification of the breast cancer susceptibility genes *BRCA1* and *BRCA2*.[4]

The tumorigenic pathway by which breast tumors arise in the mammary tissue of *BRCA1* or *BRCA2* germline mutation carriers may partly differ from each other and from their sporadic counterparts. This is well illustrated by the specific histologic features of *BRCA1*- and *BRCA2*-associated breast cancers, although there is considerable overlap in histology with sporadic tumors as well. Both *BRCA1*- and *BRCA2*-related tumors do not have a single cytoarchitectural type or uniform grade.[5-8]

Identifying differences in survival and tumor characteristics of hereditary breast cancers may have important implications. The efficacy of screening, the benefit of (contralateral) prophylactic surgery or chemoprevention, and the optimal treatment of hereditary breast cancer are all in a way related to the prognosis of these tumors. Moreover, such information is crucial for the women who are carriers of a *BRCA1* or *BRCA2* germline mutation.[9]

## FAMILY HISTORY AS A PROGNOSTIC FACTOR

A positive family history for breast cancer is an established risk factor for the disease. It has also been extensively

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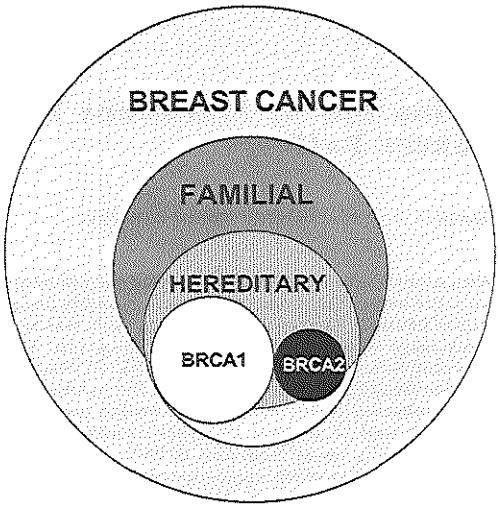


Fig 1. Relationship between *BRCA1*- and *BRCA2*-associated, hereditary, familial, and sporadic breast cancer in a given population.

investigated as a prognostic feature, but results are conflicting. A positive family history for breast cancer has been correlated with better, similar, and worse prognosis relative to nonfamilial breast cancer.[10] This may readily be explained by the fact that, apart from being ill-defined, familial breast cancer is a heterogeneous entity. Methodological differences too are likely to play a role in the diverging results from these studies.

Depending on how familial breast cancer is defined, up to 20% of the women diagnosed with breast cancer have at least one relative who is also affected by the disease.[11] Fig 1 gives a simplified diagram of how *BRCA1*- and *BRCA2*-associated breast cancer relate to hereditary, familial, sporadic, and thus overall breast cancer. Because breast cancer is not an infrequent disease in Western countries, a significant proportion of the familial breast cancer cases will result from chance clustering alone as well as shared environmental factors and not a predominant genetic susceptibility.

Far from all cases of hereditary breast cancer are explained by *BRCA1* and *BRCA2*. A number of genes are known in which germline mutations give rise to an increased susceptibility for breast cancer (see review in Martin and Weber[12] ), like the *p53* gene in the Li Fraumeni syndrome[13] and *PTEN* in Cowden's disease.[14] Although these other susceptibility genes only explain a small proportion of hereditary breast cancer, there is compelling evidence that other major breast cancer susceptibility genes (*BRCAX*) in non *BRCA1/2* breast cancer must exist.[8,15-17]

To further complicate matters, population-based studies have shown that not all women with hereditary breast cancer caused by *BRCA1* or *BRCA2* will have a close (first-degree) relative affected by the disease.[18-20] These patients are represented in Fig 1 by the part of the diagram of "Hereditary" not overlapping with "Familial" breast cancer patients. This fact can simply be the result of the composition of a family (for instance, paternal inheritance in combination with a small family and predominant male sibships). On the other hand, these population-based studies have raised questions of whether the initial breast cancer risks given for carriers of a *BRCA1* or *BRCA2* germline mutation may have been too high, due to the fact that these were estimates based on high-risk families with multiple women affected by breast cancer.[21]

The final reason why family history is a poor substitute for investigating the role of genetic susceptibility as a prognostic factor is the fact that the ratios of *BRCA1* versus *BRCA2* and *BRCA1/2* versus the total group of hereditary and familial breast cancer will differ between specific populations. This is best illustrated by the example of the Icelandic population in which the vast majority of proven hereditary breast cancer families are caused by one single mutation in *BRCA2* (999del5).[22] In the end, the prevalence of breast cancer and exogenic causes of breast cancer will also influence the proportions in a population, as shown in Fig 1.

## SIMILARITIES AND DISTINCTION BETWEEN *BRCA1* AND *BRCA2*

Besides their gene names, a number of similarities exist between *BRCA1* and *BRCA2* and their associated phenotypes when these genes are mutated. Both genes were found as a result of an extensive genome-wide search in families with multiple early-onset breast cancer patients, so not surprisingly, germline mutations in both confer strongly elevated lifetime risks for breast cancer.[23] Again not surprisingly, a young age at onset and an elevated risk for bilateral breast cancer are characteristics shared by both *BRCA1* and *BRCA2*.

Perhaps the best reason to consider breast cancer associated with either one of these tumor suppressor genes as a single entity is the fact that of the nuclear proteins for which *BRCA1* and *BRCA2* encode, both are postulated to play a role in DNA damage–response pathways and interact with each other and the RAD51 protein, thus confirming their function in some of the same cellular pathways.[24] In addition to this, both genes are very rarely somatically mutated in nonhereditary breast cancer.[25-28] It is therefore

Table 1. Steroid Receptor Status (%) and 5-Year Survival (%) in Dutch *BRCA1*- and *BRCA2*-Associated Breast Cancer Patients and Controls Matched for Age and Year of Diagnosis[36,37]

| | *BRCA1* | Matched Controls | *BRCA2* | Matched Controls |
|---|---|---|---|---|
| ER-positivity | 36 | 65 | 93 | 84 |
| PgR-positivity | 30 | 66 | 100 | 77 |
| Relapse-free survival | 49 | 51 | 52 | 52 |
| Overall survival | 63 | 69 | 74 | 75 |
| Contralateral breast cancer* | 19 | 5 | 12 | 2 |

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor.
*5-year rate (%) of metachronous contralateral breast cancer.

not uncommon to see epidemiologic studies that combine carriers of *BRCA1* and *BRCA2* germline mutations and consider them as a single group.[29-32]

From a biologic point of view, the differences between the two genes are of great interest. Risk calculations for premenopausal *BRCA2* carriers with regard to breast cancer turn out to be somewhat lower than for *BRCA1*. However, the risk for *BRCA2* carriers continues to increase after menopause, whereas that of *BRCA1* carriers levels off.[23] In line with these calculations, we recently reported a lower risk with regard to contralateral breast cancer in postmenopausal *BRCA1* carriers.[33] It is tempting to relate these differences to hormone-dependent pathways, and in this light, the distinction with regard to the presence of steroid receptors between *BRCA1* and *BRCA2* tumors is striking[34-37] (Table 1). In our own updated experience, we currently know the steroid receptor status from 26 *BRCA2*-associated breast tumors, of which 92% (n = 24) are estrogen receptor–positive and 95% (20 of 21) are positive for progesterone receptor. In contrast, only one third of the *BRCA1* tumors are steroid receptor–positive. This difference in steroid receptor status might be of special importance with regard to chemoprevention of breast cancer with antiestrogens.[38]

Differences between *BRCA1* and *BRCA2* also exist with respect to histology.[6,7,39,40] Although both *BRCA1* and *BRCA2* tumors have been found to be more often of high grade as compared with age-matched controls, multivariate analysis shows that in *BRCA2* this is mostly due to the formation of less tubular structures in a tumor, whereas *BRCA1* tumors have high mitotic counts.[7] Both *BRCA1* and *BRCA2* have a higher proportion of their tumor perimeter with a continuous pushing margin, a feature that they share with breast carcinomas of the medullary type. Medullary carcinomas in this context are of special interest because they have been associated with a more favorable prognosis than ordinary ductal carcinomas of the breast.[40]

Possibly *BRCA2*-associated tumors more often have an extensive intraductal component as compared with *BRCA1* tumors.[41] An extensive intraductal component is one of the risk factors for local recurrence after breast-conserving therapy.[42] It could also point to a more indolent behavior of an in situ cancer until the invasive cancer arises, and this might point to opportunities for intervention by screening in *BRCA2* mutation carriers. For the reasons mentioned above, despite the fact that only a small proportion of all breast cancer cases are caused by germline mutations in *BRCA2*, it is of major interest to investigate these patients as a distinct group.

## *BRCA2* CARRIER STATUS AS A PROGNOSTIC FACTOR

Although some reports failed to discriminate between *BRCA1* and *BRCA2* mutations, a limited numbers of studies have looked at survival of *BRCA2* mutation carriers with breast cancer as a separate entity (Table 2). The first study to report on the outcome of *BRCA2*-associated breast cancer patients was by Sigurdsson et al,[43] who studied 42 Icelandic breast cancer patients from families with evidence for linkage to *BRCA2*. They found a significantly worse disease-free survival for *BRCA2*-associated cases as compared with sporadic controls matched for age but not for

Table 2. Five-Year Overall Survivel in *BRCA2*-Associated Breast Cancer

| First Author | Year | No. of Carriers | Survival (%) | | *P* | Possible Outcome |
|---|---|---|---|---|---|---|
| | | | Carriers | Controls | | |
| Sigurdsson[43] | 1996 | 42 | NR* | NR* | .009 | Worse (or better?)† |
| Gaffney[44] | 1998 | 20 | 73 | 70 | NS | Equal |
| Lee[45] | 1999 | 23‡ | 65 | 78 | NS | Equal |
| Verhoog[36] | 1999 | 28 | 74 | 75 | .50 | Equal |
| Loman[46] | 2000 | 54 | 72 | 83 | .06 | Worse§ |

Abbreviations: NR, not reported; NS, not significant.
*Only disease-free survival after 10 years of follow-up was reported: 40% and 55% for carriers and controls, respectively.
†Allegedly better outcome when matched for year of diagnosis.
‡First-degree relatives of carriers affected by breast cancer.
§Breast cancer–specific survival rates were 72% and 86% for carriers and controls, respectively; $P = .003$.

year of diagnosis. Most likely, not matching for year of diagnosis led to large differences in the treatment of these patients as well as differences in the distribution of stage of the disease. However, in an other study these authors investigated grade and histology of partly the same *BRCA2*-associated patients and reported a trend towards a more favorable outcome when controls were matched for year of diagnosis.[34]

Gaffney et al[44] studied survival in 20 *BRCA2*-associated breast cancer patients, also from families that previously had shown positive linkage for *BRCA2*. These patients were matched to controls from a population-based cancer registry, not only for age and year of diagnosis, but also for tumor size. Unfortunately, information about the control patients, such as surgical procedure or nodal status, was otherwise limited, but no significant difference in survival was detected.

