

# **BREAST CANCER**

## **Predisposing genes and their clinical implications**

**Hanne Meijers-Heijboer**

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**BREAST CANCER**  
**Predisposing genes and their clinical implications**

**BORSTKANKER**  
Predispositie genen en hun klinische implicaties

**PROEFSCHRIFT**

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*Op het midden van onze levensweg bevond ik me in een donker woud,  
omdat ik van de rechte weg was afgedwaald.  
Ach, hoe moeilijk is het onder woorden te brengen  
hoe woest en ruw en onbegaanbaar dat woud was!*

*Wanneer ik eraan denk, slaat mij de schrik weer om het hart  
(...). Maar om over het goede te hebben dat ik eraan trof,  
zal ik vertellen van de dingen die ik er ook gezien heb.*

Dante Alighieri

*La Divina Commedia/Inferno/ Canto I*

ned. Vertaling F. van Dooren

Voor Carel  
en  
Gratia en Eva

In herinnering aan mijn vader

# CONTENTS

<b>Scope of the thesis</b>	<b>9</b>
<b>CHAPTER 1</b>	
<b>Introduction</b>	
<b>1.1 Breast cancer</b>	<b>11</b>
<i>Epidemiology and risk factors</i>	<i>11</i>
<i>Pathology</i>	<i>13</i>
<i>Prognosis and treatment</i>	<i>14</i>
<b>1.2 Genetic susceptibility to breast cancer</b>	<b>15</b>
<i>Predisposing genes</i>	<i>15</i>
<i>Polygenic model of breast cancer susceptibility</i>	<i>18</i>
<i>Predisposing genes -- search for the unknown ones</i>	<i>19</i>
<b>1.3 BRCA1 and BRCA2 genes</b>	<b>21</b>
<i>Structure and function</i>	<i>21</i>
<i>Molecular diagnosis and mutation spectrum</i>	<i>22</i>
<i>Prevalence of the mutations</i>	<i>24</i>
<i>Cancer risks and genotype-phenotype correlations</i>	<i>25</i>
<i>Tumor and clinical characteristics of BRCA1 and BRCA2 associated breast cancer</i>	<i>29</i>
<b>1.4 Clinical aspects of familial breast cancer</b>	<b>30</b>
<i>Genetic counseling and testing</i>	<i>30</i>
<i>Breast cancer risk assessment</i>	<i>34</i>
<i>Probability of finding BRCA1 and BRCA2 mutations in clinical settings</i>	<i>35</i>
<i>Risk reducing interventions</i>	<i>36</i>
<i>Multidisciplinary approach</i>	<i>39</i>
<b>CHAPTER 2</b>	<b>41</b>
<b>Large regional differences in the frequency of distinct BRCA1/BRCA2 mutations in 517 Dutch breast and/or ovarian cancer families. <i>Eur J Cancer</i> 2001; 37:2082-90</b>	
<b>CHAPTER 3</b>	<b>59</b>
<b>Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation. <i>Lancet</i> 2000; 355:2015-20</b>	

<b>CHAPTER 4</b>	<b>75</b>
<b>Use of genetic testing and prophylactic mastectomy and oophorectomy in women with breast or ovarian cancer from families with a BRCA1 or BRCA2 mutation. <i>J Clin Oncol</i> 2003; 21:1675-81</b>	
<b>CHAPTER 5</b>	<b>89</b>
<b>Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. <i>N Engl J Med</i> 2001; 345:159-64</b>	
<b>CHAPTER 6</b>	<b>103</b>
<b>Are <i>ATM</i> mutations 7271T→G and IVS10-6T→G really high-risk breast cancer susceptibility alleles? <i>Submitted for publication</i></b>	
<b>CHAPTER 7</b>	<b>111</b>
<b>Low-penetrance susceptibility to breast cancer due to <i>CHEK2</i>*1100delC in noncarriers of <i>BRCA1</i> or <i>BRCA2</i> mutations. <i>Nature Genet</i> 2002; 31:55-9</b>	
<b>CHAPTER 8</b>	<b>123</b>
<b>The <i>CHEK2</i> 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. <i>Am J Hum Genet</i> 2003; 72:1308-14</b>	
<b>CHAPTER 9</b>	<b>135</b>
<b>General discussion</b>	
<b>REFERENCES</b>	<b>139</b>
<b>SUMMARY</b>	<b>159</b>
<b>SAMENVATTING</b>	<b>165</b>
<b>LIST OF TABLES AND FIGURES</b>	<b>173</b>
<b>LIST OF PUBLICATIONS</b>	<b>175</b>
<b>DANKWOORD</b>	<b>181</b>
<b>CURRICULUM VITAE</b>	<b>185</b>





## Scope of the thesis

Breast cancer is a major health problem in women in the Western world. It is not only a disease of individual patients but also of their families. This holds true for both the psychological and hereditary aspects of the disease. Although familial clustering of breast cancer was recognized in ancient times, it took until 1994 and 1995 that two major high-risk breast cancer susceptibility genes, BRCA1 and BRCA2, were identified.<sup>1,2</sup> These discoveries allowed for the first time to identify women at high risk of breast cancer and urged to find ways to handle these risks clinically.

In the various articles which form the basis of this thesis we first studied the frequency of mutations of BRCA1 and BRCA2 in clinically ascertained breast cancer families (Chapter 2). Subsequently, we investigated the demand of patients and their healthy relatives of genetic testing and we analyzed the choices of women with a mutation with respect to preventive measures, i.e. prophylactic mastectomy and oophorectomy (Chapters 3 and 4). The efficacy of prophylactic bilateral mastectomy in healthy women with a BRCA1 or BRCA2 mutation was studied prospectively (Chapter 5).

Only a small fraction of breast cancer susceptibility is explained by mutations of BRCA1 or BRCA2 or other known genetic risk factors. The second part of this thesis deals with the search for additional breast cancer susceptibility genes. Chenevix-Trench et al.<sup>3</sup> recently reported that two mutations of the Ataxia Telangiectasia Mutated gene (ATM) confer high risks of breast cancer comparable to mutations of BRCA1 and BRCA2. As described in Chapter 6 we could not confirm these results in a series of 961 breast cancer families without a mutation of BRCA1 or BRCA2. Intriguingly, in our genome-wide linkage search for *high*-risk breast cancer alleles we identified the 1100delC mutation of the CHEK2 (cell cycle checkpoint kinase 2) gene as the first *low*-risk breast cancer susceptibility allele (Chapter 7). In several CHEK2 1100delC families we observed an increased frequency of also colorectal cancer. We therefore investigated the frequency of CHEK2 1100delC in colorectal cancer families with and without breast cancer, and vice versa. We provided evidence for the existence of a breast cancer and colorectal cancer syndrome using CHEK2 1100delC as a genetic tag (Chapter 8). We further showed that CHEK2 1100delC acts in synergy with an as yet unknown risk factor or factors, exemplifying the recently proposed polygenic model of breast cancer susceptibility.



# CHAPTER 1

## Introduction

Clinical genetic services developed since the beginning of the seventies of the last century with the increasing insight in the cytogenetic and molecular basis of congenital clinical malformations and genetic diseases and the development of new diagnostic techniques. These services were mainly focused on postnatal and prenatal diagnosis of chromosomal abnormalities and genetic metabolic diseases and genetic counseling of parents at increased risk of a handicapped child.<sup>4-9</sup> In 1983 DNA linkage for Huntington's disease was established which for the first time enabled predictive genetic testing for an incurable disease clinically expressing in adulthood. Ten years later the responsible gene defect was identified and several other presymptomatic DNA tests became available for rare late onset diseases such as myotonic dystrophy and autosomal dominant cerebellar ataxias.<sup>10,11</sup>

A major change in clinical genetic practice evolved during the mid-nineties when mutations were found in major susceptibility genes involved in breast cancer (BRCA1 and BRCA2) and colorectal cancer (MSH2, MLH1 and MSH6). This enabled the identification of individuals at high risk of relatively common diseases. Unlike to Huntington's disease and other late-onset neurological genetic disorders, here genetic test results (presence or absence of a mutation) influenced clinical management. Multidisciplinary family cancer clinics were initiated where oncologists, surgeons, gynecologists, clinical geneticists and other specialists closely collaborate. This multidisciplinary care has contributed to an increase in the life expectancy of high risk individuals by preventive measures such as mastectomies and oophorectomies<sup>12-21</sup> and recurrent colonoscopies and timely surgical intervention.<sup>22-24</sup>

The current thesis represents a contribution to the research in the field of hereditary breast cancer from a clinical genetic perspective in close collaboration with oncology, surgery and other specialties.

## 1.1 Breast cancer

### *Epidemiology and risk factors*

Cancer of the breast is one of the most feared of human illnesses. Its high incidence rate, the severe physical and psychological impact of local and systemic treatment, and the threat of a fatal course of the disease all contribute to this. Worldwide, breast cancer is the most frequent type of cancer among women with each year nearly one million new cases and 375.000 related deaths. Breast cancer is the leading cause of cancer mortality in women in Europe and the second in the United States after lung cancer.<sup>25,26</sup> Fortunately, mortality rates are presently

declining in many Western countries as a result of the combined effect of an increased awareness, earlier diagnosis due to breast cancer screening programs, encouragement of prompt investigation of palpable lumps, and improved treatment by the use of hormonal and chemotherapeutical agents.<sup>25,27</sup>

Breast cancer incidence rates have been increasing in most Western countries from 1950 onwards. The incidence rates vary as much as fivefold between countries and are low in East and Southeast Asia and are particularly high in Western countries. E.g., the incidence rate in Japan is approximately 20% of the rate in the United States. Interestingly, immigrants were shown to acquire the pattern of cancer risk of their new country.<sup>28,29</sup> Irrespective of geographical location, incidence rates may also correlate with ethnic origin and show age-specific patterns. The incidence rates in the United States, for example, are 20-40% higher in white women than in African-American women,<sup>30</sup> but rates are higher in young (under age 45 years) African-American women than in young white women.<sup>31</sup>

In the Netherlands in 1998 10,317 new invasive breast cancers were diagnosed and 3542 women died due to the disease.<sup>32</sup> The breast cancer incidence rate has approximately doubled among all age groups in the Netherlands during 1958-1992, but has stabilized thereafter. The cumulative lifetime risk of breast cancer for Dutch women is currently 9%. About one quarter of breast cancer patients is diagnosed under the age of 50 years.

Major risk factors for breast cancer are gender (male vs. female = 1:150), age (incidence rates double approximately each decade of life until menopause, after which the rate increases more slowly), carrier status of a high-risk breast cancer susceptibility allele, and a family history of breast cancer. Other risk factors include prior invasive or non-invasive breast cancer, benign disease of the breast (atypical hyperplasia), dense tissue on mammography, no full-term birth, advanced age at first childbirth, early age at menarche, advanced age at menopause, use of hormone replacement therapy (HRT), in-utero exposure to diethylstilbestrol, low socio-economical status, alcohol consumption, leanness (risk factor for premenopausal breast cancer only), overweight (risk factor for postmenopausal breast cancer only), exposure to ionizing radiation in young adolescent women, and frequent disruption of the diurnal sleep-wakefulness rhythm. No consensus exists whether use of hormonal contraceptives is a risk factor for breast cancer development.<sup>33,34</sup> Protective factors for breast cancer include breast-feeding, intake of carotenoids, foliate, and soy/phytoestrogens (the latter reported for Chinese women only), physical activity, and in-utero exposure to placental dysfunction (Table I represents a list of most of presently known risk factors for breast cancer; see also reviews).<sup>35-37</sup>

**Table 1** Risk factors for breast cancer

---

**Demographic factors**

- Female sex
- Increasing age
- Living in Western countries
- Low socio-economical status

**Family history**

- Carrier of a germline mutation of a cancer susceptibility gene
- Positive family history of breast cancer

**Endocrine factors**

- No full-term pregnancy
- Advanced age at first childbirth
- Younger age at menarche
- Older age at menopause
- No breast feeding of offspring
- Usage of Hormone Replacement Therapy
- Exposure to diethylstilbestrol in utero
- Few physical activity
- No exposure to placental dysfunction

**Physical characteristics**

- Dense tissue at mammography
- Atypical hyperplasia of the breast
- Leanness (for premenopausal breast cancer)
- Overweight (for postmenopausal breast cancer)

**Exogenous factors**

- Alcohol use
  - Exposure to ionizing radiation at young adolescent age
  - Low intake of carotenoids, folate, soy/phytoestrogens
- 

*Pathology*

Breasts (Latin; mamma; Greek:  $\mu\alpha\sigma\tau\omicron\sigma$ ) are the classifying organs for mammalian vertebrates and produce milk as post-natal nutrition for the offspring. The primordia of the mammary glands appear in human embryos of 8 mm as a paired thickening of the epidermis between the axillae and inguinal folds. These thickenings are called the mammary crests or 'milk lines'. Normally, only one limited region of the mammary crests gives rise to a breast. However, mammary tissue sometimes develops at ectopic sites, in particular along the milk lines, and cancer may develop also in this tissue. A breast consists of 10 to 15 major duct systems, each of which drains separately to the nipple. Each ductal system is subdivided into lobules that are

the functional units of the gland. Cancers of the breast usually develop from the glandular epithelium in the terminal duct and lobular units and are adenocarcinomas. Breast cancers are classified by their histological patterns and cytological characteristics. Ductal carcinomas, sometimes mixed with lobular carcinoma, compose 80% of all invasive breast cancers. Ductal carcinoma *in situ* and lobular carcinoma *in situ* lesions (DCIS and LCIS, respectively) are confined to the intraductal space. Some lesions are acknowledged risk factors of subsequent invasive breast cancer in the same area in the breast and are therefore considered precursor lesions (atypical hyperplasia, DCIS). Invasive breast cancer spreads primarily through the lymphatics to regional lymph nodes. Hematogenous spread may occur to distant sites, most commonly to the bones, lungs and pleurae, liver, adrenals, ovaries, skin and brain.

In current practice breast cancer patients are classified in four stages based on the clinical and pathologic extent of the disease according to the TNM system, where T refers to tumor size, N to the presence of metastases in the local regional lymph nodes, and M to distant metastases, i.e. beyond the ipsilateral supraclavicular lymph nodes. The histological grading system according to Bloom and Richardson comprises the parameters a) degree of glandular differentiation, b) degree of nuclear atypia, and c) mitotic index. Breast tumors are classified by the combined score of these parameters, as well differentiated (grade I), moderately differentiated (grade II), or poorly differentiated (grade III).<sup>38</sup>

### *Prognosis and treatment*

At present half of all women diagnosed with breast cancer will survive the disease without recurrence. Roughly one-third of patients, however, will die of metastases of the primary cancer within 10-15 years from diagnosis whereas another 15% will have recurrence of the disease after 15 years. Patients thus can never be completely reassured. For the individual patient, however, the prognosis varies depending on several clinical and cell biological factors.

A wealth of prognostic and predictive factors has been established in breast cancer, many of which are interdependent. A prognostic factor is defined as a biologic or clinical measurement associated with a good or bad prognosis in the absence of adjuvant hormonal or chemotherapeutical treatment. A predictive factor is defined as any measurement associated with response or lack of response to a particular therapy. The main prognostic and predictive clinical factors in breast cancer are age (young age being associated with a worse prognosis), tumor size, status of axillary lymph nodes, histological subtype, histological grade, and estrogen and progesterone hormone receptor status. Many cell biological and genetic tumor characteristics are also prognostic and predictive factors, such as growth factors, receptors, proteases, overexpression of oncogenes, deletion or mutation of tumor suppressor genes<sup>39</sup> and, as was shown recently, the gene-expression profile of the tumor.<sup>40,41</sup>

The treatment modalities offered to a newly diagnosed breast cancer patient are determined by the expected prognosis and treatment outcome. The tumor is generally surgically removed either by mastectomy, or lumpectomy followed by radiation therapy of the affected breast.<sup>38</sup> Both surgical interventions are accompanied by ipsilateral removal of the sentinel node (that is the first axillary node to receive drainage from the tumor), or multiple axillary nodes. Depending on the age of the patient, tumor stage, grade and hormone receptor status, patients receive adjuvant treatment with endocrine therapy and/or chemotherapeutical agents.<sup>38</sup>

## 1.2 Genetic susceptibility to breast cancer

### *Predisposing genes*

Cancer is a genetic disease involving mutations of multiple genes. Environmental and endogenous genotoxic agents continuously cause mutations in the genome, but usually they are efficiently repaired or lead to cell death. If these repair mechanisms fail and cell division of the affected cell is not halted, invasive cancer may evolve.<sup>42</sup> Most of the mutations involved in carcinogenesis are acquired at the cellular level over time. However, mutations may also be inherited through the germline. Depending of their role in cellular function mutation carriers have a small, moderate or large increase of the risk to develop cancer. A hallmark of germline mutations of cancer susceptibility genes is familial aggregation of the disease. About 13% of breast cancer patients have one or more first-degree relatives with breast cancer.<sup>43</sup> The increase of breast cancer risk for female first-degree relatives of breast cancer patients is an estimated two-fold averaged across all ages.<sup>43</sup> This excess of familial risk provides the upper estimate of the hereditary effect that has to be explained.<sup>44</sup>

Familial aggregation of breast cancer was already noticed in pre-Mendelian times, but was first accurately documented by the French surgeon Paul Broca in 1866.<sup>45</sup> Numerous reports and studies on familial aggregation of breast cancer have been published since, but it took a century to make clinicians really aware of the potential hereditary nature of cancer.<sup>46-48</sup>

The first evidence for Mendelian inheritance of an susceptibility allele was provided in 1988 using complex segregation analysis.<sup>49</sup> They estimated that 4% of breast cancers was attributable to an autosomal dominant allele. Two years later, the first breast cancer susceptibility gene (designated BRCA1; Breast Cancer 1) was located on chromosome17q using molecular genetic linkage analysis in early-onset breast cancer families.<sup>50</sup>

**Table 2** Clinical features of breast cancer susceptibility genes

---

*General*

	Early age at diagnosis (< 45 years)
	Bilateral breast cancer
	Excess of affected relatives (at any age)
BRCA1 and BRC2 germline mutations: HBC and HBOC	Ovarian cancer of epithelial origin at any age
	Breast and ovarian cancer in a single individual
	Male breast cancer
	Primary peritoneal cancer and fallopian tube cancer
	Prostate cancer
	Pancreatic cancer
TP53 germline mutations: Li-Fraumeni syndrome	Breast cancer at very early age (< 25 years)
	Sarcoma
	Brain tumors
	Leukemia / lymphoma
	Adrenal cortical cancers
PTEN germline mutations: Cowden syndrome	Breast cancer at very early age (< 25 years)
	Benign or malignant thyroid disease
	Meningioma
	Leiomyoma
	Endometrial cancer
	Skin lesions (multiple facial papules, acral and palmoplantar keratoses, multiple skin tags, facial trichilemmomas, subcutaneous lipomas)
	Hamartomas of the colon
	Benign breast disease
STK11/LKB1 germline mutations: Peutz Jeghers syndrome	Pigmented macular spots of the lips, buccal mucosa, conjunctiva, periorbital area, and digits
	Intestinal hamartomatous polyps)
	Small bowel cancer
	Sex-cord tumors
	Melanoma
	Pancreatic cancer
	Gastric cancer
CHEK2 1100delC germline mutation: HBC and HBCC	Colorectal cancer

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Abbreviations: HBC: Hereditary Breast Cancer; HBOC: Hereditary Breast and Ovarian Cancer; HBCC: Hereditary Breast and Colorectal Cancer



The BRCA1 gene was identified by positional cloning in 1994.<sup>1</sup> The second breast cancer susceptibility gene was located on chromosome 13q within the same year<sup>51</sup> and the BRCA2 (Breast Cancer 2) gene was cloned in 1995.<sup>2</sup> Among Caucasian women 16% of the excess of familial breast cancer risk is attributable to pathogenic germline mutations of either the BRCA1 or BRCA2 gene.<sup>52</sup>

Apart for mutations of BRCA1 and BRCA2, germline mutations of the high-risk cancer susceptibility genes TP53, PTEN and STK11/LKB1 have been associated with breast cancer. Mutations of those genes cause complex cancer syndromes including breast cancer, namely Li-Fraumeni syndrome,<sup>53</sup> Cowden syndrome,<sup>54</sup> and Peutz Jeghers syndrome,<sup>55,56</sup> respectively (see Table 2). Germline mutations of these genes are very rare, and are not found in patients with breast cancer in the absence of the other clinical stigmata of these cancer syndromes.<sup>64-70</sup> Breast cancer may also be part of the hereditary cancer syndromes Hereditary Non-Polyposis Colorectal Cancer syndrome<sup>71-73</sup> that is caused by germline mutations of the mismatch repair genes MSH2, MLH1 and MSH6; Familial Atypical Multiple Mole syndrome that is caused by germline mutations of the CDKN2 gene;<sup>74,75</sup> and, hereditary gastric cancer that is caused by germline mutations of the E-Cadherin gene,<sup>76-78</sup> but data are still sparse and conflicting. Noteworthy, somatic mutations of the E-Cadherin are present in the majority of breast cancers of the lobular type<sup>79</sup> but no germline mutations of this gene have been reported in familial clustering of lobular breast cancer.<sup>80,81</sup> Germline mutations of the androgen receptor gene on the X chromosome were reported to confer an increased risk of male breast cancer,<sup>82,83</sup> but no evidence exists that female mutation carriers are at increased risk.

In a collaborative study we report on the discovery of a low-risk breast cancer susceptibility gene, namely the CHEK2 (cell cycle checkpoint kinase 2) gene on chromosome 22q (Chapter 7). CHEK2 is the human ortholog of yeast Cds1 and Rad53<sup>84,85</sup> and has a role in the p53-pathway (see for review on the functions of CHEK2 Bartek et al.).<sup>86</sup> In view of its functional properties the gene was initially selected as a candidate gene for Li-Fraumeni syndrome. Mutational screening in Li-Fraumeni families lacking TP53 mutations showed the truncating 1100delC germline mutation in one family.<sup>87,88</sup> An association of CHEK2 with Li-Fraumeni syndrome was not confirmed by others<sup>89-91</sup> and us (Chapters 7 and 8). In fact, CHEK2 1100delC appears to be a low-risk breast cancer allele, conferring only a two-fold increase in breast cancer risk in women (Chapter 7). CHEK2 1100delC was present in about 1.1% of the general population, 1.4% of breast cancer patients unselected for family history, and 4.2% of index cases of multiple-case families (Chapter 7). The frequency of CHEK2 1100delC among families with a BRCA1 or BRCA2 mutation was not increased when compared to the general population (0.3%). As CHEK2 binds and regulates BRCA1,<sup>92</sup> the biological mechanisms underlying the elevated risk of breast cancer in CHEK2 mutation carriers thus might already be subverted in carriers of BRCA1 mutations. CHEK2 1100delC

was estimated to account for 1% of overall breast cancer incidence and for 0.5% of the excess of familial risk (Chapter 7). Similar results were then reported for Finnish breast cancer families, thus confirming our observations.<sup>93</sup> Variants of CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility.<sup>89,94,95</sup>

Several other candidate breast cancer susceptibility genes have been proposed, including RAD51,<sup>96</sup> BACH1,<sup>97</sup> BARD1,<sup>98</sup> and ATM.<sup>3,99,100</sup> None of these reports have, however, thus far convincingly been confirmed in large series of breast cancer families.<sup>101-104</sup> In particular, the ATM mutations 7271T→G and IVS10-6T→G have been suggested to confer breast cancer risks comparable to mutations of BRCA1 or BRCA2 in one and two families, respectively.<sup>3</sup> However, in a collaborative study we show no evidence for ATM as a high-risk breast cancer susceptibility gene (Chapter 6).

Ample data on candidate low-risk breast cancer susceptibility genes and genetic modifiers of breast cancer risk are available in the literature.<sup>105-108</sup> However, most of the reported associations between genetic variants and breast cancer are controversial or inconclusive due to conflicting results, methodological drawbacks, small sample size and/or lack of replication by others. A few genetic variants have been proposed to modify BRCA1 and BRCA2 associated cancer risks. These include, for breast cancer, the lengths of triplet repeats in the androgen-receptor<sup>109</sup> and AIB1<sup>110</sup> genes and polymorphisms in the progesterone-receptor<sup>111</sup> and (for BRCA2) RAD51<sup>112</sup> genes. Rare alleles at the HRAS1 minisatellite locus have been suggested to be associated with ovarian cancer risk in BRCA1 mutation carriers.<sup>113</sup>

At present, the roles of BRCA1, BRCA2, TP53, PTEN, STK11/LKB1, and CHEK2 in breast cancer susceptibility have been firmly established. Altogether, mutations of these genes account for about one-quarter of the excess of familial risk that is observed in first-degree relatives of breast cancer patients.<sup>44,114</sup>

### *Polygenic model of breast cancer susceptibility*

The lack of finding new high-risk breast cancer genes since BRCA1 and BRCA2 fuels questions on their existence. Segregation analyses have indicated that the remaining of breast cancer susceptibility may partially be explained by dominantly inherited risk and/or recessively inherited risk<sup>115,116</sup> or a mainly polygenic model of inheritance.<sup>116,117</sup> In the polygenic model the remaining of breast cancer susceptibility results from a number of common, low-risk genes with additive and/or multiplicative effects on risk. This model also explains the differences in BRCA1 and BRCA2 cancer risks as observed in family-based studies versus population-based studies.<sup>117-119</sup> BRCA1 and BRCA2 cancer risks would be subject to modification by other risk factors, which are more prevalent in multiple case

families. In agreement with the polygenic model we reported that CHEK2 1100delC interacts with other as yet unknown risk factors with a multiplicative effect on risk (Chapters 7 and 8).

Peto and Mack recently suggested that the majority of breast cancers arise in a susceptible minority of women.<sup>120,121</sup> This theory rests on the occurrence of constant breast cancer incidence rates in twins, contralateral breasts and first-degree relatives of patients.<sup>120</sup> Interestingly, these findings are in agreement with the polygenic model of breast cancer susceptibility by Antoniou.<sup>116</sup> If the model holds true and all breast cancer susceptibility alleles could be identified, it would be possible to classify the population by genetic risk profiling into two groups: one half where 88% of breast cancers occurs and one half in which 12% of the cancers occurs.<sup>122</sup>

The theory that the majority of breast cancers arise in a susceptible minority of women seems in conflict with epidemiological studies indicating that less than 50% of breast cancer incidence in Western populations is attributable to genetic factors.<sup>123-127</sup> The theory also contrasts with the observation that breast cancer incidence rates in migrants become similar to those in the local population.<sup>28,29</sup> These observations could be explained when a significant number of breast cancer susceptibility alleles only confer increased risks in the presence of specific non-genetic risk factors, and vice versa. Interactions between genetic and non-genetic risk factors thus may play a major role in breast cancer development.

#### *Predisposing genes -- search for the unknown ones*

The number of genes to be found is highly dependent on the magnitude of the associated breast cancer risks and their frequencies. Supposing that each of the breast cancer susceptibility alleles confers only a 1.5-fold increase in risk, hundreds of such genes would be needed to account for the excess of familial risk of 2 if their individual frequencies were 1%. Only a few dozen would, however, be needed if their individual frequencies were as high as 10%. Assuming that the excess of familial risk is attributable to solely high-risk genes comparable to BRCA1 and BRCA2, only 4 to 5 of such genes would suffice.<sup>44</sup> The final results of the international Breast Cancer Linkage Consortium and others on their search for high-risk loci using genome-wide linkage searches in hundreds of families with multiple early-onset patients are still awaited.

Some candidate loci have been proposed, but not confirmed (13q21 locus<sup>128,129</sup>; 8p12-p22 locus<sup>130,131</sup>). Noteworthy, genome-wide linkage searches increasingly lack power to identify high-risk loci in case these are rare, e.g. are causal in less than 25% of families under study. Identification of low-risk alleles generally will not be possible by genome-wide linkage analysis because they do not result in multiple case families. Low-risk alleles are easier to identify using either family-based or population-based case-control studies. For the association-based approach the goal is to detect linkage disequilibrium between a

susceptibility locus and a genetic marker and hence the marker must be very close to the susceptibility locus. This approach thus rests on the assumption that the low-risk allele has arisen only once, early in the history of the population. It is estimated that about 50,000-100,000 single nucleotide polymorphisms (SNPs) are needed to allow for such a genome-wide association search<sup>132-135</sup>. With that number of SNPs at hand and when rapid genome-wide sequencing becomes available and affordable, any genetic variant with a frequency of 1% or more that confers an increased risk of 1.5-2 times may become detectable.<sup>135-137</sup>

In the nearest future, the search for new genes and risk alleles will focus on plausible candidates based on their function, structure, or location in the genome (see reviews on techniques that can be applied to find cancer genes).<sup>138,139</sup> Functional candidates are those that could affect a pathway in carcinogenesis (DNA damage response, detoxification of carcinogens, steroid hormone metabolism, and immune surveillance). Among the many attractive candidates are genes involved in Fanconi anemia<sup>140</sup>, and the genes 53BP1<sup>141-143</sup>, MDC1<sup>144,145</sup>, and ATR.<sup>146-148</sup>

Another way forward is to cluster breast cancer families into subgroups most likely to represent single-gene disorders. One approach is the stratification of breast cancer families based on a molecular profile of the tumors.<sup>149</sup> Restricting gene searches to genetically isolated populations also serves this end.<sup>150</sup> Stratification by familial cancer phenotype has been powerful in unraveling genetic heterogeneity among breast cancer families. The recognition of the association of familial breast cancer and early onset of the disease,<sup>151</sup> ovarian cancer,<sup>152</sup> sarcoma,<sup>153</sup> thyroid cancer,<sup>154</sup> and colorectal cancer<sup>155</sup> preceded long the identification of the genes involved in these complex cancer phenotypes. Early onset of the disease was the 'tag' for selecting breast cancer families that were more likely to carry a BRCA1 mutation.<sup>50</sup> For BRCA2, male breast cancer was the 'tag' that identified breast cancer families at higher risk of a mutation of this gene.<sup>156</sup> We identified a subset of breast cancer families characterized by a complex pattern of both breast cancer and colorectal cancer using CHEK2 1100delC as a genetic 'tag' (Chapter 8). Results suggest that the CHEK2 1100delC allele interacts with an as yet unknown cancer risk allele or alleles (Chapters 7 and 8). Thus restricting to families with the combined breast cancer and colorectal cancer phenotype, and/or CHEK2 1100delC may facilitate the identification of hitherto unknown risk alleles for breast cancer and/or colorectal cancer within these families. Likewise, at present low-risk genes and modifiers of risk are searched for by stratifying families and/or individuals for the presence of a BRCA1 or BRCA2 germline mutation. Rare associations of breast cancer with cancer types other than those observed in the known cancer susceptibility syndromes may have escaped clinical recognition thus far. Future research will provide more insight into the occurrence of other cancers in breast cancer families not due to mutations of the known breast cancer susceptibility genes, and will possibly enable their subclassification. In this respect, data

obtained from population-based studies on the occurrence of other cancers in breast cancer patients and their relatives are also of interest since they may point towards a genetic association.<sup>123,157-165</sup>

The identification of cancer susceptibility genes may also be facilitated by research in animals. The availability of animal models (e.g. breast cancer models in rats)<sup>166-171</sup> and the possibilities of experimental modification on defined genetic backgrounds enable the identification of cancer risk genes and modifiers of risk in these species.<sup>172-174</sup> This may reveal valuable candidates to test in human populations.

### 1.3 BRCA1 and BRCA2 genes

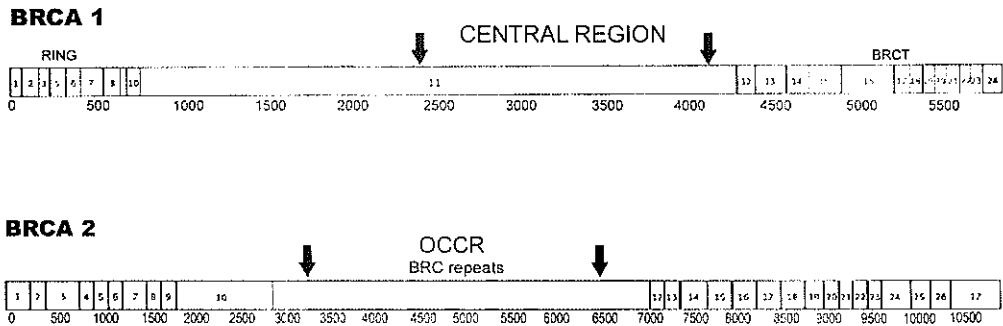
#### *Structure and function*

The BRCA1 gene is located on chromosome 17q21. The gene entails 24 exons that encode a nuclear protein of 1863 amino acids that is ubiquitously expressed. The N-terminus of the BRCA1 protein contains a zinc-binding module (the 'RING' domain) that has been implicated in protein ubiquitination. Its C-terminus contains two tandemly repeated 'BRCT' domains that are thought to be involved in DNA repair processes (Figure 1).