The study by Lee et al[45] was unique because, so far, it is the only population-based study on survival performed by recruiting volunteers from Ashkenazi Jewish descent. The figures for overall survival of *BRCA2*-associated cases in this study are those of breast cancer patients who tested positive for the presence of the Ashkenazi *BRCA2* founder mutation, 6174delT, in addition to those of affected relatives who were not tested. The design of the study was that of a combined *BRCA1/2* study with an inferred mutation carrier status using the kin-cohort method.[21] In this way, no data were available on clinical presentation and stage of the disease. No significant prognostic differences were detected.

At the Daniel den Hoed Cancer Center, we investigated tumor characteristics and survival of 28 breast cancer patients from the first 14 consecutive families with an identified *BRCA2* germline mutation.[35] Despite a somewhat larger tumor size at time of diagnosis, no difference in survival could be demonstrated in comparison with controls matched for age and year of diagnosis. Interestingly, the axillary lymph node status was not more often positive. The stage-adjusted recurrence and death rates of *BRCA2*-associated breast cancer patients versus sporadic breast cancer patients were nonsignificantly better (hazards ratio = 0.84 and 0.59, respectively; $P = .61$ and .19).

The recent study of Loman et al[46] has the largest number of *BRCA2*-associated breast cancer patients (n = 54) in a single study, and the patients were identified through oncogenetic counseling. In their study, the patients with *BRCA2*-associated breast cancer had a significantly worse disease-specific survival. To a great extent, this was due to the fact that significantly more *BRCA2*-associated cases presented with axillary lymph node involvement or stage IV breast cancer at the time of diagnosis. After adjusting for stage, the breast cancer–specific survival was no longer significantly worse for the *BRCA2*-associated cases (relative risk = 1.6; 95% confidence interval, 0.85 to 3.1). Differences in tumor biology were given as the most likely explanation for the more aggressive clinical presentation of *BRCA2*-associated breast cancer. The authors also investigated the possibility that families with more breast cancer deaths might have preferably been included in their study, but this seemed to be an unlikely explanation. This is not surprising, because selection bias is generally believed to lead to the inclusion of families with breast cancer patients who have a better prognosis.

## METHODOLOGICAL ASPECTS

Studying survival in *BRCA1/2*-associated breast cancer is further complicated by a number of methodological problems. Probably the best way to investigate the prognostic relevance of *BRCA2* germline mutations is in a population-based group of *BRCA2*-associated breast cancer patients.[48] However, to obtain sufficiently large numbers of patients, thousands of breast cancer patients would have to be tested for the presence of *BRCA1/2* mutations in a prospective manner. Apart from the long time it would take for results from such a study to emerge, the costs of screening for mutations in *BRCA1/2* in unselected breast cancer patients would probably be too high. The most straightforward results are to be expected from relatively easy studies in populations in which founder mutations are prevalent, like the Ashkenazi Jewish and Icelandic populations. This could be done in a historical cohort by looking for mutations in archival paraffin-embedded tissue blocks.[48] To establish carrier status, these samples would only have to be tested for a limited number of mutations.

However, studying a limited number of mutations may have its drawbacks as well. Some reports initially stated that tumor grade was related to the position of the mutation in the *BRCA1* gene but this has remained unconfirmed.[5] However for BRCA2 mutations such a genotype-phenotype correlation is not inconceivable.[49] Moreover these mutations are studied against a specific genetic and environmental background which may also influence survival of both cases and controls.

On hypothetical grounds, studying survival in self-ascertained families is said to lead to selection for longevity of cases.[50] This bias would result first from families with more living affected breast cancer patients being more likely to be (self) referred to a family cancer clinic, and, second, patients alive at the time of study being more likely to be included in the study sample. Missing data and lack of adequate follow-up or histopathologic confirmation of individuals

who died early and are therefore not included in the study sample might also play an important role.

In support of this, we found that after exclusion of breast cancer patients who were the first family members to visit our family cancer clinic (ie, the probands), there was a trend toward a worse outcome for patients with *BRCA1*-associated breast cancer.[37] It is of interest that this phenomena was observed in *BRCA1*-associated families but not after exclusion of probands in *BRCA2*-associated families. Moreover, including the *BRCA2*-associated patients who were identified by constructing a pedigree but for whom insufficient pathology or follow-up were available did also not show a trend toward selection for longevity.[36] One might hypothesize that selection toward longevity in family-based breast cancer patients is more readily apparent when carriage of a germline mutation is a strong adverse prognostic factor.

However, the notion that a family comes to the attention merely on the basis of living breast cancer patients is, within the setting of our family cancer clinic, a misconception. Most probands themselves are unaffected by breast cancer and visit our family clinic because of obvious familial clustering of breast cancer. Whether the breast cancer patients are alive is of little influence on the individuals who will seek oncogenetic counseling. Moreover, genetic testing can sometimes be offered without living affected family members being available for mutation analysis, that is by performing mutation analysis in a number of unaffected first-degree relatives.

Finally, studies that have reported adverse pathobiologic characteristics in *BRCA1*- and *BRCA2*-associated breast cancer were liable to the same sort of bias, because the tumors that have been investigated are family-based in a manner similar to that of patients in survival studies.[8] Despite this disadvantage, these studies were able to demonstrate a higher tumor grade and poor prognostic characteristics in tumors from these patients, whereas if this selection bias had played a significant role, then the prognostic better tumors would have been ascertained.

In conclusion, patients with hereditary breast cancer resulting from a *BRCA2* mutation have not been conclusively shown to have a different prognosis when compared with age-matched sporadic breast cancer patients. Although breast cancer associated with *BRCA2* mutations as well as sporadic breast cancer and breast cancer associated with *BRCA1* mutations is a heterogenic group, such cases have unique features that may be of importance for the prevention, screening, and, ultimately, the treatment of breast cancer in women with *BRCA2* germline mutations. Studying the outcome and natural history of *BRCA2*-associated breast cancer may prove to be of great value for understanding the molecular genetics and tumorigenesis of this group of tumors and in this way help to better understand breast cancer as a whole.

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# Chapter 8

## Ipsilateral breast tumor recurrence in hereditary breast cancer following breast conserving therapy.

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*Background*
The overall rate of an ipsilateral breast tumor recurrence after breast conserving therapy ranges from 1-2% per year. Risk factors for ipsilateral recurrence include young age but data on the impact of BRCA1/2 mutations or a definite positive family history for breast cancer are scarce. In families with BRCA1/2 germline mutations or a clearly established family history we investigated the risk of ipsilateral recurrence in conservatively treated breast cancer patients.

*Methods*
87 hereditary breast cancer patients, identified through our Family Cancer Clinic, were diagnosed between 1980 and 1995 and conservatively treated in our institution (cases). 26 Patients from this group were BRCA1/2 mutation carriers (21 BRCA1, 5 BRCA2), while the remaining 61 patients came from high-risk breast (ovarian) cancer families (≥ 3 first degree relatives with breast and/or ovarian cancer) hereafter referred to as "unspecified HBC". They were compared in a 1:2 ratio to 174 sporadic breast cancer patients (controls) treated with breast conserving therapy in our institution, after matching for age at onset and year of diagnosis. Median follow up was 6.1 years for the cases and 6.0 years for controls.

*Results*
Patient and tumor characteristics were similar in the cases and controls. The subgroup of BRCA1/2-associated BC was characterized by a younger age at onset, a trend towards a larger tumor size and more contralateral breast cancer as compared with the unspecified HBC group and controls. An ipsilateral recurrence was observed in 19 hereditary (21.8%) vs 21 sporadic (12.1%) patients (p=0.14). In the hereditary patients more recurrences occurred elsewhere in the breast (4/19 = 21% versus 2/21 = 9.5%) therefore suggestive of new primaries. In the actuarial outcomes the 2 year ipsilateral recurrence rate was not different, but at 5 and 10 years this rate was twice as high in the hereditary patients as compared to the controls (14% vs 7% and 30% vs 16%, respectively; p=0.05). The recurrence rate elsewhere in the breast, suggestive of new primaries, began to diverge at 10 yrs, becoming non-significantly higher at 15 yrs in the hereditary patients (24% vs 3%; p=0.08). Post-local relapse survival, and 2- and 5-year overall survival were not different between the cases and controls.

*Conclusion*

Hereditary breast cancer (defined by the presence of BRCA1/2 mutations or by a strong family history) may be associated with a higher frequency of ipsilateral recurrence following breast conserving therapy. These recurrences appear to consist of early recurrences (2-5 years) at the site of the original tumor and of late new primaries (≥ 5 years). These data are helpful in informing women with a genetic susceptibility and must be taken into account when additional "risk reducing" surgery after primary breast conserving therapy is considered. Moreover, since ipsilateral recurrence in hereditary breast cancer patients frequently is a late event there is an indication for long-term follow-up.