The BRCA2 gene is located on chromosome 13q12 and contains 27 exons that also encode a nuclear protein of 3418 amino acids. BRCA2 contains a series of eight repeated 'BRC' sequences in the central part of the protein. There is however no nucleotide homology between the BRCA1 and BRCA2 genes. Both BRCA1 and BRCA2 are poorly conserved across species, but recently putative orthologs of BRCA1 have been identified in plants and rice.<sup>175-177</sup>

The BRCA1 and BRCA2 proteins have each been implicated in several biological processes, including DNA repair and recombination, regulation of gene transcription and chromatin remodeling, checkpoint control of the cell cycle, centrosome amplification, and for BRCA1, protein ubiquitination. A role for both proteins in DNA repair is deduced by their interaction with several proteins that have been implicated in DNA damage response pathways. Interactions of BRCA1 and/or BRCA2 proteins has been reported among others with p53, CHEK2, RAD51, the Rad50/NBS1/MRE1 complex, the BASC complex including ATM, BLM, SMH2, MSH6, MLH1, and BACH1, BARD1, BAPI, SWI/SNF, p300/CBP, several Fanconi anemia proteins, and each other. Current thoughts are that DNA damage induces rapid phosphorylation of the BRCA1 protein at a region that is rich in 'SQ motifs' and is located N-terminal to the BRCT domain. Several kinases have been shown to phosphorylate BRCA1, including the ATM, ATR, CDK2 and CHEK2 proteins.

Thus far it is unknown why cancer predisposition associated with BRCA1 and BRCA2 mutations manifest in epithelial tissues such as breast and ovary. See also reviews on structure and function of BRCA1 and BRCA2 and interacting proteins.<sup>174,178-185</sup>



**Figure 1** Schematic presentation of BRCA1 and BRCA2

Indicated are exons, nucleotide positions, functional domains RING, BRCT tandem repeats (BRCA1) and BRC repeats (BRCA2). Boundaries of the BRCA1 Central Region are nucleotide 2401 and 4190 (Thompson et al., *Cancer Epidemiol Biomarkers Prev* 2002; 11:329-36). Boundaries of the BRCA2 Ovarian Cancer Cluster Region (OCCR) are nucleotides 3059-4075 and 6503-6629 (Thompson et al., *Am J Hum Genet* 2001; 68:410-9).

*Molecular diagnosis and mutation spectrum*

A variety of mutation detection techniques can be used to identify mutations of the BRCA1 and BRCA2 genes. Exon 11 of BRCA1 and exons 10 and 11 of BRCA2, respectively, encode about half of the respective proteins. Because of their large size, these exons are most conveniently analyzed by protein truncation tests (PTT)<sup>186</sup> and are generally prioritized over the other exons. The remaining exons are often analyzed by heteroduplex analysis, such as single-stranded confirmation polymorphism analysis (SSCP) or denaturing gradient gel electrophoresis (DGGE), direct sequencing, diagnostic PCR's for specific large rearrangements, or multiplex ligation-dependent probe amplification (MLPA).<sup>187</sup>

The department of Clinical Genetics at the Erasmus MC performs the molecular analyses of the BRCA1 and BRCA2 genes for breast cancer families that meet the clinical criteria for molecular screening and that are registered at the Rotterdam Family Cancer Clinic (Chapter 2). At least two independent DNA samples with unique identification are isolated from each individual tested. Screening of the complete coding sequences and intron-exon boundaries is performed using PTT, DGGE, SSCP, pyrosequencing, PCR's for specific large deletions known to be present in the Dutch population<sup>188</sup> and MLPA. To this end 40 PCR reactions for the BRCA1 gene and 57 PCR reactions for the BRCA2 gene are performed using a robot system. After the identification of an abnormal pattern in the PTT, DGGE, SSCP or MLPA analysis, the abnormal fragment is directly sequenced. Pathogenic mutations are independently confirmed by analysis of the duplicate DNA sample. Currently, mutational screening of both genes takes about 4 months and costs € 1224 (€ 612 per gene).

Myriad Genetic Laboratories in the United States offers mutation detection of BRCA1 and BRCA2 commercially. Test results are available within three weeks after arrival of the samples and analysis of the complete coding regions and intron-exon boundaries costs \$ 2760 for both genes.

At present, more than 1200 distinct germline sequence variants are listed for each gene at the Breast Cancer Information Core (BIC) database of the National Human Genome Research Institute (<http://research.nhgri.nih.gov/bic/>). In each gene, over 200 distinct pathogenic mutations have been reported.<sup>189</sup> Both BRCA1 and BRCA2 mutations are evenly scattered throughout the gene. All mutations that are predicted to disrupt transcription or to encode a truncated protein are thought to be pathogenic when the disruptions occur before the end of exon 23 of BRCA1 or the beginning of exon 27 of BRCA2, respectively.

Of 424 reported distinct pathogenic mutations, 85% were nonsense mutations or frameshift mutations and 12.5% occurred in the analyzed noncoding introns.<sup>189</sup> The distinction between a pathogenic missense mutation and a neutral polymorphism is difficult in the absence of a functional assay that can unequivocally distinguish wild-type from mutant alleles. As much as 13% of persons analyzed for BRCA1 and BRCA2 mutations are currently thought to carry such an 'unclassified variant'.<sup>189</sup> At present pathogenicity for only a few of them has been firmly established, e.g. BRCA1 C61G and BRCA1 C64G. It is to be expected that a proportion of the as yet unclassified variants is in fact pathogenic in view of the estimated sensitivity of 63% of molecular screening methods for BRCA1.<sup>190</sup>

The most common reported pathogenic BRCA1 mutations in the international NHGRI BIC database are: 185delAG, 5382insC, and C61G; and for BRCA2: 6174delT, 6503delTT, and 3061del4. There is a different spectrum in the Netherlands of the most frequently found mutations, in particular for BRCA1. The 5 most frequent Dutch mutations of BRCA1 are IVS21-36del510, 2804delAA, IVS20+1G>A, IVS12-16443del3835, and 2312del5. These

mutations represent 44% of a total of 718 registered Dutch BRCA1 mutation positive families. 31% of 210 registered Dutch BRCA2 mutation positive families carry 6503delTT, S1882X, 6174delT, 1538del4 or 5579insA (data were kindly provided by F. Hogervorst, Netherlands Cancer Institute, Amsterdam, coordinator of a collaborative effort of Dutch DNA diagnostic laboratories; 2003). The spectrum of mutations of BRCA1 and BRCA2 among breast cancer families registered at the Rotterdam Family Cancer Clinic is described in Chapter 2.

### *Prevalence of mutations*

The prevalence of germline BRCA1 and BRCA2 mutations has been determined among several population-based and hospital-based series of breast patients unselected for family history of breast cancer (reviewed by Liede and Narod).<sup>191</sup> Among Caucasians, the frequency of BRCA1 and BRCA2 mutations is 3% among breast cancer patients diagnosed below age 70 years, 6% among patients diagnosed below 50 years, and 0.23% among the general population at birth.<sup>52</sup> BRCA1 mutations are more prevalent than BRCA2 mutations in some populations, e.g. in the Netherlands (see Chapter 2), while in other countries mutations of BRCA2 outnumber those of BRCA1, e.g. the UK.<sup>52</sup> Specific mutations of BRCA1 and BRCA2 have been described to occur at high frequency in specific ethnic groups or in geographically confined populations. For example, 2.4% of unselected Ashkenazi Jewish individuals carries either BRCA1 186delAG, BRCA1 5382insC or BRCA2 6174delT<sup>192,193</sup> and 0.6% of the Icelandic population carries the BRCA2 999del5 mutation.<sup>194,195</sup> We describe a specific mutation of BRCA1, IVS12-1643del3835, and of BRCA2, 5579insA, that have a high prevalence among breast cancer patients from the West-Brabant and Zuid-Beveland population, respectively (Chapter 2). De novo mutations of BRCA1 and BRCA2 have only rarely been documented.<sup>196-198</sup>

Although the patterns of inheritance of both BRCA1 and BRCA2 mutations are autosomal dominant, the gene mutations are functionally recessive. In BRCA1 or BRCA2 associated tumors, one mutant allele is inherited through the germline and inactivation of the other allele occurs through an acquired mutation at the cellular level. To date, only one individual has been reported to be biallelic mutant for BRCA1 in her germline, by inheritance of the same mutation from each parent (deletion of AA at position 2800).<sup>199</sup> This woman developed breast cancer at the age of 32 years and had no malformations or other diseases. Based on data in Brcal knockout mice it is assumed that biallelic BRCA1 germline mutations in humans will mostly result in embryonic lethality.<sup>174,200</sup> Thus far, in the Ashkenazi Jewish population no individuals have been reported who were bilallelic mutant for the founder mutations of BRCA1 known to be present in this population.<sup>189</sup> Based on their high



prevalence this was to be expected. Therefore, the genotypes 185delAG/185delAG, 185delAG/5382insC, and 5382insC/5382insC likely result in prenatal lethality in humans.

Recently, biallelic germline variants of BRCA2 have been reported in patients without breast cancer but with the recessive disorder Fanconi anemia.<sup>140</sup> Fanconi anemia is characterized by specific birth defects, progressive bone-marrow failure and cancer susceptibility, in particular acute myeloblastic leukemia in childhood. The BRCA2 germline variants were described in two patients with the FANCD1-type, one with the FANCB-type and two patients with unassigned FA-type. One patient with the FANCD1-type carried a protein truncating mutation of BRCA2 in both alleles (of exon 11 and of the start of exon 27). Both of these mutations are considered to increase breast cancer risk (BIC database). Brca2 deficiency in knockout mice results in early embryonic lethality provided that disrupting mutations produce truncations that occur upstream of exon 11.<sup>201,202</sup> Partial viability is obtained when a truncated Brca2 product retains Rad51 interacting BRC repeat sequences.<sup>203,204</sup> Brca2 mutant mice that lack exon 27 homozygously appear developmentally normal, but have a shortened life span due to increase in carcinogenesis.<sup>205</sup> Thus humans with biallelic mutations with one of them located near the end of the gene likewise may escape prenatal death. Of note, thus far no individual homozygous for the Ashkenazi Jewish BRCA2 6174delT founder mutation of exon 11 has been reported.<sup>189</sup> At the Rotterdam Family Cancer Clinic one couple has been registered who were both carriers of the 5579insA protein truncating mutation of exon 11 of BRCA2. They had two daughters and reported to have had three miscarriages. One of the daughters was shown heterozygous for the BRCA2 mutation, while the other had not inherited the mutation of either parent. These data suggest that also biallelic germline BRCA2 5579insA results in embryonic lethality in humans.

Individuals with a germline mutation of both BRCA1 and BRCA2 have also been reported. In particular, several of such double-mutant individuals have been observed in the Ashkenazi Jewish population.<sup>189,206-209</sup> So far no other clinical phenotype has been observed in these individuals than that seen in carriers of a BRCA1 or BRCA2 mutation only.

#### *Cancer risks and genotype-phenotype correlations*

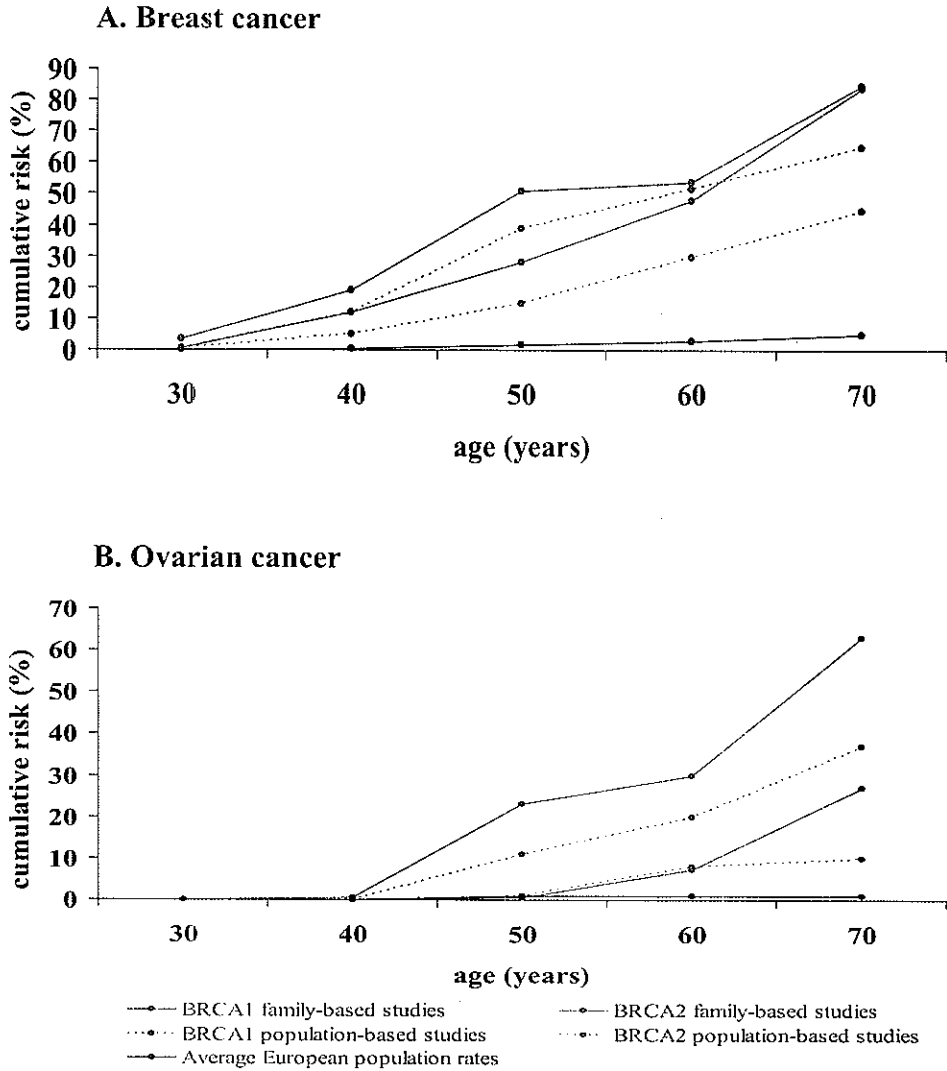
Women who carry a germline mutation of BRCA1 and BRCA2 have an increased risk of breast cancer from age 20-25 years onwards, and of ovarian cancer from age 30-35 years onwards. Considerable variation is observed among the reported risk figures, but confidence intervals are often rather wide and the apparent differences should thus be viewed with some caution.<sup>190,208,210-225</sup> For practical purposes, in family cancer clinics the risk estimates from the Breast Cancer Linkage Consortium (BCLC) are most useful as they rely on multiple-case families, large datasets and robust methodologies. Within the setting of multiple-case families, the cumulative risk of breast cancer at age 70 years in BRCA1 and BRCA2 mutation

carriers was 85% and 84%, respectively, and of ovarian cancer 63% and 27%, respectively (Figure 2).<sup>190,213</sup>

Breast and ovarian cancer risks associated with BRCA1 and BRCA2 mutations are age-related. The breast cancer incidence rate in BRCA1 mutation carriers increases with age up to age 45-49 years and remains roughly constant thereafter.<sup>119</sup> In BRCA2 mutation carriers, the rate increases progressively with age. The ovarian cancer incidence rates in BRCA1 and BRCA2 mutation carriers are still low (< 0.5%) under age 40 years and 50 years, respectively, but reach clinically relevant levels from then on.<sup>119</sup> The average age at breast cancer diagnosis is about 42 years for BRCA1, and 48 years for BRCA2 mutation carriers, and at ovarian cancer diagnosis about 52 years and 62 years, respectively (Chapter 3).<sup>212</sup> Few data are available on the risk of breast and ovarian cancer above age 70 years. It seems likely that ovarian cancer risks are still importantly increased above that age for both BRCA1 and BRCA2 mutation carriers. One study showed no increased breast cancer risk above age 70 years in BRCA2 mutation carriers.<sup>214</sup>

For both BRCA1 and BRCA2 it has been shown that cancer risks are influenced by the position of the mutation within the gene sequence.<sup>224,226-228</sup> Women with a mutation in the central region of the BRCA1 gene (nucleotides 2401 to 4190) were shown to have a lower breast cancer risk than women with mutations outside this region (Figure 1, page 22). The ovarian cancer risk associated with mutations upstream of nucleotide 4191 was higher than that associated with mutations downstream of nucleotide 4191.<sup>228</sup> For BRCA2, mutations in the central region of the gene (designated 'OCCR', for ovarian cancer cluster region; nucleotides 3059-4075 to nucleotides 6503-6629) were associated with a higher risk of ovarian cancer than mutations outside this region, whereas mutations in the OCCR were associated with a lower breast cancer risk than mutations outside the OCCR (Figure 1, page 22).<sup>224</sup> The cumulative risk of breast cancer and ovarian cancer for mutations within the OCCR were 46% and 20% at age 70 years, respectively, and for non-OCCR mutations 33% and 11%, respectively.<sup>224</sup>

Cancer risk figures obtained from family-based studies are generally higher than those obtained from population-based studies.<sup>118</sup> A meta-analysis on 22 population-based and hospital-based studies confirmed that the cumulative lifetime risks based on the later design are lower than those based on multiple-case families.<sup>119</sup> The differences were smaller, however, than suggested. In this meta-analysis, the cumulative risk of breast cancer at age 70 years in BRCA1 and BRCA2 mutation carriers was 65% and 45%, respectively, and of ovarian cancer 39% and 11%, respectively (Figure 2).<sup>119</sup>



**Figure 2.** Cumulative risks of breast cancer (A) and ovarian cancer (B) in women with a BRCA1 or BRCA2 mutation according to family-based studies (Easton et al., *Am J Hum Genet* 1995; 56:265-71; Ford et al. *Am J Hum Genet* 1998; 62:676-89) and a meta-analysis of population-based studies (Antoniou et al. *Am J Hum Genet* 2003; 72:1117-30).

These differences in risks related to study design may be explained by the model of polygenic breast cancer susceptibility, where other genetic factors may modify BRCA1 and BRCA2 associated cancer risks.<sup>117,229</sup> Women with the same mutations thus may differ in their risk profiles, depending on their genetic background. The family history remains therefore an important factor in translating standard risk estimates to individual patients and obvious atypical cancer phenotypes within families with a BRCA1 or BRCA2 mutation require adaptation of cancer risk estimates.

Information on specifically the three Ashkenazi Jewish founder mutations was obtained in several population-based studies. The two founder mutations 185delAG and 5382insC of BRCA1 reside outside the central region of BRCA1 (Figure 1, page 22). The cumulative breast cancer risks associated with BRCA1 185delAG and BRCA1 5382insC varied between 36% at age 85 years to 60% at age 70 years, and for ovarian cancer from 16% to 37% at age 70 years.<sup>208,220,221,223,230</sup> The Ashkenazi Jewish founder mutation 6174delT of BRCA2 resides within the OCCR (Figure 1, page 22), and in population-based studies cumulative risks at age 70 years for breast cancer of 26% to 56%, and for ovarian cancer of 16% to 21% were obtained.<sup>208,220,221,223,230</sup> The data indicate that the Ashkenazi Jewish founder mutations of BRCA1 and BRCA2 confer similar cancer risks when compared to other mutations of the same gene regions.

Women with a BRCA1 or BRCA2 mutation have increased risks of second primary cancers. Their risk of a second primary breast cancer is 50%-60% at age 70 for mutations of both genes.<sup>211,213</sup> The risk of ovarian cancer after breast cancer is 40% for BRCA1 mutation carriers and 15% for BRCA2 mutation carriers.<sup>211,213</sup> Especially BRCA2 mutation carriers but also BRCA1 mutation carriers have increased risks of several cancers at other sites than female breasts and ovaries. The cumulative risks at age 70 years, however, do not exceed 10% for any single cancer type.<sup>211,231</sup> Risks were increased of peritoneal cancer<sup>17,18</sup> and fallopian tube cancer in women,<sup>211,212</sup> of breast cancer in men,<sup>212,214</sup> of prostate cancer below age 65 years and of pancreatic cancer in both sexes.<sup>211,231</sup> For specifically BRCA1 mutation carriers risks were increased of cancer of the uterine body and cervix.<sup>231</sup> For specifically BRCA2 mutation carriers, risks were increased of prostate cancer also above age 65 years, and, for both sexes, of gallbladder and bile duct cancer, stomach cancer, and malignant melanoma.<sup>211</sup> Colorectal cancer was initially reported to occur at increased frequency in families linked to the BRCA1 locus.<sup>216</sup> However, analysis of the largest series of BRCA1 mutation positive families to date did not confirm the association of BRCA1 with colorectal cancer.<sup>231</sup>

To further analyze the association of mutations of BRCA1 and/or BRCA2 with cancer at other sites, the prevalence of mutations of these genes was investigated in population-based and hospital-based series of patients with these other cancers, and in families with clustering of these cancers. Increased frequencies of mutations of BRCA1 and BRCA2 were observed in

series of patients with cancer of the fallopian tube, peritoneal cancer, breast cancer in men, and pancreatic cancer thus confirming their association. 11% of a series of 44 unselected women with cancer of the fallopian tube carried a BRCA1 mutation and 5% a BRCA2 mutation.<sup>232</sup> The frequency of BRCA1 and BRCA2 mutations in women with peritoneal cancer was similar to that observed in women with ovarian cancer.<sup>233,234</sup> In a population-based series of 94 men with breast cancer from the UK, the carrier frequency of BRCA1 and BRCA2 mutations was 0% and 8%, respectively.<sup>235</sup> Of about 100 male breast cancer patients of Ashkenazi Jewish origin, 4% carried either BRCA1 185delAG or 5382insC, and 15% carried BRCA2 6174delT.<sup>236</sup> A BRCA2 mutation was found in 17% of 29 families in which three or more relatives were affected with pancreatic cancer.<sup>237</sup> In a series of 102 unselected patients with pancreatic cancer, 1% carried a BRCA1 mutation, and 3% a BRCA2 mutation.<sup>238</sup>

Conflicting results have been obtained for prostate cancer. BRCA1 and BRCA2 mutations have a limited role in familial prostate cancer as was shown by three studies in totally 78 prostate cancer families.<sup>239-241</sup> The frequency of the local founder mutation in Iceland was increased in men with prostate cancer below age 65 years (2.7% vs. 0.6% in that general population).<sup>242</sup> An increased frequency of BRCA2 mutations was also observed in the UK in men with early-diagnosed prostate cancer ( $\leq 55$  years; 2.3%).<sup>243</sup> However, in Ashkenazi Jewish men with prostate cancer no increased prevalence of the BRCA1 and BRCA2 founder mutations known to be present in this population has been observed.<sup>244-247</sup> The risk of prostate cancer in BRCA1 and BRCA2 mutation carriers warrants further investigation in diverse populations.

#### *Tumor and clinical characteristics of BRCA1 and BRCA2 associated breast cancer*

BRCA1 and BRCA2 associated breast tumors differ from other breast cancers in a few aspects.<sup>248,249</sup> Both BRCA1 and BRCA2 associated tumors are more often poorly differentiated; BRCA1 associated tumors tend to have high mitotic counts and BRCA2 associated tumors have few tubules. Both BRCA1 and BRCA2 associated breast tumors have a higher proportion with continuous pushing margins as opposed to sporadic breast tumors. BRCA1 associated tumors have more lymphocytic infiltration, are more often hormone receptor negative<sup>250-252</sup> and are more often of the medullary subtype as opposed to sporadic breast tumors.<sup>248,249,253,254</sup> BRCA2 associated breast tumors are predominantly hormone receptor-positive, similar to sporadic breast tumors.<sup>252,255,256</sup> In BRCA1 and probably also in BRCA2 associated breast tumors, p53 proteins are often overexpressed, while HER-2/neu protein overexpression is rare.<sup>250,252,257</sup>

Gene expression profiles and comparative genome hybridization of breast tumors have revealed differences between BRCA1 associated breast tumors, BRCA2 associated breast

tumors and sporadic breast tumors.<sup>40,258-261</sup> The degree of aneuploidy is higher in BRCA1 associated breast tumors when compared to sporadic breast cancers.<sup>250,258,262</sup> Loss of heterozygosity (LOH) of the wildtype allele is almost invariably seen in BRCA1 and BRCA2 associated breast tumors.<sup>263-266</sup> LOH is also often seen at the BRCA1 and BRCA2 loci in sporadic breast cancer, but the retained allele is almost never mutated.<sup>267-269</sup> Silencing of the BRCA1 gene by promoter hypermethylation, however, does occur in a significant proportion of sporadic breast tumors suggesting that BRCA1 is also involved in sporadic breast tumorigenesis.<sup>270-275</sup> Interestingly, the expression profile of a breast tumor showing aberrant hypermethylation of the BRCA1 promoter region concurred with the profiles of breast tumors of BRCA1 germline mutation carriers.<sup>259</sup> This suggests that carcinogenesis through germline mutation or hypermethylation of BRCA1 follows similar routes. Hypermethylation of the BRCA2 promoter was not observed for sporadic breast tumors.<sup>276</sup>

The prognosis of BRCA1 and BRCA2 associated breast cancer has been studied extensively (see reviews).<sup>277-280</sup> Despite their predominantly poorly differentiated histology, BRCA1 and BRCA2 breast cancer patients generally have the same prognosis when compared to sporadic breast cancer patients. However, patients with relatively small, node-negative BRCA1 associated breast tumors may have a worse prognosis.<sup>281,282</sup> This phenomenon has been proposed to reflect an increased sensitivity for chemotherapy of BRCA1 and BRCA2 deficient tumors.<sup>282-284</sup> BRCA1 and BRCA2 associated breast cancers have an increased rate of local recurrence or of local new primary tumors.<sup>285,286</sup> There is no increased radiation sensitivity in BRCA1 or BRCA2 carriers.<sup>287</sup>

The effect of some (potential) non-genetic risk factors of breast cancer has been analyzed for women with a BRCA1 or BRCA2 mutation. One study suggested that smoking reduces the risk of breast cancer among BRCA1 and BRCA2 mutation carriers.<sup>288</sup> BRCA1 and BRCA2 mutation carriers with children were shown to have an increased risk of breast cancer by age 40 years when compared to mutation carriers without children, whereas the age at the first pregnancy did not influence breast cancer risks.<sup>289</sup> BRCA1 mutations carriers who used oral contraceptives before age 30 years, or who used them for five or more years may have a modestly increased risk of early onset breast cancer.<sup>290</sup>

#### **1.4 Clinical aspects of familial breast cancer**

##### *Genetic counseling and testing*

Genetic counseling concerns diseases with a potential genetic etiology. The World Health Organization (WHO) defines genetic counseling as the 'provision of accurate, full and unbiased information in a caring, professional relationship that offers guidance, but allows individuals and families to come to their own decisions'.<sup>291</sup> According to WHO counseling is

essential before any genetic testing is carried out. The American Society of Clinical Oncology has published general guidelines for genetic testing for cancer susceptibility (Table 3).<sup>292</sup> Until recently, genetic counseling was 'non-directive'. The counselor's task was to facilitate the decision-making process and to support the final decisions of individuals and their families. Genetic counseling has become less non-directive in case of cancer susceptibility and more similar to approaches in general medicine, where the doctor recommends beneficial treatment or interventions.<sup>291</sup>

**Table 3** Basic elements of informed consent for germline DNA testing for cancer susceptibility according to the American Society of Clinical Oncology (from *J Clin Oncol* 1996; 14:1730-36).

1	Information on the specific test being performed
2	Implications of a positive and negative result
3	Possibility that the test will not be informative
4	Options for risk estimation without genetic testing
5	Risk of passing a mutation to children
6	Technical accuracy of the test
7	Risk of psychological distress
8	Risk of insurance or employer discrimination
9	Confidentiality issues
10	Options and limitations of medical surveillance and screening following testing
11	Fees involved in testing and counseling

To date, in Western countries genetic counseling and testing is common in the clinical management of families with multiple breast cancer patients. In the Netherlands clinical geneticists and/or genetic nurses provide genetic counseling for breast cancer susceptibility at eight departments of clinical genetics at university hospitals and at the Netherlands Cancer Institute. Within the same organizational context of clinical genetic centers DNA testing is carried out which ensures short communication lines between experts responsible for the molecular analysis and those involved in genetic counseling. The Dutch health insurers fund genetic counseling, genetic testing, and the various risk management strategies. Discrimination of employees on grounds of genetic susceptibility is prohibited by law in the Netherlands. Since 1995 the Association of Insurance Companies (The Hague) have agreed on a moratorium which ensures that a clients' genetic susceptibility is not taken into account in case of insurances for disablement up to € 30.000 per year and life-insurances up to € 150.000.

Men and women over 18 years are eligible for genetic counseling for breast cancer susceptibility (Chapter 2). Testing of children or young-adolescents is not performed to preserve their autonomy and because test results do not have medical implications at these ages. Prerequisites for meaningful genetic counseling are accurate information on the patients' disease and a detailed family history. Counselees are requested to provide pedigree information on cancer occurrence up to at least three generations. Cancer diagnoses are verified with written informed consent of individuals involved. Pedigrees with inherited breast cancer typically show breast cancer of women in multiple generations (ages at diagnosis between 25 and 60 years), while men are not affected but may have daughters with cancer. Based on the pattern of cancer occurrence within a family, the clinical geneticist should be able to define which cancer susceptibility gene may be mutated and estimate probabilities for the presence of such a gene mutation. Family data on cancer occurrence also allow the estimation of cancer risks independent of genetic tests.

Evaluating and communicating the magnitude of risk is an important and difficult issue. A woman's understanding of risk varies depending on her education and life experiences.<sup>293</sup> The way in which risks are expressed by counselors also impinges upon comprehension. Tailored print materials contribute to information and knowledge, and to the accuracy of the perceived risk.<sup>294</sup>

Family matters are of great concern in the counseling of cancer susceptibility. The cooperation in genetic testing of a (sometimes distant) relative of a counselee is often needed to establish a genetic diagnosis within a family. On the other hand such a diagnosis might have major clinical implications for many (also distantly related) female relatives who are not aware of the genetic disease within their family. An important task of a counselor is to assist the counselee in handling these matters respectfully within the family and to provide adequate genetic counseling to each individual relative involved. Sometimes there is pressure within the family in favor of being tested or not. Like in general medicine, leading principles here are to avoid harm or, at least, minimize harm to individuals; to maximize benefits to the health of individuals; and to respect the self-determination of individuals.<sup>291</sup> More than 90% of women believe that genetic information on the presence of a mutation of BRCA1 or BRCA2 in an individual should be shared with close relatives.<sup>295,296</sup> Of note, the WHO states that doctors have to inform individuals about their ethical duty to communicate with their relatives that they might be at genetic risk.<sup>291</sup> According to the WHO, if an individual refuses to inform their at-risk relatives it is considered ethically justified if the counselor directly contacts them, especially in cases where effective and affordable treatment or preventive measures are available. At the Rotterdam Family Cancer Clinic individuals rarely are reluctant to inform their relatives (see also Chapters 3 and 4). In case of disrupted family relationships individuals may however object to contact relatives, but most of them then allow their counselor to



contact their relatives directly and provide their addresses. At the Rotterdam Family Cancer Clinic we are aware of one ‘uninformed’ close relative within a period of ten years who developed breast cancer years after her father was identified as a BRCA1 mutation carrier. She had distant metastases at the time of diagnosis and died at the age of 38 years.