Key words: breast cancer, ipsilateral breast tumor recurrence, breast conserving therapy, hereditary breast cancer, BRCA1, BRCA2, historical cohort study

## Introduction

Breast conserving surgery with subsequent radiation therapy (breast conserving therapy) has become the treatment of choice in women with early stage breast cancer. It was found to be equivalent to mastectomy regarding distant disease-free and overall survival.[1-4] However, following breast conserving therapy, recurrences in the preserved ipsilateral breast occur at an average rate of 1-2% per year, accumulating to approximately 15% after 10 years.[1,2,5,6] While early recurrences most likely represent outgrowth of residual disease, therefore occurring in the vicinity of the original tumor, late recurrences are suggestive of a new primary tumor. Accordingly the latter are found more often in an area distinct from the site of the primary breast tumor.[7,8]

Breast cancer patients who develop an ipsilateral recurrence have an increased risk of developing distant metastatic disease indicative of the prognostic value of an ipsilateral recurrence.[1,9-11] However, subanalyses show that early ipsilateral recurrences, as compared to late ipsilateral recurrences, are associated with a worse outcome.[7-9] Recurrences in the skin probably are a separate entity and are also associated with a worse outcome.[12,13] Finally, although scientifically difficult to assess, besides the adverse outlook, breast cancer patients face intense emotional turmoil and psychological distress at the time of a recurrence.

A number of risk factors for ipsilateral recurrence following breast conserving therapy have been reported, including the extensive presence of ductal carcinoma in situ, vascular invasion, multifocality of the primary tumor, large tumor size, microscopically involved margins of the excision and young age of the patient.[10,14,15] Whether or not a positive family history for breast cancer or proven genetic susceptibility is also a risk factor has already been the subject of a number of studies.[16-27] However many of these studies are hampered by incomplete data on the family pedigree, small sample size, selection of specific populations (resulting in specific BRCA1/2 mutations) and short follow-up. Notwithstanding the fact that some of the above mentioned limitations also apply to the present study, we feel that more data on this topic are warranted because of the clinical importance. Therefore, we investigated the patient and tumor characteristics as well as the outcome (with special interest for ipsilateral breast tumor recurrence) in a well defined group of

hereditary breast cancer patients as compared to sporadic breast cancer patients, treated with breast conserving therapy.

## Patients and methods

*Patient cohorts*

From the cancer registry of our institute we retrospectively selected subjects fulfilling the following criteria:

1) women with early stage infiltrating breast cancer (BC) being treated by breast conserving therapy (BCT);
2) BC diagnosis between 1980 and 1995;
3) treatment performed at the Erasmus MC-Daniel den Hoed Cancer Center either from diagnosis on or within 3 months after diagnosis when patients were referred for postoperative radiation therapy.

The Daniel den Hoed cancer registry includes data from more than 10,000 breast cancer patients treated in the hospital since 1980. Within this cohort, 87 hereditary BC (HBC) patients were identified that were also known at the Family Cancer Clinic of our institute. HBC patients were selected that belonged either to BRCA1/2 mutation families or hereditary breast (ovarian) cancer (HB(O)C) families with ≥ 3 first degree relatives with breast and/or ovarian cancer. This hereditary group consisted of 26 BRCA1/2 mutation carriers (21 BRCA1; 5 BRCA2), while in 61 patients the diagnosis of HB(O)C was based upon pedigree data and as yet no mutations were identified (unspecified HBC). BRCA1/2 mutation analysis is described elsewhere.[28,29]

From the initial cohort, eligible "sporadic" BC patients were then selected and frequency-matched in a 1:2 ratio, for age at onset and period of diagnosis (5-year periods), with the hereditary cases, totaling 174 sporadic BC patients. Since the registry contains information on malignancies in family members, patients with a positive family history for BC were excluded from the sporadic group.

*Breast conserving therapy*

BCT generally consisted of wide local excision of the tumor with an attempted margin of healthy tissue of at least 1 cm after fixation, axillary lymph node dissection, and postoperative radiation therapy. All patients received whole breast irradiation to a

total dose of 45-50 Gy, given in fractions of 1.8 or 2 Gy, five times a week. A boost dose of 16-20 Gy was delivered to the tumor bed using either photons or electrons. Radiation therapy of the axillary and supraclavicular lymph nodes (46 Gy) was considered by indication in patients with extensive axillary involvement, defined as four or more metastatic lymph nodes with either involvement of the highest level, extracapsular extension and/or loose tumor deposits in the fat.

Adjuvant systemic therapy was given in lymph node positive patients. In general, pre-menopausal women were treated with 6 cycles of CMF, 4 cycles of FAC or AC chemotherapy, without subsequent tamoxifen. Postmenopausal patients received tamoxifen, 40 mg daily, for a period of 2-5 years, initially irrespective of the hormonal receptor status. Follow up consisted of 3-monthly evaluation the first two years, 6 monthly during year 3 to 5, and yearly evaluation thereafter. At each visit a history was taken, and physical examination was carried out. Mammography was performed once a year. Other tests were performed on indication.

*Data collection*

Detailed patient data were abstracted from the hospital records onto standardized forms. Information was collected on: age at onset of BC; menopausal status; tumor characteristics (location and extent of the tumor, histologic type, differentiation grade, presence and extent of DCIS, microscopic tumor margin involvement, hormonal receptor status); TNM status; local and systemic therapy. Central review of histology was not performed.

The main endpoint of this analysis was the occurrence of ipsilateral breast tumor recurrence (IBTR) as a first failure or simultaneously (within 3 months) with distant recurrence of the disease. IBTR was defined as recurrence of tumor within the initially treated breast tissue or overlying skin. The time interval from initial surgery to IBTR and its exact location were recorded: at or near the scar (in the vicinity of the original tumor), elsewhere in the breast (quadrant distinct from the site of the primary tumor), or diffuse in the breast/skin.

Table 1 Distribution of patient and tumor characteristics for BRCA1/2-associated, unspecified hereditary and sporadic breast cancer patients

| | Hereditary breast cancer | | | | | | Sporadic breast cancer | | |
|---|---|---|---|---|---|---|---|---|---|
| | BRCA1/2 n=26 (%) | | Unspecified HBC n=61 (%) | | Total n=87 (%) | | n=174 (%) | | p-value* |
| **Age at diagnosis (yrs)** | | | | | | | | | |
| Mean | 38.7 | | 48.9 | | 45.9 | | 46.1 | | 0.87 |
| Range | 27-54 | | 28-78 | | 27-78 | | 23-76 | | |
| **Follow up (yrs)** | | | | | | | | | |
| Median | 5.7 | | 6.4 | | 6.1 | | 6.0 | | 0.78 |
| Range | 1.5-14.9 | | 1.6-14.9 | | 1.5-14.9 | | 0.4-16.3 | | |
| **Menopausal status** | | | | | | | | | |
| Pre- | 21 | (80.8) | 29 | (47.5) | 50 | (57.5) | 105 | (60.0) | 0.85 |
| Peri- | 1 | (3.8) | 5 | (8.2) | 6 | (7.0) | 10 | (5.8) | |
| Post- | 3 | (11.5) | 25 | (41.0) | 28 | (32.2) | 50 | (28.7) | |
| Unknown | 1 | (3.8) | 2 | (3.3) | 3 | (3.5) | 9 | (5.2) | |
| **Tumor size** | | | | | | | | | |
| ≤ 2 cm | 14 | (53.8) | 38 | (62.3) | 52 | (60.0) | 121 | (69.5) | 0.09 |
| 2-5 cm | 8 | (30.8) | 9 | (14.7) | 17 | (19.5) | 35 | (20.1) | |
| > 5 cm | 0 | (0.0) | 0 | (0.0) | 0 | (0.0) | 1 | (0.6) | |
| Unknown | 4 | (15.4) | 14 | (22.9) | 18 | (21.0) | 17 | (9.8) | |
| **Lymph node involvement** | | | | | | | | | |
| Yes | 20 | (76.9) | 41 | (67.2) | 61 | (70.0) | 117 | (67.0) | 0.54 |
| No | 4 | (15.4) | 16 | (26.2) | 20 | (23.0) | 49 | (28.0) | |
| Unknown | 2 | (7.7) | 4 | (6.6) | 6 | (7.0) | 8 | (5.0) | |
| **Histologic type of tumor** | | | | | | | | | |
| Ductal | 25 | (96.0) | 49 | (80.0) | 74 | (85.0) | 147 | (84.5) | 0.92 |
| Lobular ± ductal | 0 | (0.0) | 2 | (3.3) | 2 | (2.3) | 6 | (3.4) | |
| Medullary | 1 | (3.8) | 1 | (1.6) | 2 | (2.3) | 7 | (4.0) | |
| Mucinous | 0 | (0.0) | 3 | (4.9) | 3 | (3.4) | 4 | (2.3) | |
| Other | 0 | (0.0) | 4 | (6.6) | 4 | (4.6) | 5 | (2.9) | |
| Unknown | 0 | (0.0) | 2 | (3.3) | 2 | (2.3) | 5 | (2.9) | |
| **Differentiation grade** | | | | | | | | | |
| I | 0 | (0.0) | 4 | (6.6) | 4 | (4.6) | 3 | (1.7) | 0.47 |
| II | 4 | (15.4) | 12 | (19.7) | 16 | (18.4) | 29 | (16.7) | |
| III | 15 | (57.7) | 32 | (52.5) | 47 | (54.0) | 92 | (52.9) | |
| Unknown | 7 | (26.9) | 13 | (21.3) | 20 | (23.0) | 50 | (28.7) | |