Genetic testing for breast cancer susceptibility may imply a lifelong diagnosis and has wide implications. The decision for genetic testing should therefore only be taken after thorough reflection. Counselors should notify that recent stressful events, e.g. a recent cancer diagnosis or death in a relative might temporarily increase anxiety and promote decisions towards genetic testing disproportionately. However, in practice genetic testing can safely be performed at first consultation when counselees have already completed the decision-making at that time. This situation often occurs within families in which the hereditary nature of breast cancer has been known for long and counselees have been well informed by their relatives. When presymptomatic genetic testing for late-onset diseases became feasible in the late eighties, adverse psychological implications of this procedure were initially feared.<sup>10</sup> Research and clinical practice have now shown that the psychological problems posed by presymptomatic genetic testing for any late-onset disease are relatively small after adequate counseling.<sup>11,297,298</sup> Serious adverse effects or regret were also rarely observed after BRCA1 and/or BRCA2 presymptomatic testing at a follow-up of 6 months and one year.<sup>299-305</sup> In one study individuals refraining from BRCA1 and BRCA2 testing even fared less well than individuals who decided for testing and where shown carrier or noncarrier of a mutation.<sup>306</sup>

Main reasons for genetic testing for breast cancer susceptibility are for women defining their cancer risks in order to make decisions on surveillance and preventive strategies, and, for women and men, to assess the cancer risks of their children.<sup>299,307</sup> The interest in genetic testing at the Rotterdam family cancer clinic was 87% in women who already had had breast cancer and/or ovarian cancer and belonged to families in which eventually a BRCA1 or BRCA2 mutation was identified (Chapter 4). The test rate in unaffected women with a 50% and 25% pre-test genetic risk of a BRCA1 or BRCA2 mutation was 57% and 27%, respectively, and 22% in men with a 50% pre-test genetic risk of a mutation (Chapter 3).

The appreciation of genetic testing for mutations of BRCA1 and BRCA2 by women was similarly high in other countries.<sup>299,308-312</sup> Important reasons to refrain from genetic testing are the absence of reimbursement of costs and potential discrimination by employers and insurers upon mutation identification.<sup>299,307</sup> 69% of US citizens opted for fee-free BRCA1 and BRCA2 testing in contrast to 22% of individuals who had to pay themselves for the test.<sup>312</sup>

BRCA1 and BRCA2 mutation carrier status may influence reproductive choices in women and men.<sup>299</sup> Prenatal diagnosis for BRCA1 and BRCA2 aiming elective termination of a female fetuses with a germline mutation is a controversial ethical issue.<sup>313-316</sup> In the Netherlands prenatal testing for these mutations is available after extensive counseling at

some departments of clinical genetics and gynecology.<sup>316</sup> Experience indicates that parents want to discuss this option only incidentally.

Some caveats can be mentioned in genetic counseling and testing of breast cancer susceptibility. Within a family more than one cancer susceptibility allele may be present, and, if unrecognized, may result in faulty risk calculations. Therefore a genetic diagnosis should preferably be sought in all patients with breast cancer and ovarian cancer within a family (and sometimes also in patients with other cancers). Proof should be sought through which lineage a mutation is inherited. Otherwise relatives may undergo presymptomatic testing for a mutation for which in fact they are not at risk. In small families and families with predominantly men the presence of a breast cancer susceptibility allele may easily remain unrecognized. When providing risk estimates, it is crucial to record the facts and assumptions they are based on.

Medical records may be destroyed ten years from diagnosis and as a consequence cancer diagnosis in preceding generations can often not be confirmed. Breast cancer is usually correctly reported by relatives due to its unmistakable localization and surgical treatment sequelae,<sup>317,318</sup> but likely not so for ovarian cancer. Within a family with only breast cancer patients, death of unknown cause of a female relative at ages 40-55 years adds to the probability of finding a BRCA1 or BRCA2 mutation as carriers typically may die because of ovarian cancer at these ages.

The issue of autonomy of individuals is at stake within families with a known mutation when presymptomatic genetic testing is requested by adult children while their putative transmitting parent is unwilling to be tested. A similar problem is faced when only one half of a monozygotic twin demands genetic testing. In both situations the genetic test result of the counselee (may) implies (imply) the genetic status of the unwilling half of the twin (parent). These situations do occur and harbor a high risk of harm to both parties. Intensive counseling *before* genetic testing of both parties is often helpful, resulting in mutual understanding and consensus on the way to handle the situation.

Breast cancer susceptibility affects main themes of life. The genetic counseling and testing for breast cancer susceptibility challenges the skills of counselors.

#### *Breast cancer risk assessment*

Women with a family history of breast cancer have on average about a two-fold increased risk of developing breast cancer when compared to women without such family history.<sup>43</sup> More accurately assessed breast cancer risks are desirable for optimal decision-making concerning genetic testing, surveillance and risk reduction interventions. Two models are often used to estimate a woman's risk to develop breast cancer.

The *Gail model* provides cumulative risk estimates of invasive and non-invasive breast cancer by decade. The model is based on the major predictors of risk identified in the Breast Cancer Detection Demonstration project.<sup>319</sup> The model includes the woman's age, age at menarche, age at first offspring, number of previous breast biopsies, presence of atypical hyperplasia of the breast, and number of first-degree relatives with breast cancer. The Gail model does not consider breast cancer among second-degree relatives nor ages at diagnosis of breast cancer, resulting in a less optimal assessment of breast cancer risk for families with multiple affected women diagnosed at young ages and among two or more generations.

The *Claus model* provides cumulative risk estimates for women with a family history of breast cancer. This model uses empirical data from the Cancer and Steroid Hormone (CASH) study, and assumptions of the prevalence of high-risk breast cancer susceptibility genes.<sup>320</sup> The model includes the woman's age, the number of first- and second-degree relatives with breast cancer, and their age at breast cancer diagnosis. The Claus model does not consider non-genetic risk factors, and can maximally accommodate two affected family members, whereas the number of unaffected female relatives is not considered. Breast cancer risk estimates including the presence of ovarian cancer in first-degree relatives have also been published.<sup>321</sup>

With increasing numbers of families tested negatively for BRCA1 and BRCA2 mutations, there is a growing need for detailed breast cancer risk estimates for women from families excluded for mutations of BRCA1 and BRCA2. Neither the Gail model nor the Claus model includes BRCA1 or BRCA2 mutation status. Women from families excluded for BRCA1 and BRCA2 mutations appear to have lower breast cancer risks than women from unselected breast cancer families.<sup>215,322,323</sup> A statistical modeling analysis predicted that the lowering in breast cancer risks may be most pronounced for women with multiple affected relatives.<sup>322</sup> In women with two affected first-degree relatives the increase in breast cancer risk fell from 14 times to 2.3 times when BRCA1 and BRCA2 mutation positive families were excluded. In contrast, in women with only a single affected first-degree relative, the increase in risk only slightly changed from 2.3 times to 2.0 times when BRCA1 and BRCA2 mutation positive families were excluded.<sup>322</sup> According to a recent study on 788 breast cancer patients diagnosed under age 40 years, breast cancer risks in first-degree relatives fell by maximally 20% when patients with mutations of BRCA1 and BRCA2 were excluded.<sup>323</sup>

#### *Probability of finding BRCA1 and BRCA2 mutations in clinical settings*

Strong predictors for the presence of BRCA1 and BRCA2 mutations in breast cancer families are early age at diagnosis of breast cancer or a family history of both breast cancer and ovarian cancer.<sup>324-326</sup> Co-occurrence of male breast cancer in a family and Ashkenazi Jewish ancestry further enhance the probability of finding a BRCA1 or BRCA2 mutation

substantially.<sup>324,325</sup> Based on these parameters, probabilities to identify a mutation may well rise to over 50%. Noteworthy, about all families with inherited patterns of both breast cancer and ovarian cancers can be explained by BRCA1 and BRCA2 mutations.<sup>190,322</sup>

Genetic testing for BRCA1 and BRCA2 mutations is currently still laborious and careful case selection for genetic testing is therefore desirable, for example by using a threshold of 10% probability to identify such a mutation. To that end local probabilities (Chapter 2), or the periodically updated probabilities produced by the clinical testing service of Myriad Genetics Laboratories may be used as guidelines (<http://www.myriadtests.com/provider/mutprev.htm>). Several computer programs have been developed that estimate probabilities based on data provided by healthcare professionals, including the validated BRCAPRO model (<http://astor.som.jhmi.edu/brcapro>).<sup>327,328</sup> The BRCAPRO model considers the complete structure of the family pedigree including the number of breast cancers, ovarian cancers, male breast cancers, and bilateral breast cancer as well as the ages at diagnosis of breast cancer and ovarian cancer, and Ashkenazi Jewish ancestry.

#### *Risk reducing interventions*

Women from breast cancer families may reduce their risk of (death by) breast cancer by breast self-examination, clinical breast examination, mammography, magnetic resonance imaging (MRI), prophylactic bilateral mastectomy, bilateral oophorectomy, and/or chemoprevention. Women with a family history of ovarian cancer or who carry a BRCA1 or BRCA1 mutation may reduce the risk of (death by) ovarian cancer by vaginal ultrasonography of the ovaries, assessment of serum CA 125 levels, and/or prophylactic bilateral salpingo-oophorectomy (BSO) (see reviews).<sup>329-331</sup>

According to current Dutch guidelines surveillance of women from breast cancer families consists of 6-monthly clinical breast examination (CBE), annual mammography and instructions for monthly breast self examination (BSE). Surveillance of the breasts starts at age 25 or 35 years depending on family history and genetic test results. While population-based randomized trials repeatedly showed that mammography screening can reduce breast cancer mortality by 20-30% in women aged 40-70 years, the value of screening or intensive surveillance in women below the age of 40 years and/or with a family history of breast cancer is unproven. Several small reports about the efficacy of mammography and clinical examination in women with a family history have been published with inconsistent results, most likely due to differences in delineating family history, screening scheme and modality.<sup>332,333</sup> Screening results appeared to be especially unfavorable in proven carriers of a BRCA1 or BRCA2 mutation (Chapter 5).<sup>334</sup> In two studies as much as half of the breast cancers diagnosed in BRCA1 or BRCA2 mutation carriers while under regular mammographic and CBE surveillance were detected by the women themselves in between

two screening visits (Chapter 5).<sup>335</sup> This high rate of interval cancers probably relates to the high-grade nature of BRCA1 and BRCA2 associated breast cancers, and/or lower mammography sensitivity of these tumors due to their specific histological characteristics.<sup>248,336</sup>

Several reports suggest that MRI is a better screening modality in women with a BRCA1 or BRCA2 mutation and more in general in women at high risk.<sup>337-341</sup> To firmly establish the added value of MRI as a screening instrument, large prospective studies are needed with independent assessment of MRI and mammography. These studies are presently conducted in various countries including the Netherlands.<sup>341</sup> In the Netherlands, the costs of breast MRI is currently 3 times that of mammography. Importantly, adherence to breast surveillance programs of mutation carriers aged 25-70 years who do not opt for prophylactic mastectomy is very high in our experience. Others reported an adherence to mammography upon receipt of a positive BRCA1 or BRCA2 test result of 59% and 82%, respectively.<sup>342,343</sup>

Patients with ovarian cancer including early stage disease were reported to have specific serum proteomic patterns.<sup>344</sup> Likewise, proteomic profiling of serum of women at increased risk of breast cancer may potentially facilitate early diagnosis of breast cancer in the future.<sup>345,346</sup>

We showed prospectively that prophylactic bilateral mastectomy dramatically reduces the incidence of breast cancer in unaffected women with a BRCA1 or BRCA2 mutation (Chapter 5). Earlier, similar results were obtained in a large retrospective study of 639 unaffected women from breast cancer families of whom 18 women were later shown to carry a pathogenic BRCA1 or BRCA2 mutation.<sup>15,21</sup> Mastectomy of the contralateral breast in breast cancer patients from breast cancer families also significantly reduced the incidence of a second primary contralateral breast cancer.<sup>20,347</sup> Mastectomy, however, never reliably removes all breast tissue and breast cancer may develop incidentally in residual tissues. Subcutaneous mastectomy which preserves the nipple and areolar complex is not recommended because a substantial amount of breast tissue remains (see review).<sup>348</sup> Some dozens of breast cancers have been diagnosed after incomplete/subcutaneous prophylactic mastectomy, but to date no primary breast cancers have been reported after complete mastectomy aiming prophylaxis in high-risk women.<sup>348</sup> Although some women may still prefer subcutaneous mastectomy for cosmetic reasons, it should be realized that the sensory function of the remaining tissue is lost. Prophylactic mastectomy may also fail in its goal to prevent breast cancer related death when metastatic breast cancer disease develops from small tumors missed at pathological examination of the mastectomy specimens (Menke-Pluymers, personal communication).<sup>21,349</sup> Pathological examinations of prophylactic mastectomy specimens from women at high risk of breast cancer have revealed clinically occult invasive

cancers.<sup>20,21,335,350-353</sup> Careful pre-operative screening of the breasts and meticulous pathological review are thus indicated to achieve optimal care.

At the Rotterdam Family Cancer Clinic the demand for prophylactic bilateral and/or contralateral mastectomy in clinically unaffected and affected women with a BRCA1 or BRCA2 mutation was 51% and 35%, respectively (Chapters 3 and 4). Worldwide large variation seems to exist in the appreciation of prophylactic mastectomy by women with a mutation of BRCA1 and BRCA2 and their doctors.<sup>354</sup> Thus far only for the UK and Northern America systematic analyses of the actual rate of prophylactic surgery in mutation carriers have been reported. The demand for prophylactic mastectomy in the UK was 61% of 31 mutation carriers;<sup>355</sup> in a single cancer center in the US 23% of 214 mutation carriers;<sup>355</sup> and in a multi-center study in Northern America 3% of 29 mutation carriers.<sup>356</sup> In France the demand for prophylactic mastectomy is likely to be low which seems also related to a more general psychologically negative attitude towards mastectomy which is also present in various other countries and cultures.<sup>357</sup>

Bilateral salpingo-oophorectomy (BSO) at premenopausal age lowers the risk of breast cancer in the general population and also in BRCA1 and BRCA2 mutation carriers with about 50%.<sup>17-19</sup> BSO also prevents ovarian cancer and cancer of the fallopian tubes and is often performed in BRCA1 and BRCA2 mutation carriers around age 40 years.