(Table 1 continued)

| | Hereditary breast cancer | | | | | | Sporadic breast cancer | | |
|---|---|---|---|---|---|---|---|---|---|
| | BRCA1/2 n=26 | (%) | Unspecified HBC n=61 | (%) | Total n=87 | (%) | n=174 | (%) | p-value* |
| **Microscopic margin** | | | | | | | | | |
| Free | 15 | (57.7) | 28 | (45.9) | **43** | **(49.4)** | **97** | **(55.8)** | 0.32 |
| Involved | 1 | (3.8) | 6 | (9.8) | **7** | **(8.1)** | **10** | **(5.8)** | |
| Doubtful | 1 | (3.8) | 13 | (21.3) | **14** | **(16.1)** | **16** | **(9.2)** | |
| Unknown | 9 | (34.6) | 14 | (22.9) | **23** | **(26.4)** | **51** | **(29.3)** | |
| **In situ component** | | | | | | | | | |
| No | 15 | (57.7) | 26 | (42.6) | **41** | **(47.1)** | **62** | **(35.6)** | 0.18 |
| Moderate | 5 | (19.2) | 13 | (21.3) | **18** | **(20.7)** | **36** | **(20.7)** | |
| Extensive | 2 | (7.7) | 10 | (16.4) | **12** | **(13.8)** | **24** | **(13.8)** | |
| Unknown | 4 | (15.4) | 12 | (19.7) | **16** | **(18.4)** | **52** | **(29.9)** | |
| **Radiotherapy** | | | | | | | | | |
| Axillary nodes | 2 | (7.7) | 9 | (14.7) | **11** | **(12.6)** | **13** | **(7.5)** | 0.26 |
| Regional nodes | 1 | (3.2) | 8 | (13.1) | **9** | **(10.3)** | **25** | **(14.4)** | 0.34 |
| **Systemic treatment** | | | | | | | | | |
| Chemotherapy | 5 | (19.2) | 10 | (16.4) | **15** | **(17.2)** | **33** | **(19.4)** | 0.23 |
| Tamoxifen | 1 | (3.8) | 2 | (2.7) | **3** | **(3.4)** | **4** | **(2.3)** | 0.36 |
| **Breast tumor recurrence** | | | | | | | | | |
| At/near scar | 2 | (7.7) | 9 | (14.7) | **11** | **(12.6)** | **12** | **(6.9)** | 0.14 |
| Elsewhere | 1 | (3.8) | 3 | (4.9) | **4** | **(4.5)** | **2** | **(1.1)** | |
| Diffuse/skin | 1 | (3.2) | 1 | (1.6) | **2** | **(2.3)** | **7** | **(4.0)** | |
| Unknown | 0 | (0.0) | 2 | (3.3) | **2** | **(2.3)** | **0** | **(0.0)** | |
| Total | 4 | (15.4) | 15 | (24.6) | **19** | **(21.8)** | **21** | **(12.1)** | |
| **Contralateral breast cancer** | | | | | | | | | |
| No | 20 | (76.9) | 55 | (90.2) | **75** | **(86.2)** | **163** | **(93.7)** | 0.06 |
| Synchronous | 2 | (7.7) | 1 | (1.6) | **3** | **(3.5)** | **4** | **(2.3)** | |
| Metachronous | 4 | (15.4) | 5 | (8.2) | **9** | **(10.3)** | **7** | **(4.0)** | |

*p-value for the difference between the total group of hereditary breast cancer versus the sporadic group

*Statistical analysis*

Chi-square tests were used to compare patient and tumor characteristics of hereditary and sporadic BC patients. In addition, characteristics of the 21 identified BRCA1 gene mutation carriers and 61 other hereditary patients were analyzed separately. Kaplan Meier survival probabilities were calculated and differences between curves were tested by the logrank test. The simultaneous effect of several characteristics on the IBTR and other endpoints was investigated by Cox's proportional hazards method. This was done separately for BRCA1 and unspecified HBC versus sporadic cases. Covariates were added to the model if a change of more

than 10% in the hazard ratio of either BRCA1 or unspecified HBC patients versus sporadic cases was seen. All analyses were performed with STATA and SPSS for windows software.

## Results

*Patient and tumor characteristics*

Patient and tumor characteristics of the cases are summarized in Table 1. There were no clear differences between the hereditary and sporadic cohort with respect to TNM status, histologic type, differentiation grade, microscopic margin involvement, extent of in situ component, radiation and systemic therapy. Subgroup analysis of the 21 BRCA1 mutation carriers and the 61 as yet unspecified HBC patients, showed a different age at onset of the disease, with a mean age of 38.7 years for BRCA1 mutation carriers in contrast with 48.9 years for the unspecified hereditary patients ($p=0.003$). Furthermore, there was a trend for smaller tumor size ($p=0.07$) and more estrogen receptor (ER) positive tumors ($p=0.07$) in the unspecified hereditary patient group as compared to BRCA1 mutation carriers (data not shown).

*Ipsilateral breast tumor recurrence*

An IBTR occurring as first event was observed 40 times, 21 times in sporadic (12.1%) and 19 times in hereditary BC patients (21.8%) respectively. Four recurrences (4/26; 15%) occurred in BRCA1/2 mutation carriers and 15 (15/61; 24%) in unspecified HBC patients. As is shown in Table 1, more recurrences at/near the site of the primary tumor were found in the hereditary group, especially in unspecified hereditary patients (12.6 vs 6.9%). Furthermore, a recurrence in a quadrant distinct from the site of the primary tumor (defined "elsewhere") being suggestive of a new primary, occurred more often in the hereditary group (4.5 vs 1.1%). More IBTR's presented as diffuse/skin locations in the sporadic group. Overall, however, the differences were not statistically significant. There was no association between the location of a local recurrence and both the microscopic margin involvement and the extent of an in situ component (data not shown).

Table 2: Ipsilateral breast tumor recurrence rates for hereditary breast cancer (n=87) versus sporadic breast cancer patients (n=174) in relation to site of the recurrence.

| | Hereditary breast cancer | | | | Sporadic breast cancer | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2 yr | 5 yr | 10 yr | 15 yr | 2 yr | 5 yr | 10 yr | 15 yr | p-value |
| Overall ipsilateral recurrence rate | 0.03 | 0.14 | 0.30 | 0.49 | 0.04 | 0.07 | 0.16 | 0.20 | 0.05 |
| Recurrence rate at/near the scar | 0.02 | 0.10 | 0.16 | 0.23 | 0.02 | 0.04 | 0.10 | 0.14 | 0.12 |
| Recurrence rate elsewhere in the ipsilateral breast | 0 | 0 | 0.08 | 0.24 | 0 | 0.01 | 0.03 | 0.03 | 0.08 |

The recurrence rate as a function of time, i.e. period elapsing between initial therapy and IBTR, is demonstrated in table 2 and figure 1 and 2. At 2 years after primary therapy the rate of IBTR was identical in both groups. At 5 years, however, it was twice as high in the hereditary as in the sporadic cohort (14% versus 7% respectively). This was mainly due to recurrences in the vicinity of the primary tumor, with a rate of 10% versus 4% respectively. The IBTR rate at 10 and 15 years in the hereditary cohort remained twice as high (30% and 49%) as in the sporadic group (16% and 20%). Now, in the hereditary group there were mainly recurrences elsewhere in the breast (p=0.08). Overall, the difference in recurrence rate is borderline statistically significant (p=0.05, figure 2).

Figure 1: Ipsilateral breast tumor recurrence by cohort; total group of hereditary breast cancer patients and sporadic breast cancer patients

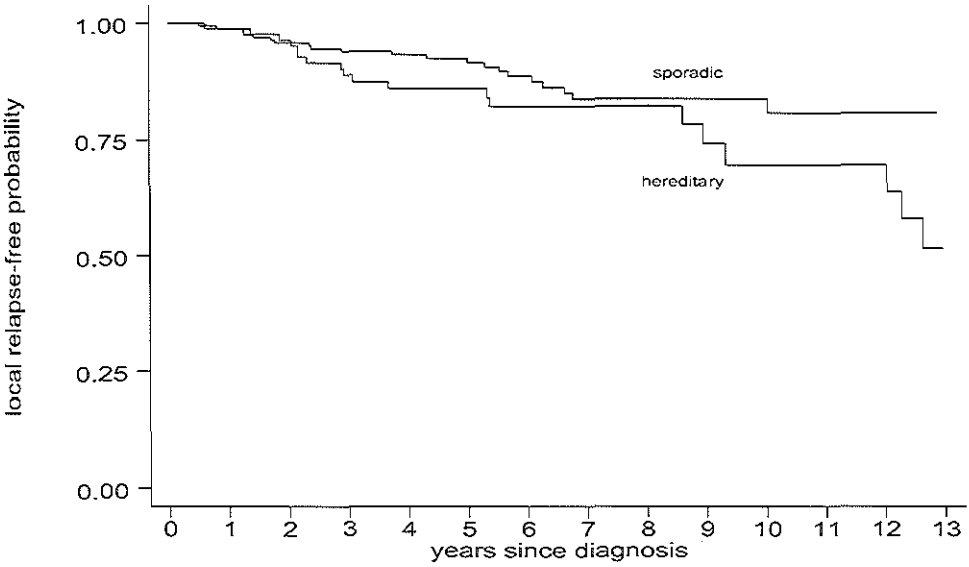
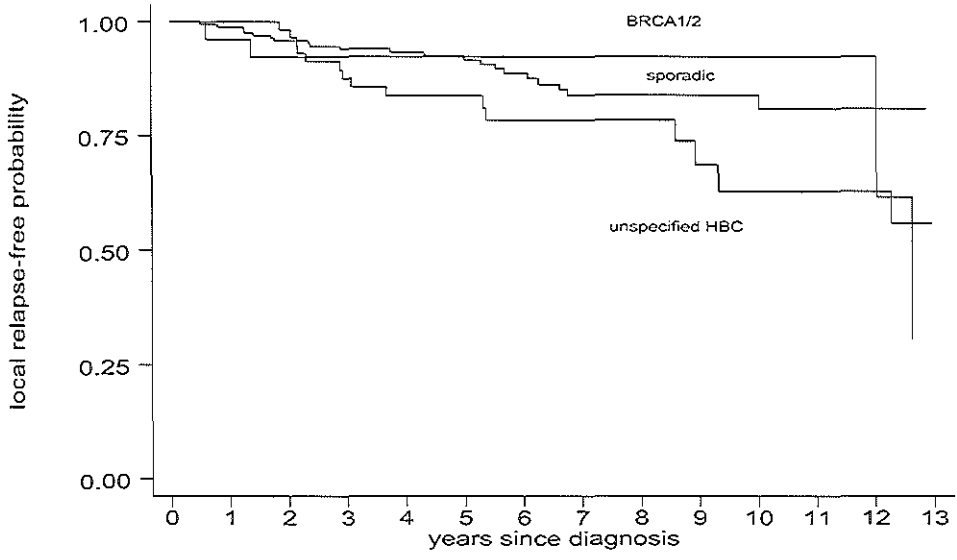



Figure 2: Ipsilateral breast tumor recurrence by cohort; unspecified hereditary, BRCA1/2-associated patients and sporadic breast cancer patients



*Contralateral breast cancer (CBC)*

The incidence of CBC was increased in the hereditary cohort as compared to the sporadic group (13.8% versus 6.3% respectively, p=0.06). This was most pronounced in BRCA1/2 mutation carriers with synchronous bilateral BC occurring in 2 cases (7.7%), and metachronous CBC in 4 patients (15.4%, table 1). The time span to CBC occurrence was variable, ranging between 2 and 7 years for sporadic, and between 1 and 12 years for HBC patients (mean 5 and 3.7 years respectively).