Tamoxifen, a selective estrogen receptor modulator, was the first drug shown to reduce significantly the incidence of breast cancer in healthy women by about 50%.<sup>358</sup> Conflicting results were obtained on the efficacy of this drug in high-risk women, in particular in BRCA1 mutation carriers.<sup>359-362</sup> Chemoprevention trials on the efficacy of tamoxifen and other hormonal agents in women with a mutation of BRCA1 or BRCA2 are underway. Poor recruitment of high-risk women for chemoprevention trials, however, seems to hamper these studies significantly.<sup>355</sup>

Medical and/or psychological side effects accompany all risk reducing interventions. Surveillance will inevitably result in false-positive findings, which causes unnecessary anxiety and additional non-invasive and invasive procedures. Prophylactic mastectomy is accompanied by a complication rate of up to 30% depending on the type of surgery and follow-up period.<sup>348,363,364</sup> A significant mental health or body image problem in women upon prophylactic mastectomy was not observed. Women only rarely indicated to regret this procedure though a proportion of women reported negative changes in feelings of sexual attractiveness, femininity, and looks.<sup>365-372</sup> Prophylactic oophorectomy at premenopausal ages induces the onset of postmenopausal symptoms in the absence of the use of HRT. Bilateral premenopausal oophorectomy clearly has an effect on mood and sexuality but use of HRT largely negates these adverse side effects. It is unknown whether the use of hormone replacement therapy (HRT) subsequent to BSO increases the risk of breast cancer for women

with a BRCA1 or BRCA2 mutation. In one study, however, HRT use did not negate the effect of decreased breast cancer risk by bilateral oophorectomy in BRCA1 mutation carriers.<sup>19</sup>

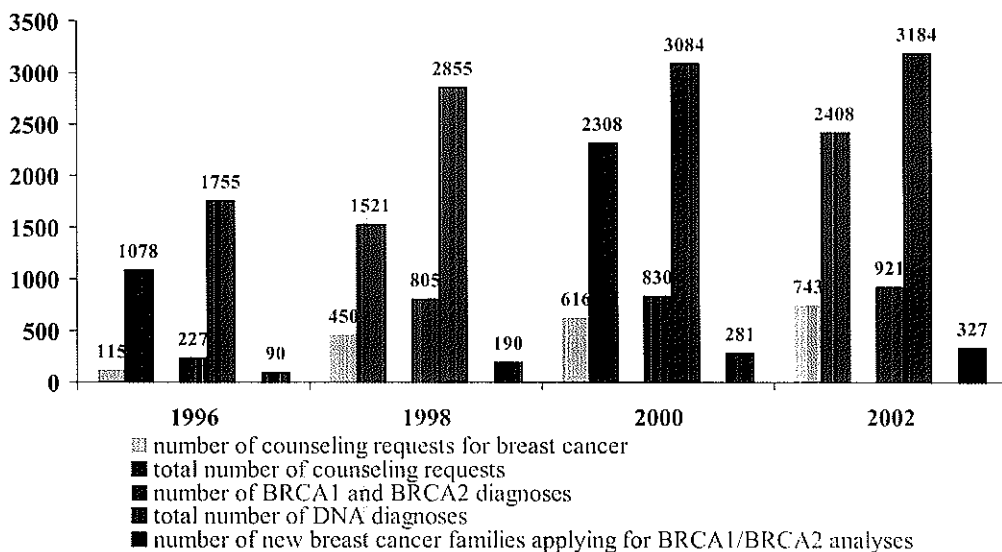
### *Multidisciplinary approach*

The management of families at high-risk of breast cancer requires a multidisciplinary approach in view of its clinical genetic, oncological, surgical, gynecological and psychological aspects, and at present multidisciplinary care is provided at many centers throughout the Western world. In Rotterdam a multidisciplinary Committee on Hereditary Tumors at the Erasmus MC started to coordinate this care and associated research since 1991. The multidisciplinary approach ensures short communication lines between the various specialists and uniformity in advices and information, which is essential in the provision of optimal care to patients and families. The number of families applying for genetic counseling and BRCA1 and BRCA2 testing grew rapidly during the years after the identification of these genes, and grew more slowly since 1998 (Figure 3). As shown, thus far there is no decrease in the number of new breast cancer families applying for genetic counseling and testing. At the end of 2002, 1500 distinct breast cancer families have been registered at the department of Clinical Genetics, Erasmus MC, and in 300 of these families a pathogenic mutation of BRCA1 or BRCA2 has been found. The organizational impact of the identification of the BRCA1 and BRCA2 genes is illustrated by the fact that in 2002 29% of all diagnostic DNA testing concerns breast cancer susceptibility and 31% of all genetic counseling requests (Figure 3).

The need of a patients' support group for women with a BRCA1 or BRCA2 mutation was evaluated in 109 mutations carriers known at the Rotterdam Family Cancer Clinic and was shown to be high.<sup>373</sup> A national patients support group was founded and incorporated in the Dutch Breast Cancer Patient Support Group (de Nederlandse Borstkanker Vereniging NBV). Individuals with a BRCA1 or BRCA2 mutation and individuals who are at risk for such a mutation coordinate this national patients support group. They provide a telephone network for individual patient contact, supply information on hereditary breast cancer (e.g. by flyers, publications, interviews, and a national patient information conference) and represent their interests at a national and international level (e.g. in Europa Donna; <http://www.europadonna.org>). The group is advised by specialists in the field of cancer genetics and oncology.

In the future increasing number of genes will be identified that play a role in the susceptibility of other common late-onset diseases that are amenable to beneficial intervention, such as other forms of cancer, cardiovascular disease and diabetes. The multidisciplinary approach as applied in the clinic for patients and families with breast cancer may serve as a model for the development of optimal care for patients and families with other

common diseases. Clinical genetic centers have a vast knowledge and experience with chromosomal, molecular and clinical diagnostics of many genetic diseases, and with the genetic counseling and testing of patients and their families. Of note, the Dutch government requires by law that centers provide comprehensive genetic services in order to be eligible for funding. In the Netherlands such centers are only located at university hospitals, although there are affiliated outdoor clinics at a few regional hospitals. With increasing involvement of clinical genetics in other common diseases, genetic services will soon be required on a structural basis at larger regional hospitals as well. It will be the task of the departments of clinical genetics of the university hospitals to organize such services and to guarantee their quality in the future.<sup>374</sup>



**Figure 3** Organizational impact of the identification of BRCA1 and BRCA2 at the department of Clinical Genetics, Rotterdam (data were kindly provided by Drs. Rob Verhage).



## 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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#### **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%) out of 138 families with breast and ovarian cancer (HBOC), in 43 (13%) of the 339 families with breast cancer only (HBC), and 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 8 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.**

## **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, Ashkenazi 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 cancer 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 Southwestern part of the Netherlands.

## **PATIENTS AND METHODS**

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

A series of families with clustering of breast and/or ovarian cancer was referred for oncogenetic 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.

**Table 1** Minimal criteria for BRCA1/BRCA2 mutation analysis

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- 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\* relatives affected by breast cancer, one of them diagnosed before the age of 45 years
  - Two first- or second-degree\* 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\* relatives affected by ovarian cancer
  - Three first- or second-degree\* relatives affected by either breast cancer or ovarian cancer
- 

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

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

In view of the results with respect to the geographic 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 (Figure 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 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 Figure 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.



<b>BRCA1</b>			<b>BRCA2</b>		
★	185insA	n=6	●	IVS20+1G>A	n=10
✚	1411insT	n=7	◆	IVS21-36del1510	n=8
▼	2804delAA	n=8	●	5579insA	n=6
●	IVS12-1643del3835	n=17	+	6503delTT	n=7
○	Other BRCA1/2 mutations found ≤ 5 times each; n=41				

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

#### *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

**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<sup>a</sup>

	0 BC below 40 years	1 BC below 40 years	≥ 2 BC below 40 years	Total
<b>HBC<sup>b</sup></b>				
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%)
<b>HBOC<sup>c</sup> with 1 OC</b>				
Total	47	30	17 <sup>b</sup>	94
Mutations				
BRCA1	7	12	12	31
BRCA2	1	3	0	4
Either gene	8 (17%)	15 (50%)	12 (71%)	35 (37%)
<b>H(B)OC<sup>c,d</sup> with ≥ 2 OC</b>				
Total	27	12 <sup>b</sup>	16 <sup>b</sup>	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.

<sup>a</sup>Only mutations detected with the routinely applied set of mutation-detection methods are shown.

<sup>b</sup>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.

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 IVS-20+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.

#### *Statistical analysis*

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

## **RESULTS**

#### *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

<b>Family characteristics</b>	<b>No of families</b>	<b>BRCA1 (%)</b>	<b>BRCA2 (%)</b>	<b>Either gene (%)</b>
<i>HBC</i>				
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)
<i>HOC</i>				
≥ 2 patients with OC	11	3 (27)	1 (9)	4 (36)
<i>HBOC</i>				
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)
<b>Total</b>	<b>517</b>	<b>98 (19)</b>	<b>21 (4)</b>	<b>119 (23)</b>

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. Figures 2a and 2b 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 4** Number of families for each mutation and frequency of cases of breast cancer (BC) and ovarian cancer (OC) per mutation

<b>BRCA1</b>	Exon	No of families	No of BC/OC*	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)
<b>Total</b>		<b>98</b>	<b>431</b>	<b>337</b>	<b>120</b>

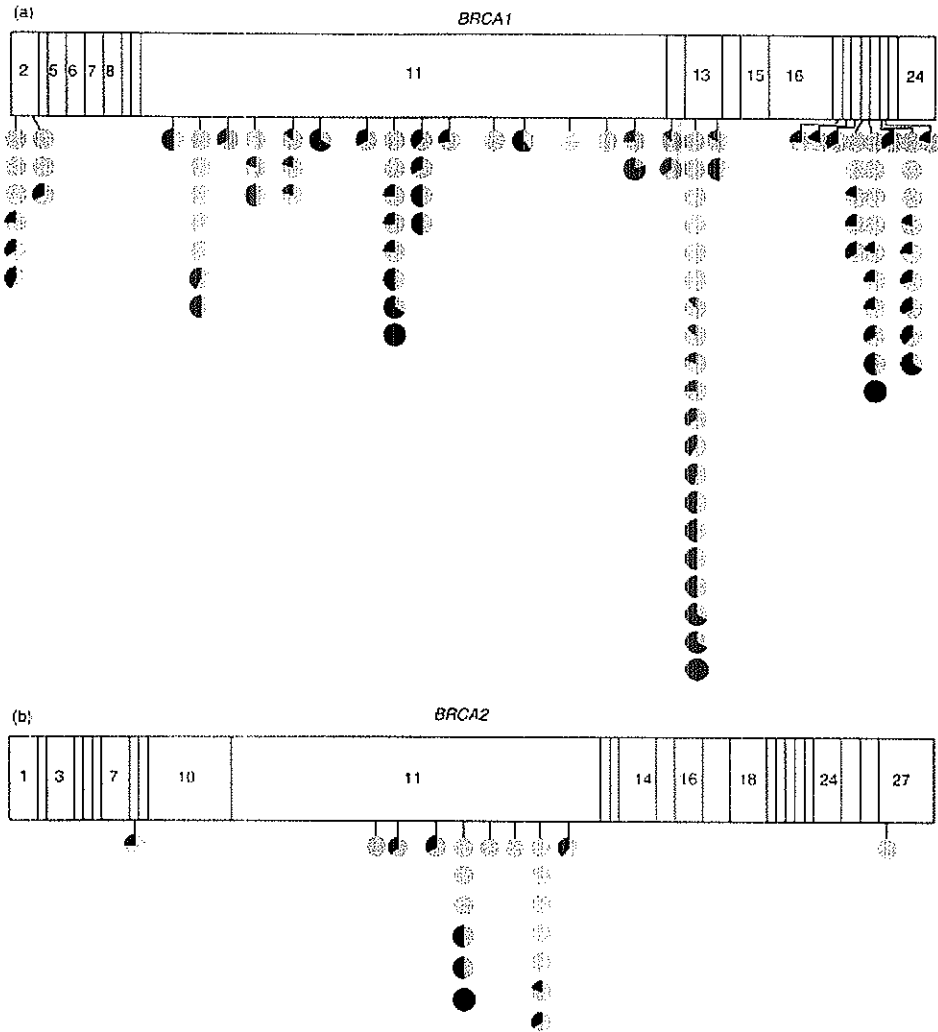
**Table 4** – continued –

<b>BRCA2</b>	Exon	No of families	No of BC/OC*	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
<b>Total</b>		<b>21</b>	<b>78</b>	<b>66</b>	<b>19</b>

\* Figures of breast and ovarian cancer do not add up because of cases with both breast and ovarian cancer.

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 diagnosed with breast cancer at ages 40-49 years and at ages  $\geq 50$  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 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 40-49 years in families with HBC (data not shown).

The proportion of BRCA1/BRCA2 mutations that was detected varied from 6% (11/172) in 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).



**Figure 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.

### *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 Figure 1. Five pairs of 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 Figure 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.

### *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 diagnoses, 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.

## **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] (Figure 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 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 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 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.

## 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/](http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/) (for BRCA1 and BRCA2 mutations); Dutch database: <http://ruly70.medfac.leidenuniv.nl/~devilee/Lab/b1n15.htm>; <http://ruly70.medfac.leidenuniv.nl/~devilee/Lab/b2n15.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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#### 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% (198/411) of women and 22% (59/271) 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% (35/68) opted for bilateral mastectomy and 64% (29/45) 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.

## 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 4 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 own 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.

## **PATIENTS AND 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 3 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  $\geq 20$  years and 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 subjects 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 counsellors (index individuals) were asked to inform all adult first- and second-degree relatives of the patients with breast and/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 of 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 of 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 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 (OR) and 95% confidence intervals (CI) 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 in three age-groups: < 40 years; 40-54 years; and  $\geq$  55 years.

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 i.e., 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 the 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.

**Table 1** Characteristics of studied families

Gene	Number of families	Breast cancer		Ovarian cancer	
		Number of patients (no. per family)	Mean age at diagnosis (SD)	Number of patients (no. 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

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

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

Variable	Number tested (%)	Univariate odds ratio (95% CI)	P	Multivariate odds ratio (95% CI)	P
<b>Women</b>					
<i>Age</i>					
<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
<i>Parenthood*</i>					
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
<i>Genetic risk</i>					
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
<b>Men</b>					
<i>Age</i>					
<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)	0.79
<i>Parenthood†</i>					
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

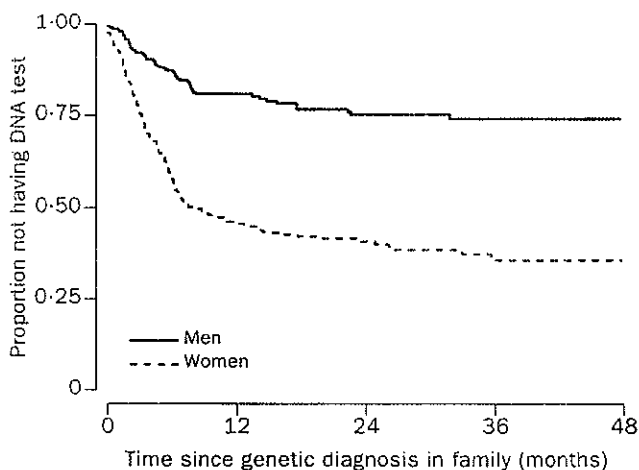
DNA testing was utilised by 38% (257/682) of eligible unaffected risk carriers. The median follow-up after the disclosure of the individual DNA test result was 21 months (range 10-61) in 68 women eligible for prophylactic mastectomy and 24 months (11-61) in 45 women eligible for oophorectomy 48% (198/411) women opted for testing and 22% (59/271) men (odds ratio for difference between sexes 3.21 (95% CI 2.27-4.51);  $p < 0.001$ ; table 2). In women, significant predictors for utilisation of DNA testing in the univariate analysis were age (OR for  $\geq 50$  years vs  $< 50$  years 0.54, CI 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 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% (90/108) vs 27% (23/86), OR 12.74, 95% CI 6.40-25.35] whereas at the age of  $\geq 50$  years the test rates in women and men were almost the same [40% (31/78) vs 30% (30/100), OR 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.

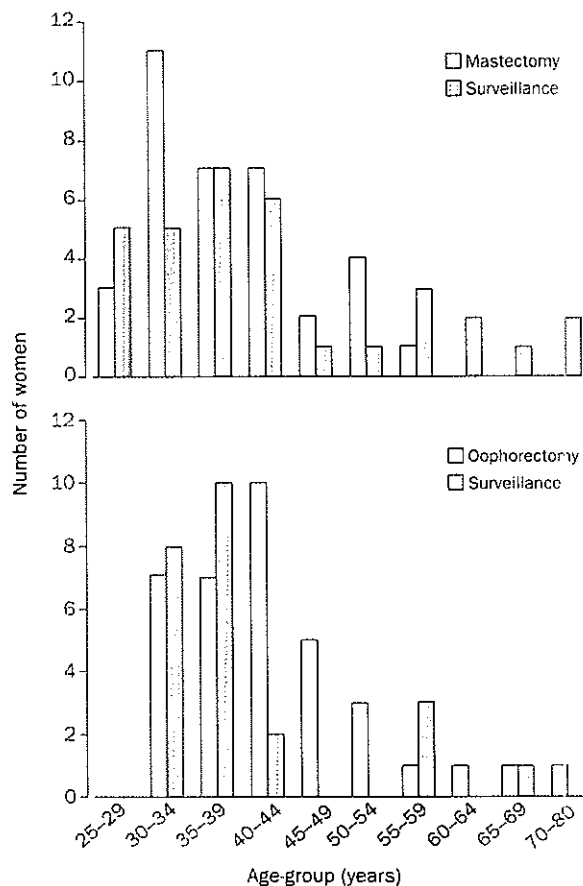


Number at follow-up				
Men	271	220	120	31
Women	275	127	63	23

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

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% (21/38); women aged 40-54

years, 62% (13/21); and women aged  $\geq 55$  years 11% (1/9). There was a tendency towards mastectomy in younger (< 55 years) women. In the 30-35 year age-group, 69% (11/16) opted for prophylactic mastectomy (figure 2). The oldest woman to choose prophylactic mastectomy was 55 years of age at time of surgery.