*Outcome after IBTR*

At 2 years, the post local-relapse survival was not different between the groups, being 75% (C.I. 50-89%) for hereditary, and 74.6% (C.I. 52-88%) for sporadic patients (p= 0.91). Overall survival (OS) at 2 and 5 years was 96% (C.I. 90-99%) and 78% (C.I. 68-85%) for hereditary patients, and 98% (C.I. 94-99%) and 82% (C.I. 76-87%) for sporadic BC patients respectively. However, the OS at 10 and 15 years was lower in the hereditary group, being 59% and 42% respectively, versus 73% and 60% respectively for the sporadic group (p=0.08).

*Subgroup analysis for various endpoints*

In Table 3, multivariate hazard ratios (HR) for ipsilateral recurrence and other endpoints are presented separately for BRCA1 carriers and unspecified HBC patients versus sporadic cases. After correction for age at onset and tumor size, no increased risk of ipsilateral recurrence was found for mutation carriers, whereas a significantly increased risk for unspecified HBC patients was found (HR 2.31; p=0.02).

OS did not differ between unspecified HBC patients and sporadic patients; for BRCA1-mutation carriers a non-significantly worse OS was found (HR 1.76; p=0.22). The CBC risk was significantly increased for BRCA1 carriers (HR 5.17; p=0.01) and non-significantly for unspecified HBC versus sporadic cases (HR 2.01; p=0.24).

Table 3: Hazard ratios (HR) adjusted for age at diagnosis and tumor size with 95% confidence intervals (CI) for BRCA1-associated and unspecified hereditary breast cancer patients as compared to sporadic patients

| | BRCA1-associated BC | | Unspecified HBC | |
|---|---|---|---|---|
| **Event** | HR (95% CI) | p-value | HR (95% CI) | p-value |
| Ipsilateral recurrence | 0.69 (0.16-2.95) | 0.61 | 2.31 (1.18-4.52) | 0.02 |
| Death of all causes | 1.76 (0.72-4.30) | 0.22 | 0.88 (0.42-1.87) | 0.74 |
| Contralateral breast cancer | 5.17 (1.48-18.1) | 0.01 | 2.01 (0.63-6.44) | 0.24 |

## Discussion

This study was prompted by the question of both clinicians and patients whether breast conserving therapy is appropriate for hereditary breast cancer. On the one hand a potential higher risk of ipsilateral recurrence might be expected in hereditary breast cancer because:

1) the residual ipsilateral breast tissue (as well as the contralateral breast) is genetically predisposed for developing breast cancer.
2) both BRCA1 and BRCA2 are involved in DNA-damage repair while radiation therapy causes double strand DNA-breaks, therefore possibly inducing secondary malignancies more readily in BRCA1/2-mutation carriers.

The latter could apply for the treated breast as well as the contralateral breast due to scattered radiation.[30] On the other hand tumor characteristics of hereditary patients, like the less frequent presence of an extensive intraductal component and more frequent pushing borders in BRCA1-associated breast cancer,[31] may lead to surgical excision with free margins and following decreased risk of ipsilateral recurrence. Finally the possibility has been raised that instead of inducing new primary tumors, radiation therapy could prevent the development of these tumors by "sterilizing" the remaining breast tissue.[25]

In our study a higher frequency of ipsilateral recurrence after breast conserving therapy was observed in the hereditary group as compared to the sporadic group, becoming evident from 5 years after the primary diagnosis. Although non-significant, when subdivided into ipsilateral recurrences at or near the site of the original tumor and recurrences elsewhere in the breast, the latter appeared to develop after a longer follow up period (10 years and more). Furthermore, the higher incidence of CBC was confirmed, especially in BRCA1/2 mutation carriers. No significant reduction of breast cancer in the irradiated breast was seen as compared to contralateral breast cancer (data not shown). Post-local-relapse, and 2- and 5-year overall survival were not significantly different, but there was a trend for a worse 10- and 15-year overall survival in the hereditary group. Cautious interpretation of these results is necessary in view of the relative short median follow up (6.1 and 6.0 years, respectively, for hereditary and sporadic patients).

In the light of a higher risk of ipsilateral recurrence, the trend for a worse survival in the hereditary group is not surprising because, as mentioned before, this is an important predictor of a worse distant disease-free survival. However the long time interval to ipsilateral recurrence as observed in the hereditary group is an indicator of a relative favorable outcome.[7] A recent study by Huang and colleagues attempted to classify ipsilateral recurrences as either true recurrence or new primary tumor by comparing the site of the recurrence and histology.[32] They showed an improved 10 year overall survival rate for the group of patients with an ipsilateral recurrence that was presumed to be a new primary. Interestingly in this study the mean time for patients with a new primary was 7.3 years vs 5.2 years for patients with a true recurrence and patients with a new primary were more likely to develop CBC.

The relationship between ipsilateral recurrence and genetic susceptibility has been studied by several groups yielding variable results. The methodology used in these studies is diverse and therefore also in the way by which comparison groups were selected. This may be one of the reasons for the varying results. Many studies failed to find an association between a positive family history for breast cancer and an increased risk of ipsilateral recurrence.[16-19,21,27] The most important limitation of these studies is the lack of an exact definition of a positive family history indicative for inherited breast cancer.

The above mentioned failure to detect a correlation between family history and ipsilateral recurrence underscores the importance of studying patients with a well defined family history. Because the medical registration at our institute is set up in such a way that cases with a positive family history can be distinguished we truly selected sporadic breast cancer patients. Our definition of HB(O)C families is in agreement with what is generally accepted as being indicative for a predominant genetic susceptibility. Though the number of patients we studied was large enough to make separate analyses for BRCA1/2-associated cases and unspecified hereditary breast cancer, we are aware that the latter group is heterogeneous with respect to the underlying susceptibility genes.

Regarding proven BRCA1/2-mutation carriers, data are more scarce (table 4). In a first analysis of Ashkenazi Jewish breast cancer patients (diagnosed before the age of 42 years; breast conserving therapy in 9 BRCA1/2 mutation carriers versus 25 sporadic cases, with short follow up) Robson and colleagues did not find an increased rate of ipsilateral recurrence.[20] By extending the group of patients and prolonging the follow-up period, however, they found an increased rate of ipsilateral recurrence and contralateral breast cancer at 5 and 10 years in the BRCA1/2 mutation group,[22] which is in accordance with our findings.

Table 4: Ipsilateral breast tumor recurrence rate following breast conserving therapy in hereditary breast cancer patients

| | | No of patients | | 5 yr-IBTR* (%) | | 10yr-IBTR* (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| First author | Year | unspecified hbc† | BRCA1/2 | HBC‡ | Controls | HBC‡ | Controls | P | Median Follow-up |
| Robson[22] | 1999 | - | 28 | 14.9 | 4.5 | 22.0 | 6.9 | 0.25 | 10.3 yrs |
| Pierce[25] | 2000 | - | 71 | 2 | 4 | - | - | 0.8 | 7.5 yrs |
| Eccles[24] | 2001 | 36 | 36 | - | - | 18 | 21 | 0.4 | 7.0 yrs |
| Haffty[26] | 2002 | - | 22 | 22 | 18 | 41 | 19 | 0.007 | 12.7 yrs |
| Present study | | 61 | 26 | 14 | 7 | 30 | 16 | 0.05 | 6.1 yrs |

*IBTR: ipsilateral breast tumor recurrence rate

† Unspecified HBC: patients with a significant family history of breast (and/or ovarian) cancer; as yet no BRCA1/2 mutations identified

‡ Total group of BRCA1/2 mutation carriers and unspecified HBC patients

Haffty and colleagues report in a recently published case-control study on the outcome after breast conserving therapy in 22 BRCA1/2 mutation carriers and 105 sporadic cases with a diagnosis of breast cancer at the age 42 years or younger.[26] The median follow up in this study is 12.7 years. The rate of ipsilateral recurrence at 5 and 10 years was found to be increased in the genetic cohort as compared to the sporadic cohort, which became evident after 5 years of follow-up. The distinctly different location and histological features of 9 out of 11 ipsilateral recurrences were suggestive of new primary breast tumors. Likewise, the rate of contralateral breast cancer was increased in the genetic cohort. These data are well in accordance with our findings.

The data of Pierce and colleagues reporting on the results of breast conserving therapy in 71 BRCA1/2 mutation carriers versus 213 sporadic patients are in a way different, but not contradictory.[25] With a median follow-up of 5.3 and 4.6 years, the 5-year local control rates were 98% and 96% for the hereditary and sporadic groups respectively, while the actuarial survival at 5 yr was 86% and 91% respectively. We believe that data on ipsilateral recurrence after more than 5 years are of special relevance in this matter. Interestingly, in their study, the median time to local recurrence of the 3 patients in the genetic cohort was 8.2 years compared to 3.1 years in the sporadic cohort. No difference was seen in site of the recurrence within the breast between the two groups.

Following a different approach, but again well in line with the present findings, Turner and colleagues tested 52 breast cancer patients with ipsilateral recurrence following breast conserving therapy.[33] Eight BRCA1/2 mutations were detected (15%), and 6 of these were encountered in the 15 patients under the age of 40 (40%). In the 15 matched control patients under the age of 40 without an ipsilateral recurrence only 1 mutation was found (6.6%). Moreover median time to ipsilateral recurrence was 7.8 years compared to 4.7 years for the patients without BRCA1/2 mutations. By clinical and histologic criteria they concluded that the ipsilateral recurrences in BRCA1/2 mutation carriers were second primary tumors that had developed in the conservatively treated breast.

The group of Eccles did not find an increased rate of ipsilateral recurrence in hereditary cases as compared to controls, although their population is quite comparable to ours.[24] Strikingly, their results show an ipsilateral recurrence frequency of 24% in the control cases, much higher than the rate in controls in the

studies of Robson, Haffty and ours and that reported in the literature, being approximately 15% or less. It might be possible that in their study (no genetic testing for BRCA2 was performed and patients with a borderline family history and a calculated heterozygote risk of less than 20% were classified as having a negative family history), some hereditary cases were assigned to the control group, confounding their results.

The results presented here and those from other studies prove once again that the residual breast tissue in BRCA1/2 mutation carriers remains at risk for developing new primary tumors, i.e. both the ipsilateral and contralateral breast. This is essential information to discuss with identified BRCA1/2 mutation carriers at the moment of diagnosis of a primary breast tumor. These women (generally participating in screening programs) together with their attending physicians have to choose between undergoing uni- or bilateral mastectomy (i.e. contralateral prophylactic mastectomy) or the "standard" breast conserving therapy. Since they have already opted for surveillance instead of bilateral prophylactic mastectomy this may mean that these women are more motivated to choose breast conserving therapy instead of mastectomy.

Many more women will have been diagnosed with breast cancer and treated with breast conserving therapy in the past and later on became identified as carrier of a BRCA1/2 mutation. The fact that an ipsilateral recurrence more often manifests itself after longer follow-up, indicates that decisions concerning additional "risk reducing" surgery in a breast cancer patient with a BRCA1/2 mutation should not be taken hastily. Careful monitoring of both the remaining breasts is indicated which may provide time for both physicians and patients to make a well considered decision while taking into account the stage and prognostic factors of the primary breast cancer.

The data from this study must also be seen in the light of other preventive measures for which these women may opt. Prophylactic oophorectomy has been shown to reduce the risk of breast cancer by about 50% in carriers of BRCA1/2 mutations.[34,35] Presumably this will also apply for contralateral breast cancer and the ipsilateral recurrences which are new primary tumors. In the presently studied group only a few women received adjuvant tamoxifen. Knowledge about the effects of tamoxifen on ipsilateral recurrence and contralateral breast cancer are growing.[36] However especially with regard to hereditary breast cancer these effects may appear

to be different. Significant with regard to this aspect, tamoxifen only decreases the risk of estrogen receptor-positive contralateral breast tumors,[37] while the majority of contralateral breast tumors in BRCA1-carriers is expected to be estrogen receptor negative.

**In conclusion**: in this study, we found an increased risk of ipsilateral breast tumor recurrence after breast conserving therapy in hereditary breast cancer. These ipsilateral recurrences seemed to consist of early recurrences in the vicinity of the primary tumor in the period of 2 to 5 years, and late new primary tumors, more than 5 years after initial therapy. Furthermore, the increased risk of developing contralateral breast cancer, both in BRCA1/2-associated and in unspecified hereditary breast cancer was confirmed. These data may facilitate clinical decisions in a BRCA1/2 mutation carrier, specifically when additional "risk reducing" surgery is considered. Finally the conducted follow-up strategy in these women should make allowance for the fact that ipsilateral recurrence in their case frequently is a late event. A careful monitoring schedule, also beyond 5 years after diagnosis and treatment, remains indicated in hereditary breast cancer.

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# Chapter 9

## Summary, general discussion and concluding remarks

## Summary, general discussion and concluding remarks

This study began shortly after the identification of BRCA1 in 1994 (Miki et al, 1994). Since then an abundance of data on BRCA1, the subsequently identified BRCA2 and hereditary breast cancer emerged. Without being comprehensive, the introduction (chapter 1) summarizes the background of the results described in this thesis.

The families and patients investigated for BRCA1/2-germline mutations, were mostly identified through the Rotterdam Family Cancer Clinic. As in other populations, strong founder effects for BRCA1- and BRCA2-mutations are present in the Dutch population. General predictive factors enabling the detection of a mutation in families with clustering of breast and/or ovarian cancer are introduced in chapter 2. Some founder mutations originate from specific geographic regions in the Netherlands in the vicinity of the Rotterdam Family Cancer Clinic. Similar observations were obtained for different mutations in other Clinical Genetic Services.

Young or recent mutations are generally assumed as explaining regional differences (chapter 2). The more ancient founder mutations, as they were shown to be by haplotype-sharing, occur in various populations including the Dutch. The BRCA1-mutation 5282insC is an example. It originates from eastern-europe over 900 years ago and spread all over Europe (Szabo and King, 1997). Within the context of this study, a founding ancestor of an observed regional clustering might be a single germline mutation carrier originating from outside that region. Accordingly that mutation might be endemic in other populations. Finally, two identical mutations might also have occurred independently and haplotype analysis can show their separate origins.

In parallel to the Dutch situation "regional founders" have been encountered elsewhere (Sarantaus et al, 2000; Fricker et al, 2000), but this phenomenon can only be established when a considerable number of families from such a region is genetically tested. The Netherlands offer an opportunity for such tests, as it has a nation-covering network of family cancer clinics and clinical genetic centers and also an (increasing) awareness of the genetic aspects of breast cancer among clinicians and patients. This resulted in a relative high number of families that were referred for BRCA1/2-mutation analysis. If this is not the case - as is too be expected in many other countries - geographical clustering or founder mutations will remain undetected.

In chapter 3 the uptake of presymptomatic genetic testing and prophylactic surgery were assessed. In sharp contrast with other genetic diseases such as Huntington disease (where treatment is impossible and only informed decisions to handle ones' life and offspring are possible) (Tibben et al, 1992), almost half of the women at risk for HBOC requested presymptomatic testing. Of the unaffected women, identified as mutation carriers, the majority (51%) opted for prophylactic mastectomy and/or oophorectomy. All these women were offered genetic testing as an option of routine clinical care.

The clinical characteristics of BRCA1-related breast cancer are analyzed in chapter 4. Disease-free and overall survival of 49 breast cancer patients from 19 consecutive families with a BRCA1-mutation were compared with 196 patients with sporadic breast cancer, matched for age and year of diagnosis. Similar recurrence and death rates were found. At that time the only other study showed a better survival for BRCA1-related breast cancer patients, when compared to similarly matched controls from a population cancer registry (Porter et al, 1993). These BRCA1-associated breast cancer patients came from families identified by linkage analysis. Linkage analysis is more readily conclusive in families with a greater number of living affected persons (from whom DNA is most easily obtained) and thus leads to unintentional selection for longevity. Such bias was largely obviated in the present study, as mutation-analysis (in case of deceased affected patients) was offered to multiple non-affected relatives having 50% genetic risk (chapter 5). Moreover, next-of-kin of deceased patients often acted as probands (i.e. the initial person or "index case" by whom a family is identified). Families identified in this way seem less prone for selection for longevity. However when probands were affected by breast cancer, they appeared to be characterized by a better survival as was shown by an analysis that excluded these patients (chapter 4).

The completeness and reliability of family history data on diagnoses and pathology reports of living and deceased cancer patients is crucial to the studies reported here. In the Netherlands, recently the legal storage period of medical records and pathology specimens became 10 years. This means that similar studies will be greatly hampered at present, since they often rely on data and specimens stored for decades after the patient's death.

The survival and tumor characteristics of the first 28 BRCA2-associated breast cancer patients are described in chapter 5. Again, their recurrence- and death rates were similar as in a control group of 112 sporadic breast cancer patients matched for age and year of diagnosis. Chapter 6 discusses the prognostic value of BRCA2 mutations (as known by 2000). There is a wide range of prognostic implications observed, either based upon family history for breast cancer and/or carriership of a BRCA1/2 germline mutation. This divergence is discussed.

The tumors of BRCA1 (but not BRCA2) mutation carriers showed more often a steroid-hormone receptor negative status. The few available data at that time were confirmed. This observation also explains the predicted and later observed insensitivity for chemoprevention (King et al, 2001). On the other hand it leaves unexplained, why oophorectomy significantly reduces breast cancer risk in BRCA1-mutation carriers (Kauff et al, 2002). The role of steroid hormones is also illustrated by the decreasing relative risk for breast cancer of postmenopausal BRCA1-mutation carriers (Ford et al, 1994; Antoniou et al, 2003). In chapter 7 it is shown that this reduction is also reflected in a reduced risk for contralateral breast cancer in a BRCA1 carrier with a first diagnosis of breast cancer after the age of 50 years.

Finally, in chapter 8, ipsilateral breast cancer recurrence in BRCA1/2-associated breast cancer patients as well as patients with a family history indicative of hereditary breast cancer were compared to a control group of sporadic breast cancer, all treated with breast conserving therapy and again matched for age and year of diagnosis. In that preliminary study, intriguing, though in part, non-significant differences were observed. The different therapeutic considerations on the primary treatment of hereditary breast cancer are discussed. The value observed in the last decades of breast conserving therapy for sporadic breast cancer cannot be directly applied to carriers of a deleterious BRCA1/2-germline mutation: both their breasts are one single, paired organ in which every cell has a cancer-prone mutation.