**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.

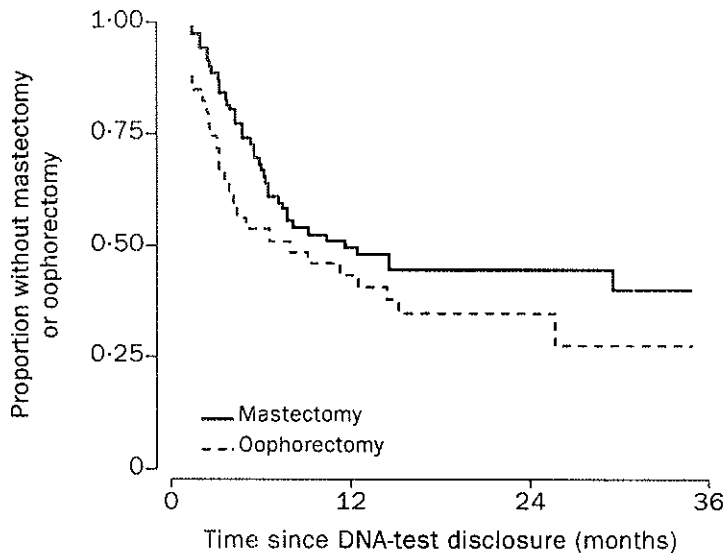
**Table 3** Choices of unaffected carriers

	<b>Mastectomy*</b>		<b>P</b>	<b>Oophorectomy†</b>		<b>P</b>
	Number with mastectomy (%)	Univariate odds ratio (95% CI)		Number with oophorectomy (%)	Univariate odds ratio (95% CI)	
<b>Age</b>						
<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
<b>Parenthood</b>						
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
<b>Total</b>	35/68 (51)	..	..	29/45 (64)	..	..

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

Having children was a significant predictor towards the choice for prophylactic mastectomy: 61% (33/54) of unaffected women with children chose prophylactic mastectomy versus 14% (2/14) childless women (OR 9.43, 95% CI 1.92-46.4;  $p=0.006$ ; table 3). Combining both predictors of age and parenthood 70% (28/40) 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 mutation carriers 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 4 women took more than 9 months to decide for prophylactic mastectomy (11, 13, 28 and 33 months, respectively). Breast reconstruction was simultaneously done in all but one woman.



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

The choice of surveillance or prophylactic oophorectomy in 60 unaffected mutation carriers (aged  $\geq 30$  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 of 35 years, seven out of 15 women younger than 35 preferred to have this surgical intervention simultaneously performed with their prophylactic mastectomy (figure 2). Overall 36 (60%) of these 60 unaffected carriers had prophylactic oophorectomy.

Of unaffected eligible mutation carriers ( $\geq 35$  years of age) 64% (29/45) 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 (OR 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 mutation carriers had a prophylactic oophorectomy (figure 3). Of all 29 unaffected mutation 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).

## 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-prior 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 in outcomes: 32% (10/31) [11] and 62% (59/95) [12] of women said they would consider prophylactic mastectomy in case they carried a mutation. Of *proven* BRCA1 mutation carriers, 35% (11/31) and 17% (2/12) expressed interest in prophylactic mastectomy and 73% (27/37) and 33% (4/12) 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 mutation carriers. 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 BRCA1/2 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 BRCA1/2 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 ultimately will 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 et al [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 7 of 575 subcutaneous mastectomy cases and 0 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 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 mutation carriers, because they experienced unresolved uncertainty and fear [29].

Studies are underway on the efficacy and morbidity of regular surveillance and prophylactic strategies for BRCA1 and 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% high-risk women based their choice for prophylactic mastectomy on a proven BRCA1/BRCA2 mutation in contrast to less than 20% before 1996.

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## CHAPTER 4

### **Use of Genetic Testing and Prophylactic Mastectomy and Oophorectomy in Women with Breast or Ovarian Cancer from Families with a *BRCA1* or *BRCA2* Mutation**

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**Purpose:** To analyze the use of genetic testing, prophylactic mastectomy, and oophorectomy among women with breast and/or ovarian cancer from families with a *BRCA1* or *BRCA2* mutation.

**Patients and Methods:** We examined prospectively the use of *BRCA1/BRCA2* testing in all women with a primary breast or ovarian cancer from a consecutive series of 112 high-risk families in which a *BRCA1/BRCA2* mutation eventually was identified. The rate of prophylactic bilateral and contralateral mastectomy and prophylactic oophorectomy was analyzed in the women who carried a *BRCA1/BRCA2* mutation and who had no metastatic disease at the time of the genetic test disclosure. We examined predictors for genetic test uptake and prophylactic surgery using univariate and multivariate analysis.

**Results:** Overall, 192 of 220 women (87%) with primary tumors underwent genetic testing. Eleven of these 192 tested women (6%) appeared not to carry the family-specific *BRCA1/BRCA2* mutation. Genetic testing occurred significantly more frequently at ages younger than 50 years ( $P = 0.04$ ) and in persons with multiple primary tumors ( $P = 0.02$ ). Among eligible women, 35 of 101 (35%) requested bilateral or contralateral mastectomy, and 47 out of 95 (49%) requested oophorectomy. Women aged younger than 50 years and women who developed their first tumor after the initial identification

of a *BRCA1/BRCA2* mutation in the family were significantly (both  $P = 0.01$ ) more likely to opt for prophylactic bilateral or contralateral mastectomy.

**Conclusion:** In a clinical setting, we show a high demand for *BRCA1/BRCA2* testing and for prophylactic surgery by women with breast and/or ovarian cancer from high-risk families.

Germline mutations in the *BRCA1* and *BRCA2* genes predispose women to breast cancer and ovarian cancer, typically at early ages [1,2]. Moreover, women with breast cancer who carry a *BRCA1* or *BRCA2* mutation are at increased risk of a second primary contralateral breast cancer and primary ovarian cancer [3,4]. Their risk of a contralateral breast cancer is 50% to 60% at age 70 years and their risk of ovarian cancer is 15% to 40% [3,4]. More specifically, we showed that the 5-year rate of metachronous contralateral breast cancer in women with a *BRCA1* or *BRCA2* mutation was 19% and 12%, respectively, whereas in age-matched control patients, this rate was 5% and 2%, respectively [5,6]. The risk mutation carriers with breast cancer have of ipsilateral events is also increased [7]. At 12 years of follow-up, 49% of mutation carriers had an ipsilateral event in contrast to 21% of patients with sporadic breast cancer [7]. Mutation carriers who have already developed breast cancer or ovarian cancer may therefore benefit from strategies that reduce morbidity or mortality. To that end, several avenues currently are being explored. Regular surveillance for breast cancer and ovarian cancer of *BRCA1/BRCA2* mutation carriers has thus far not resulted in detection of cancers at earlier stages, [8-10] although the application of magnetic resonance imaging seems to be promising [9,11,12]. The incidence of contralateral breast cancer in *BRCA1/BRCA2* mutation carriers was shown to be reduced by 50% with tamoxifen use [13], by 58% after bilateral oophorectomy [13,14], and by 60% after chemotherapeutical treatment for the first breast cancer [13]. Most effectively, the incidence of contralateral breast cancer in familial breast cancer patients (irrespective of *BRCA1* or *BRCA2* mutation status) was reduced by 95% after prophylactic contralateral mastectomy [15]. Prophylactic bilateral oophorectomy prevents ovarian cancer in women with a *BRCA1/BRCA2* mutation, but a minimum (long-term) risk of 4% of peritoneal cancer remains after this procedure [14,16].

We previously investigated predictive factors for the decision about presymptomatic DNA testing and prophylactic surgery in unaffected women from families with a *BRCA1/BRCA2* mutation [17]. Until now, no systematic evaluation has been reported on the actual use of *BRCA1/BRCA2* testing by women with breast cancer or ovarian cancer from high-risk families and on the use of prophylactic bilateral or contralateral mastectomy and prophylactic bilateral oophorectomy by women with a *BRCA1/BRCA2* mutation who previously had primary breast cancer and/or ovarian cancer. The main aims of our study were to assess these items in a setting of a family cancer clinic.

## PATIENTS AND METHODS

### *Study Participants*

We studied a consecutive series of 112 families with a *BRCA1* (n=92) or *BRCA2* (n=20) mutation identified at our Rotterdam Family Cancer Clinic before January 1, 2000. General practitioners and medical specialists had referred the families to us by since 1991. Mutational analysis of the full coding sequences and splice junctions of *BRCA1* and *BRCA2* was performed using a variety of techniques, including single-strand confirmation polymorphism, denaturing gradient-gel electrophoresis for most sequences, protein truncation test for exon 11 of *BRCA1* and exons 10 and 11 of *BRCA2*, and diagnostic polymerase chain reaction analyses for large genomic rearrangements known to be present in the Dutch population [18]. In the families under study, a protein-truncating mutation in the *BRCA1* or *BRCA2* gene was identified between 1994 and January 1, 2000. Informed consents, comprising the items from the American Society of Clinical Oncology [19], were obtained from all individuals involved in this study.

For the analysis of the use of genetic testing, all women from the 112 families were eligible that had been diagnosed with breast cancer or ovarian cancer at the time of the initial search for a *BRCA1/BRCA2* mutation in the family, or who developed breast cancer or ovarian cancer later on, but before January 1, 2000. A total of 220 women fulfilled these criteria.

For the analysis of the use of prophylactic bilateral or contralateral mastectomy and prophylactic bilateral oophorectomy, all women with breast cancer or ovarian cancer from these families that also carried a *BRCA1/BRCA2* mutation were eligible. Excluded were women with metastatic disease at the moment of personal genetic diagnosis. Metastatic disease was defined as M1 for breast cancer and as International Federation of Gynecology and Obstetrics stage III or IV for ovarian cancer. Women that previously had both breasts or both ovaries removed for reasons other than prophylaxis were excluded in the analysis of the use of prophylactic mastectomy and oophorectomy, respectively. Only women aged 35 years and older were considered eligible for prophylactic oophorectomy.

A total of 101 women were eligible for prophylactic mastectomy, and 95 women were eligible for prophylactic oophorectomy. The end point of interest of this study was January 1, 2002.

### *Data Collection*

Data on all evaluated variables were collected by personal interviews and by review of patients' medical records. Members of each family were regularly seen at our Family Cancer Clinic as part of a surveillance program. At each follow-up visit, family data on cancer

occurrence, recurrence, and vital status were updated. All genetic testing in these families was performed at our clinic. With respect to the use of prophylactic surgery, follow-up data of the mutation carriers under surveillance at our clinic were obtained by review of their medical records. We collected follow-up data on some mutation carriers who were under surveillance after breast or ovarian cancer at other clinics by means of medical letters on findings during their surveillance visits.

#### *Oncogenetic counseling and procedures*

In view of the heterogenetic origins of breast cancer and ovarian cancer, the initial search for a pathogenic *BRCA1/BRCA2* mutation in a family preferably was performed on all living women with breast and/or ovarian cancer. The initial counselee was therefore asked to contact all affected family members and to seek their participation in *BRCA1/BRCA2* mutation analysis. All women who underwent genetic testing were extensively informed by a clinical geneticist about its risks, benefits, and limitations before blood sampling according to current standards. On identification of a pathogenic *BRCA1* or *BRCA2* mutation in a family, written information on the subject was available for the counselees to distribute among their relatives. In this letter, relatives were invited to contact the clinic if they needed further information or wanted genetic testing. All women with breast cancer or ovarian cancer who carried a mutation also consulted a medical oncologist and were offered a breast and ovarian surveillance program. Breast surveillance comprised physical examination by a specialist every 6 months, annual mammography, and magnetic resonance imaging or ultrasonography, if indicated. Ovarian surveillance was initiated from the age of 35 years and consisted of physical examination by a gynecologist, vaginal ultrasonography of the ovaries once a year, and assessment of serum CA-125 concentrations once to twice a year. To any woman without evidence of metastatic disease, prophylactic mastectomy was offered at any age, and prophylactic oophorectomy was offered from the age of 35 years or older. A psychologist supported all women who considered prophylactic mastectomy. Prophylactic mastectomy was performed by standard bilateral or contralateral simple mastectomy (including the nipple) and simultaneous breast reconstruction by subpectoral implantation of silicone prostheses when requested. Postmastectomy breast cancer risk and morbidity were monitored by follow-up visits at least twice a year. Prophylactic bilateral salpingo-oophorectomy was preferentially performed by laparoscopy.

Annual postoophorectomy gynecologic follow-up was recommended, in particular to monitor the residual peritoneal cancer risk. Hormone-replacement therapy was not prescribed after prophylactic oophorectomy because these women had had breast cancer.

### *Variables and statistical analysis*

Regarding the use of personal genetic testing, the predictive value of the following variables was analyzed: age at the time of initial genetic diagnosis in the family or at the time of first personal cancer diagnosis when the cancer developed after personal DNA test disclosure (< 50 years  $\nu$   $\geq$  50 years), parenthood (no  $\nu$  yes), number of primary cancers (one  $\nu$  two or more), and type of cancer (breast cancer  $\nu$  ovarian cancer).

In the study on the use of prophylactic bilateral or contralateral mastectomy and oophorectomy, the predictive value of the following variables was analyzed: age at the time of personal genetic diagnosis (< 50 years  $\nu$   $\geq$  50 years), parenthood (no  $\nu$  yes), moment of genetic diagnosis in the family in relation to the moment of personal cancer diagnosis (post  $\nu$  prior), breast cancer tumor stage (stage I  $\nu$  stage II/III), and disease-free interval between last cancer diagnosis and personal genetic diagnosis (0 to 2 years  $\nu$   $\geq$  2 years). With respect to prophylactic mastectomy, we also analyzed the type of personal cancer (breast  $\nu$  ovarian) and the presence of bilateral breast cancer in the family (yes  $\nu$  no); for prophylactic oophorectomy, we also analyzed the presence of ovarian cancer in the family (no  $\nu$  yes).

Descriptive statistics were used to determine the rates of genetic testing and of prophylactic surgery. The predictive value of all variables was first assessed by univariate analysis, and odds ratios and 95% confidence intervals (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 with  $P$  values below 0.10 in the univariate model. All  $P$  values were two sided; values less than 0.05 were considered significant.

Kaplan-Meier survival probabilities were calculated to assess the time-dependent rate of the decisions about prophylactic surgery. Start points were date of personal DNA test disclosure in affected women or date of first personal cancer diagnosis when the cancer developed after the personal genetic diagnosis. End points were date of prophylactic bilateral or contralateral mastectomy or date of prophylactic bilateral oophorectomy, death, loss of follow-up, or diagnosis of metastatic disease.

## **RESULTS**

### *Genetic test use*

In the 112 families with a known *BRCA1* or *BRCA2* mutation, we identified 220 women that had breast cancer ( $n = 172$ ), ovarian cancer ( $n=33$ ), or both breast cancer and ovarian cancer ( $n = 15$ ). Genetic testing was used by 192 of these 220 women (87%). In the univariate analysis, young age (< 50 years) and having more than one primary cancer tended to be positively correlated with the use of genetic testing, whereas having children and type of

cancer were not (Table 1). In the multivariate analysis, the correlation of genetic testing with young age and with having multiple primary cancers reached significance ( $P = 0.04$  and  $P = 0.02$ , respectively; Table 1). The majority (89%) of the women applied for genetic testing within 3 months after the first invitation for testing. The mean time of follow-up after the initial genetic diagnosis in the family was 51 months (range, 24 to 84 months).

Interestingly, 11 of 192 tested women (6%) did not carry the family-specific *BRCA1* or *BRCA2* mutation. All eleven women had been diagnosed with breast cancer and were at risk for the family-specific mutation. The mean age at breast cancer diagnosis in all living eligible mutation carriers was 51 years ( $n = 175$ ; range, 23 to 90 years), and was 61 years in the 11 noncarriers (range, 41 to 83 years).

**Table 1** Test use in relation to age, parenthood, and number and type of cancers

Variable	Subgroup	Tested		Univariate OR (95% CI)	<i>P</i>	Multivariate OR * (95% CI)	<i>P</i>
		Number	%				
Age, years	< 50	96/105	91	1.00		1.00	
	≥ 50	96/115	83	0.47 (0.20-1.09)	0.08	0.40 (0.17-0.94)	0.04
Parenthood	No children	31/32	97	1.00			
	Children	161/188	86	0.19 (0.03-1.47)	0.11		
Number of cancers	One	139/165	84	1.00		1.00	
	> One	53/55	96	4.96 (1.13-21.6)	0.03	5.88 (1.33-25.9)	0.02
Type of cancer	Breast cancer**	113/132	86	1.00			
	Ovarian cancer	26/33	79	0.62 (0.24-1.64)	0.34		

Abbreviations: OR, Odds Ratio; CI, Confidence Interval.