Population based studies will be needed (and are underway) to clarify many questions in this thesis: such as the prognosis, the proportion of breast cancer attributable to BRCA1/2 and mutation specific risks. However analyzing BRCA1/2-mutation carriers via breast cancer families is also of great value. The collected families enabled prospective studies on the value of breast and ovarian surveillance and of prophylactic surgery. Also, the first prospective risk estimates became available for BRCA1/2 mutation carriers (Meijers-Heijboer, 2001).

In conclusion:

- The detection rate of mutations in BRCA1 and BRCA2 is highest in families with one ore more ovarian cancer cases or two or more patients with breast cancer diagnosed before the age of 40. Perhaps reassuring, in true sporadic breast cancer patients diagnosed before the age of 40 years (that is when an informative pedigree shows no other affected family members) no mutations were found.
- At a regional level differences exist in the frequency of specific mutations encountered. Since it is unlikely that this only applies to the Dutch situation, and because techniques that detect these mutations vary, cautious interpretation of data regarding the contribution of BRCA1/2 to (hereditary) breast cancer is necessary.
- In unaffected women who are at high risk of being diagnosed with breast cancer because of their family history there is a high demand for presymptomatic genetic testing for BRCA1/2 mutations and subsequent prophylactic surgery.
- Perhaps disappointing, but as is breast cancer in general, both BRCA1-and BRCA2-associated breast cancer are heterogeneous diseases with respect to the outcome. Regarding the overall prognosis of these patients, it is unlikely that large differences exist compared to sporadic breast cancer. Identifying the strength of other genetic and additional environmental factors in determining cancer-risk and outcome in an individual patient remains a major issue. The final outcome in these patients however will not only depend on the characteristics of one tumor but also on the development of other primary malignancies as a result of inherited susceptibility.
- A highly increased contralateral breast cancer risk is a feature of both BRCA1- and BRCA2-associated patients. The relative decrease in contralateral breast cancer risk for BRCA1-associated patients first diagnosed after the age of 50, together with data from other studies, suggest a strong role for steroid hormones in the development of BRCA1-associated breast cancers despite their more frequent negative steroid receptor status.
- A positive family history for breast cancer is a poor surrogate for full pedigree-data and BRCA1/2-mutation carrier status in studying the outcome of conservatively treated breast cancer.

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## Samenvatting

*Borstkanker en erfelijkheid*

Borstkanker is in Nederland de meest voorkomende maligniteit bij vrouwen. Naar schatting 1 op de 10 à 12 vrouwen wordt ergens in haar leven geconfronteerd met deze ziekte. Over het ontstaan van de ziekte is veel onbekend maar er zijn een aantal risicofactoren vastgesteld. Veel van deze risicofactoren lijken verband te houden met het feit dat de borstklier een hormoongevoelig orgaan is met als biologische taak het produceren van melk in aansluiting op de zwangerschap. Zo zijn bekende risicofactoren voor borstkanker (naast leeftijd en geslacht) een vroege leeftijd waarop de eerste menstruatie optreedt, een late leeftijd waarop een vrouw in de menopauze komt of een late leeftijd waarop zij haar eerste kind krijgt. Al deze risicofactoren echter (met uitzondering van leeftijd en geslacht), zorgen naar verhouding slechts voor een geringe toename van het risico op borstkanker.

Al heel lang is bekend dat borstkanker familiair kan voorkomen en dat een vrouw met een eerste graad bloedverwant met borstkanker een verhoogd risico heeft om de ziekte te krijgen. Afhankelijk van het aantal verwanten met borstkanker en de leeftijd waarop de ziekte zich openbaarde, kan het risico voor een zuster of dochter van een patiënt toenemen tot dicht bij de 50%. In dergelijke families volgt borstkanker een erfelijk patroon dat autosomaal dominant heet. In dat geval geeft een erfelijke factor (een gen) een sterk verhoogd risico op borstkanker (en in sommige families ook eierstokkanker). Iemand die een dergelijke erfelijke factor bij zich draagt heeft een kans van 50% om deze door te geven aan een kind. Dit geldt zowel voor mannelijke als voor vrouwelijke nakomelingen. Voor mannen heeft het dragerschap minder consequenties omdat zij veel minder vaak borstkanker krijgen dan vrouwen, maar mannen kunnen het gen wel doorgeven aan hun kinderen.

*De borstkankergenen BRCA1 en BRCA2*

In 1994 werd de structuur van een eerste 'borstkankergen' vastgesteld en minder dan twee jaar later de structuur van een tweede gen. Deze genen werden BR(east)CA(ncer)1 en BRCA2 genoemd. Borstkanker, zoals in feite alle kanker, is een genetische ziekte waarbij een aantal genen defect zijn geraakt of er een functie bij hebben gekregen. Dit zorgt ervoor dat een cel zich ongecontroleerd kan gaan

delen. De veranderingen in de betrokken genen worden mutaties genoemd, en treden stapsgewijs in een cel op. Dit is onderhevig aan het toeval en daarom kan het vele tientallen jaren duren eer een tumor zich heeft ontwikkeld. Daarom ook is kanker, in het algemeen gesproken, een ouderdomsziekte. In het geval van erfelijke borstkanker is een defect in één van deze genen reeds vanaf de geboorte aanwezig.

Chromosomen zijn de dragers van ons erfelijk materiaal, het DNA, dat codeert voor ongeveer 35.000 genen. Het BRCA1 gen ligt op chromosoom 17 en bestaat uit 22 exonen. Exonen zijn de gedeelten van een gen die coderen voor aminozuren, de bouwstenen van een eiwit. DNA is een zeer lang molecuul dat is opgebouwd uit basenparen waarvan er vier zijn; A, T, C en G. Drie opeenvolgende basenparen coderen voor een aminozuur. Een verandering in de volgorde, het aantal of het type basenparen is een mutatie. In totaal bestaat het BRCA1-eiwit uit ruim 1800 aminozuren en beslaat het hele gen ongeveer 100.000 basenparen. Ook BRCA2 is een dergelijk groot gen dat ligt op chromosoom 13.

*Borstkanker in de familie*

Mutaties kunnen zich in principe overal in het BRCA1- of BRCA2-gen voordoen en daarom is het zoeken naar mutaties in deze genen een tijdrovende en arbeidsintensieve bezigheid. Het blijkt echter dat sommige mutaties in meerdere, ogenschijnlijk niet-verwante, families voorkomen. De oorzaak hiervan is dat deze families een gemeenschappelijke voorouder (een 'founder') delen waarin deze mutatie is ontstaan. Het ontstaan van een mutatie kan vele eeuwen geleden hebben plaatsgevonden, maar aangezien verschillende populaties relatief weinig met elkaar vermengen, blijven dergelijke 'founder-mutaties' min of meer uniek voor een bepaalde bevolking. Zo wordt een aantal mutaties gevonden die relatief vaak voorkomen in de Nederlandse bevolking. Maar ook binnen de Nederlandse bevolking worden regionale verschillen gezien. Een specifieke BRCA1-mutatie (IVS12-1643del3855) en een specifieke BRCA2-mutatie (5579insA) bleken beiden te herleiden tot verschillende regio's, namelijk West-Brabant en Zuid-Beveland. Ook in een steekproef van borsttumoren van geanonimiseerde patienten uit deze regio's werden deze mutaties (relatief frequent) gevonden (***Hoofdstuk 2***).

Bij naar schatting 5-10% van alle gevallen van borstkanker speelt erfelijkheid een doorslaggevende rol, maar het aantal BRCA1- of BRCA2-gevallen draagt hieraan slechts ongeveer een vijfde bij. In families met alleen 3 tot 4 gevallen van

borstkanker en geen patiënten met eierstokkanker is de kans op een BRCA1- of BRCA2-mutatie niet groter dan 20%. Dit percentage neemt toe indien er ook eierstokkanker in de familie voorkomt of borstkanker op jonge leeftijd optreedt (onder de 40) (***Hoofdstuk 2***). Een deel van het familiair voorkomen van borstkanker kan daarnaast door toeval worden verklaard maar andere, minder penetrante genen (dwz genen die indien gemuteerd, een minder hoog risico op borstkanker geven) spelen ook een rol.

*Risico's en keuzes*

Bij het deel van de families waarin daadwerkelijk een BRCA1- of BRCA2-mutatie wordt gevonden staan de familieleden voor een aantal moeilijke beslissingen. Zij kunnen besluiten zich wel of niet te laten testen om te bepalen of zij drager zijn van de gevonden mutatie. Voor draagsters van BRCA1-mutaties begint het risico op borstkanker toe te nemen vanaf de leeftijd van ongeveer 20 tot 25 jaar en wordt het absolute risico op borstkanker geschat op 65%. Daarnaast hebben deze vrouwen ook een risico op eierstokkanker van ongeveer 40%. Voor BRCA2 gelden min of meer dezelfde getallen waarbij het risico op borstkanker mogelijk pas op latere leeftijd toeneemt en het risico op eierstokkanker lager is. De reden om zich te laten testen kan zijn dat een gezond iemand haar risico op borstkanker wil weten of juist wil weten of de mogelijkheid bestaat dat een mutatie kan zijn doorgegeven aan haar kinderen. Dit laatste is ook de reden waarom mannen een dergelijke test willen ondergaan. De vraag naar presymptomatische DNA diagnostiek (dat wil zeggen door gezonde personen) wordt beschreven in ***Hoofdstuk 3***. Van 271 mannen met een kans op dragerschap van 50% lieten 59 (22%) zich testen terwijl 158 van de 275 (57%) gezonde vrouwen hiervoor kozen.

Wanneer blijkt dat een gezonde vrouw uit een familie waarin een BRCA1- of een BRCA2-mutatie is gevonden draagster is van die mutatie, kan zij de keuze maken uit een aantal opties: geen verdere actie ondernemen, zich frequent laten controleren of zich preventief laten opereren aan borsten en/of eierstokken. Daarnaast zijn er in onderzoeksverband ook medicamenteuze mogelijkheden om het risico op borstkanker te verkleinen (zogenaamde chemo-preventie).

Aan alle keuzemogelijkheden kleven bezwaren. Zo heeft het zich regelmatig laten controleren tot doel om een borstkanker in een zo vroeg mogelijk stadium te ontdekken om zo de genezingskans te vergroten. Maar ook bij zeer kleine tumoren

bestaat een kans dat ze reeds zijn uitgezaaid. Chemo-preventie zal waarschijnlijk slechts een afname van het risico op borst- en eierstokkanker kunnen bewerkstelligen dat wil zeggen niet in alle gevallen kanker kunnen voorkómen. Geconfronteerd met de risico's blijken vooral jonge vrouwen met kinderen relatief vaak voor preventieve chirurgie te kiezen. In totaal kiest ongeveer de helft van alle gezonde vrouwen die ervoor gekozen hebben zich te laten testen en die draagster zijn, voor preventieve amputatie van de borsten (***Hoofdstuk 3***). Preventieve chirurgie en vooral het preventief amputeren van de borsten is voor een deel van de draagsters van een BRCA1/2-mutatie echter onacceptabel.

Er is geen absolute zekerheid dat iemand die zich preventief laat opereren geen kanker krijgt. Zo bestaat er een vorm van buikvlieskanker die kan optreden na het preventief verwijderen van de eierstokken. Deze vorm van kanker lijkt sterk op, en is verwant aan, eierstokkanker. Het risico op deze vorm van kanker na het preventief verwijderen van de eierstokken wordt geschat op enkele procenten. Waarschijnlijk ligt het risico op het krijgen van borstkanker na het chirurgisch preventief verwijderen van (zoveel mogelijk) borstklierweefsel nog lager. Wel is er bij BRCA1/2-mutatiedragers een licht verhoogde incidentie van sommige andere vormen van kanker vastgesteld. Daarnaast kunnen dragers van BRCA1/2 mutaties ook allerlei andere vormen van kanker krijgen zoals die ook in de algemene bevolking optreden en die in principe niet zijn gerelateerd aan het bij zich dragen van de mutatie.

*Prognose en kenmerken*

Erfelijke borstkanker openbaart zich vaak al op jonge leeftijd (dat wil zeggen voor het veertigste levensjaar) en ontstaat vaak ook in de ander borst (contralateraal borstkanker genoemd). Verder blijkt dat deze tumoren veel kenmerken hebben die duiden op een slechte prognose. Het microscopisch beeld van BRCA1- (en in mindere mate BRCA2-) tumoren laat vaak snel delende tumoren zien waarvan de cellen er sterk kwaadaardig ('slecht gedifferentieerd') uitzien. Van dergelijke tumoren is bekend dat zij vaker en eerder uitzaaiingen geven dan tumoren met een minder kwaadaardig ('goed gedifferentieerd') aspect. Daarnaast hebben BRCA1-tumoren ook kenmerken die zouden kunnen wijzen op een minder kwaadaardig gedrag. Zo hebben ze vaker een groeipatroon dat naar verhouding een betere prognose heeft als borstkanker van het meer algemene type.

Omdat borstkanker die ontstaat bij draagsters van mutaties in BRCA1 of BRCA2 verschillen toont met niet-erfelijke (zogenaamde sporadische) borstkankers is een belangrijke vraag of het ziektebeloop in deze patiënten ook verschilt. Onderzoek in de eerste families met een BRCA1- of BRCA2-mutatie gediagnosticeerd binnen de Werkgroep Erfelijke Tumoren Rotterdam, liet zien dat de overleving van borstkankerpatiënten uit deze families overeenkwam met die van sporadische patiënten van dezelfde leeftijd en periode van diagnose (***Hoofdstuk 4 en 5***). Een belangrijk verschil tussen de BRCA1-tumoren en de controles is dat de BRCA1-geassocieerde borstkankers minder vaak receptoren bleken te hebben voor oestrogenen en progesteron. Bij aanwezigheid van deze hormoonreceptoren in de tumor is er een grotere kans op gevoeligheid voor het medicijn tamoxifen. Tamoxifen is een anti-oestrogeen dat wordt gebruikt om de uitgroei van eventuele uitzaaiingen te voorkomen, dan wel te remmen. Tamoxifen wordt (in onderzoeksverband) ook gebruikt bij de preventie van borstkanker. Daarentegen zijn BRCA2-tumoren niet vaker negatief voor hormoonreceptoren (***Hoofdstuk 5 en 6***).

Een ander belangrijk verschil is de sterk verhoogde kans die BRCA1/2-borstkankerpatiënten hebben om de ziekte ook in de ander borst te krijgen. Vijf jaar na diagnose was het risico op contralateraal borstkanker in de onderzochte families 19% voor BRCA1- en 12% voor BRCA2-geassocieerde patiënten. Het bleek dat dit risico voor BRCA1-patiënten duidelijk lager was wanneer bij hen borstkanker na het 50$^{e}$ jaar was vastgesteld. Dit was bij ongeveer een kwart van de BRCA1-patiënten het geval (***Hoofdstuk 7***).

De borstsparende behandeling van borstkanker, die de eerste keuze van behandeling is bij kleinere tumoren, heeft als risico dat achtergebleven tumorcellen in de behandelde borst weer kunnen uitgroeien tot tumoren. Daarnaast blijft de mogelijkheid bestaan dat er in het resterende borstklierweefsel nieuwe tumoren ontstaan. Omdat al het borstklierweefsel bij BRCA1/2-mutatiedraagsters een verhoogde kans op het ontstaan van borstkanker heeft, was de verwachting dat bij deze vrouwen na borstbesparende behandeling de ziekte vaker in de behandelde borst zou terug keren. Om dit te onderzoeken zijn vrouwen met een sterk belaste familiegeschiedenis en BRCA1/2-geassocieerde patiënten die borstsparend waren behandeld vergeleken met sporadische borstkankerpatiënten (***Hoofdstuk 8***). In de totale groep van erfelijke patiënten bleek inderdaad dat ook na meer dan 5 jaar nog regelmatig nieuwe tumoren in behandelde borst werden ontdekt. Dit interval en het

feit dat een deel van de tumoren op een andere plaats in de borst werden gevonden dan de oorspronkelijke tumor doen vermoeden dat het hier om nieuwe, dus eigenlijke tweede tumoren gaat.

*Tenslotte*

Met de ontdekking van BRCA1 en BRCA2 zijn er borstkanker patiënten te onderscheiden met unieke kenmerken en problemen zoals de kans op contralateraal borstkanker. Daarnaast wordt een snel groeiend aantal gezonde vrouwen geïdentificeerd die draagster zijn van mutaties in BRCA1 of BRCA2. De preventie, diagnose en behandeling van borst- en eierstokkanker bij deze vrouwen vragen een geheel eigen benadering. Uit de studies beschreven in dit proefschrift kunnen de volgende conclusies worden getrokken:

- De kans op het vinden van een BRCA1- of BRCA2-mutatie is het grootst in families met één of meer gevallen van ovariumkanker of twee of meer gevallen van borstkanker onder de 40. Patiënten bij wie borstkanker voor het 40$^{e}$ levensjaar wordt ontdekt maar bij wie de ziekte sporadisch lijkt (dat wil zeggen wanneer er een informatieve stamboom is zonder andere aangedane familieleden) hebben slechts een kleine kans om een BRCA1/2-mutatie bij zich te dragen.
- Er bestaan binnen Nederland regionale verschillen in het voorkomen van specifieke BRCA1/2-mutaties. Dit wil overigens niet zeggen dat er in die regio's frequenter BRCA1/2-mutaties voorkomen!
- Ongeveer de helft van de gezonde vrouwen met een hoog risico op dragerschap uit families met een bekende BRCA1/2-mutatie wil DNA-onderzoek en bij gebleken dragerschap kiest ongeveer de helft van de vrouwen voor preventieve amputatie van de borsten.
- Borstkanker in BRCA1/2-mutatiedraagsters is niet geassocieerd met een (beduidend) slechtere prognose, maar wel met een hoog risico op contralateraal borstkanker en op de lange termijn mogelijk ook met een verhoogd risico op borstkanker in de sparend behandelde borst.

# Curriculum Vitae

Leendert Cornelis Verhoog werd op 30 augustus 1968 geboren in Spijkenisse. In 1986 behaalde hij het VWO diploma aan de Christelijke Scholengemeenschap Blaise Pascal te Spijkenisse. In hetzelfde jaar begon hij met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Na een afstudeeronderzoek naar de teratogene effecten van vitamine A-zuur op de afdeling Celbiologie en Genetica behaalde hij in 1992 zijn doctoraal examen Geneeskunde in 1994, gevolgd door zijn artsexamen. Na een korte periode werkzaam te zijn geweest als Arts Sociaal Medische Adviezen voor de Gemeentelijke Gezondheids Dienst Zuid-Holland Zuid begon hij in 1995 als arts-onderzoeker bij de afdeling Interne Oncologie van de Daniel den Hoed Kliniek. In die functie was hij lid van de Rotterdamse Werkgroep Erfelijke Tumoren en werd onder leiding van de voorzitter van deze werkgroep, Prof. Dr. J.G.M. Klijn, het werk verricht dat de basis vormde voor dit proefschrift. Hiervoor ontving hij in 2000 de Henny C. Dirven prijs en een American Society of Clinical Oncology Merit Award. Sinds 2000 is hij in opleiding tot patholoog bij de afdeling Pathologie in het Erasmus MC, locatie Josephine Nefkens Instituut, hoofd Prof. Dr. J.W. Oosterhuis, opleider Prof. Dr. Th.H. van der Kwast.

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