\* Included were all variables with univariate  $P < 0.10$ ; only variables that remained significant in the final model are shown in the table \*\*Unilateral breast cancer patients only.

#### *Use of prophylactic bilateral or contralateral mastectomy and bilateral oophorectomy*

Prophylactic mastectomy was performed in 35 of 101 (35%) eligible women, and prophylactic oophorectomy was performed in 47 of 95 (49%) eligible women. Twenty-five of 31 (81%) women eligible for both prophylactic mastectomy and prophylactic oophorectomy underwent both interventions, indicating that a decision to undergo prophylactic mastectomy correlates positively with a decision for prophylactic oophorectomy ( $P < 0.001$ ). The mean time between the patient's last cancer diagnosis and the moment of the identification of the initial *BRCA1* or *BRCA2* mutation in the family was 56 months (range, 0 to 360 months).

Several variables were analyzed for their predictive value toward prophylactic surgery (Tables 2 and 3). Women younger than 50 years of age and women who had been diagnosed as mutation carriers before they had been diagnosed with cancer more often decided to undergo prophylactic mastectomy ( $P = 0.005$  and  $P = 0.03$ , respectively). Both variables



independently predicted the decision to undergo prophylactic mastectomy in the multivariate analysis (both  $P = 0.01$ ; Table 2). The decision to undergo prophylactic oophorectomy only correlated with the tumor stage of the breast cancer; women with stage I breast cancer more often opted for this surgical intervention ( $P = 0.04$ ; Table 3).

*Time needed to decide for prophylactic mastectomy and prophylactic oophorectomy*

A total of 35 women underwent prophylactic mastectomy, and 47 women underwent prophylactic oophorectomy. The mean time interval from the personal genetic diagnosis to the moment of prophylactic mastectomy and to that of prophylactic oophorectomy was 9 and 8 months, respectively.

**Table 2** Predictive factors for prophylactic mastectomy

Variable	Subgroup	With PM		Univariate OR (95% CI)	P	Multivariate OR * (95% CI)	P
		Number	%				
Age, years	< 50	27/58	47	1.00		1.00	
	≥ 50	8/43	19	0.26	0.005	0.27 (0.10-0.73)	0.01
Parenthood	No children	4/14	29	1.00			
	Children	31/87	36	1.38 (0.40-4.78)	0.61		
DNA diagnosis	After cancer	29/93	31	1.00		1.00	
	Before cancer	6/8	75	6.62 (1.26-34.8)	0.03	8.91 (1.55-51.2)	0.01
Tumor stage breast cancer	I	13/24	54	1.00			
	II/III	19/57	33	0.42 (0.16-1.12)	0.08		
	unknown	2/10	20	-			
Time interval**	0-2 years	8/20	40	1.00			
	≥ 2 years	21/73	29	0.61 (0.22-1.69)	0.34		
Type of cancer***	Breast cancer	34/91	37	1.00			
	Ovarian cancer	1/6	17	0.33 (0.04-2.99)	0.33		
Bilateral breast cancer in family	Yes	24/58	41	1.00			
	No	11/43	26	0.49 (0.21-1.15)	0.10		

Abbreviations: PM, prophylactic mastectomy; OR, Odds Ratio; CI, Confidence Interval.

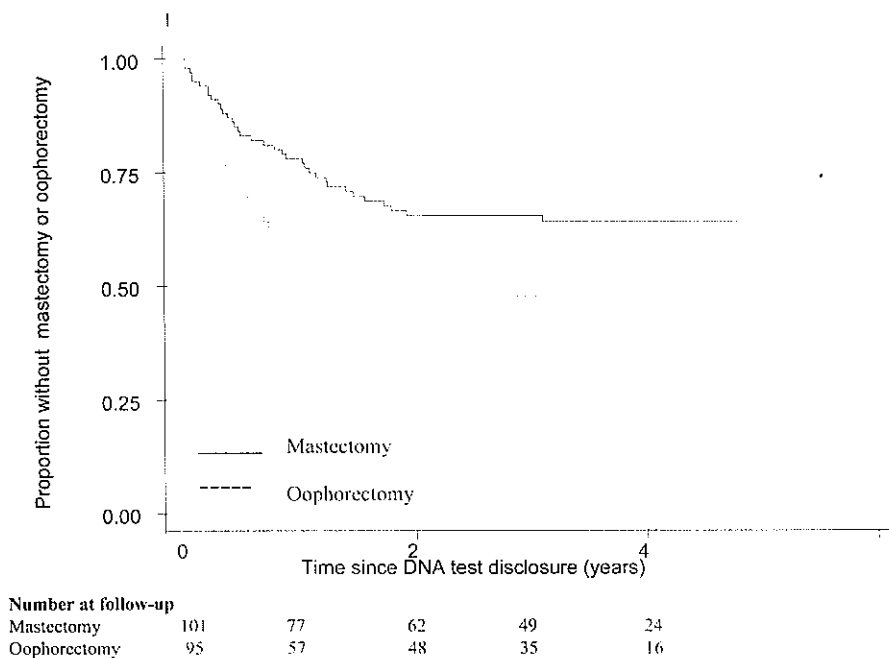
\* Included were all variables with univariate  $P < 0.10$ ; only the variables that remained significant in the final model are shown in the table \*\*Time between (last) cancer diagnosis and personal DNA diagnosis \*\*\* Excluding 4 women with both breast cancer and ovarian cancer

**Table 3** Predictive factors for prophylactic oophorectomy

Variable	Subgroup	With PO		Univariate OR (95% CI)	P
		Number	%		
Age, years	< 50	33/59	56	1.00	0.11
	≥ 50	14/36	39	0.50 (0.22-1.17)	
Parenthood	No children	5/14	36	1.00	0.27
	Children	42/81	52	1.94 (0.60-6.29)	
DNA diagnosis	After cancer	42/87	48	1.00	0.44
	Before cancer	5/8	63	1.78 (0.40-7.94)	
Tumor stage	I	18/28	64	1.00	0.04
Breast cancer	II/III	23/57	40	0.37 (0.15-0.96)	
	unknown	6/10	60	-	
Time interval*	0-2 years	11/22	50	1.00	0.85
	≥ 2 years	31/65	48	0.91 (0.35-2.40)	
Ovarian cancer in family	No	19/42	45	1.00	0.46
	Yes	28/53	53	1.36 (0.60-3.06)	

Abbreviations: PO, prophylactic oophorectomy; OR, Odds Ratio; CI, Confidence Interval

\* Time between (last) cancer diagnosis and personal DNA diagnosis

**Figure 1** Proportion without prophylactic mastectomy or oophorectomy.

Affected women opting for prophylactic mastectomy or prophylactic oophorectomy after *BRCA1/BRCA2* test disclosure.

At a follow-up of 1 and 2 years, 22% and 35% respectively, of eligible women had decided to undergo prophylactic mastectomy; only one woman had the procedure after a period of follow-up of more than 2 years (Figure 1). A similar pattern was observed with respect to the decision about prophylactic oophorectomy. At a follow-up of 1 and 2 years, 40% and 47%, respectively, of eligible women had their ovaries removed (Figure 1). After this period only two women requested this procedure.

## DISCUSSION

Women with breast cancer at a young age or with relatives with breast cancer and/or ovarian cancer may consider genetic testing for *BRCA1/BRCA2* mutations. By this means, their risks of contralateral breast cancer, ipsilateral events, and ovarian cancer can be specified, because *BRCA1/BRCA2* mutation carriers clearly have higher risks [3,4,7]. Women with breast cancer or ovarian cancer may therefore seek genetic testing for personal health management and treatment decisions, apart from concerns about the cancer risks for their children or other relatives.

We systematically evaluated the use of genetic testing, prophylactic mastectomy, and prophylactic oophorectomy in a consecutive series of 112 families with a *BRCA1/BRCA2* mutation. We found that the overwhelming majority of women with breast cancer or ovarian cancer from high-risk families are interested in genetic testing themselves or are willing to undergo genetic testing for the sake of their family members. One third of affected mutation carriers that had a relatively good prognosis decided to undergo prophylactic mastectomy, and half of them decided to undergo prophylactic oophorectomy.

These figures differ from those observed by us in unaffected women from families with a *BRCA1/BRCA2* mutation [17]. The genetic test rate in unaffected women was lower when compared with the rate in women who already had breast and/or ovarian cancer (48% [198 of 411] v 87% [192 of 220], respectively). This may be related to differences in the motives for genetic testing between affected and unaffected women. For affected women, the issue of learning the cancer risks of children and other relatives may play a more prominent role in the decision about genetic testing when compared with that in unaffected women. This may be particularly so for women with metastatic disease, because knowledge on their own mutation status has no major implications for their personal health management. In contrast, in unaffected women the decision about genetic testing may strongly correlate with the wishes these women have on personal health management in case they carry a mutation; in particular, women who consider prophylactic surgery may proceed with genetic testing. Interestingly, we observed a reverse pattern with respect to the decisions about prophylactic surgery. Unaffected women with a *BRCA1* or *BRCA2* mutation more frequently requested prophylactic

mastectomy when compared to their affected counterparts (51% [35 of 68] v 35% [35 of 101], respectively), and a similar trend was observed for prophylactic oophorectomy (unaffected v affected = 64% [29 of 45] v 49% [47 of 95], respectively). These facts may be largely explained by the aforementioned differences in the main motives for genetic testing between unaffected and affected women. From a theoretical point of view, prophylactic surgery is also more advisable to unaffected women because the potential gain in life expectancy by this procedure will be larger in this group. In affected women, the effect of prophylactic surgery on life expectancy inevitably competes with their risk of dying because of metastases of their prior cancer. Likewise, young women who decide to undergo prophylactic surgery are likely to gain more years when compared with the years gained by older women [20]. In our series, for example, young women frequently opted for prophylactic mastectomy.

Although numbers are small, as much as 75% of women who developed breast cancer after the establishment of the genetic diagnosis in their family chose to have a bilateral mastectomy with simultaneous reconstruction at the moment of personal cancer diagnosis. Thus, for women who were just diagnosed with breast cancer without a known *BRCA1/BRCA2* mutation in the family, rapid knowledge on their genetic status may also be important. Recent new strategies in high-throughput biological and genetic investigations pave the way for classifying a breast cancer as a *BRCA1*- or *BRCA2*-related cancer at the time of histological diagnosis [21-24]. In particular, women with breast cancer at a young age and women with a family history of breast cancer and ovarian cancer are at increased risk of carrying a *BRCA1* and *BRCA2* mutation. The prevalence of *BRCA1* and *BRCA2* mutations in women younger than 36 and 50 years with breast cancer is about 10% and 6%, respectively, [25], and may increase to 80% in those who also have a positive family history of breast cancer and ovarian cancer [26].

Not only are the potential reduction of cancer risk and prevention of cancer morbidity by bilateral mastectomy relevant, but the timing of this procedure is also important. Cosmetic results of breast reconstruction may be less optimal after radiation therapy of the breasts [27], which is added routinely to the treatment of breast cancer patients who undergo breast-conserving therapy. Bilateral mastectomy plus breast reconstruction may therefore be a reasonable alternative to breast-conserving therapy at the time of diagnosis of the first primary cancer for some women. The psychological effect of receiving a personal cancer and genetic diagnosis in the same time period has not been addressed yet and warrants close attention. However, we believe that the current state of knowledge and technology mandates that doctors inform and counsel just-diagnosed breast cancer patients at high risk of a *BRCA1/BRCA2* mutation about the oncogenetic issues of their disease and the related available interventions before decisions are made on the type of breast cancer treatment patients will undergo. For those patients who are interested, a rapid genetic diagnosis should

be sought so that their primary treatment for breast cancer also can be tailored toward their genetic status.

Most women opting for prophylactic surgery chose to undergo prophylactic mastectomy and/or oophorectomy within 2 years after their genetic diagnosis. The time-dependent rates of prophylactic mastectomy and/or oophorectomy indicate that most women interested in prophylactic surgery have already come forward during the period of our study. It is therefore unlikely that our reported use of prophylactic surgery will significantly increase over time.

The use of genetic testing and prophylactic surgery in our center differs from that in other countries. Factors such as potential social and financial discrimination and cultural differences in views on prophylactic surgery by patients and their doctors may result in large differences in the use and accessibility of prophylactic mastectomy and oophorectomy [28]. A recent study among United States citizens, for example, revealed that as many as 69% of eligible women opted for *BRCA1/BRCA2* testing when the tests were free of charge, as compared with only 22% who opted for testing when it was not free [29]. Dutch laws prohibit discrimination of gene mutation carriers, whether by health insurance companies or by employers, and all costs of genetic testing, surveillance, and prophylactic surgery are covered by both private and public health insurance companies. Our reported rates of genetic testing and prophylactic surgery are therefore unlikely to be confounded by financial or social constraints.

Prophylactic mastectomy is an irreversible and mutilating intervention, and therefore, issues of regret in women who had a prophylactic mastectomy are a major concern. One study found that 6% (18 of 296) of women with breast cancer who had a prophylactic contralateral mastectomy for any cause expressed regrets regarding their decision [30]. At present there are no psychological follow-up data on affected women who had a prophylactic contralateral mastectomy because of a *BRCA1* or *BRCA2* mutation carrier status. Clearly it is mandatory to monitor the medical and psychological consequences of all interventions in this group of women on the short and long term.

On the basis of current data, it is likely that prophylactic bilateral or contralateral mastectomy is most effective in reducing the risk of a second breast cancer. However, premenopausal prophylactic oophorectomy may be a good alternative for some women because this procedure not only reduces the risk of contralateral breast cancer by 50% but also prevents primary ovarian cancer [13,14,16]. Furthermore, adjuvant tamoxifen (at 20 mg/d for 5 years) not only reduces the risk of contralateral breast cancer in unselected patients by approximately 50% [31] but may also reduce the risk of contralateral breast cancer in *BRCA1/BRCA2* mutation carriers [13]. However, at present, the efficacy of chemoprevention with tamoxifen in both *BRCA1* and *BRCA2* mutation carriers is unclear [32-34]. In this respect it should be noted that *BRCA1*-related breast tumors are frequently estradiol receptor-negative [5].

The prognosis of the first tumor of women with a *BRCA1/BRCA2* mutation may ultimately overshadow the effect of subsequent interventions that aim to prevent second primary cancers. At present it is of utmost importance to establish the actual gains in life expectancy achieved by these interventions in these women. Notwithstanding, even in the absence of a gain in life expectancy, women may benefit from interventions such as prophylactic mastectomy and prophylactic oophorectomy because these interventions may reduce fear for a second primary cancer and/or reduce physical and psychological morbidity that inevitably accompanies the diagnosis of second primary cancers.

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## CHAPTER 5

### **Breast Cancer After Prophylactic Bilateral Mastectomy in Women with a *BRCA1* or *BRCA2* Mutation**

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#### **ABSTRACT**

**Background** Women with a *BRCA1/BRCA2* mutation have a high risk of breast cancer and may choose to undergo prophylactic bilateral total mastectomy. We investigated the efficacy of this procedure in such women.

**Methods** We conducted a prospective study of 139 women with a pathogenic *BRCA1* or *BRCA2* mutation who were enrolled in a breast-cancer surveillance program at the Rotterdam Family Cancer Clinic. At the time of enrollment, none of the women had a history of breast cancer. Seventy-six of these women eventually underwent prophylactic mastectomy, and the other 63 remained under regular surveillance. The effect of mastectomy on the incidence of breast cancer was analyzed by the Cox proportional-hazards method in which mastectomy was modeled as a time-dependent covariate.

**Results** No cases of breast cancers were observed after prophylactic mastectomy after a mean ( $\pm$ SE) follow-up of  $2.9\pm 1.4$  years, whereas 8 breast cancers developed in women under regular surveillance after a mean follow-up periods of  $3.0\pm 1.5$  years ( $P=0.003$ ; hazard ratio, 0; 95 percent confidence interval, 0–0.36). The actuarial mean five-year incidence of breast cancer among all women in the surveillance group was  $17\pm 7$  percent. On the basis of an exponential model, the yearly incidence of breast cancer in this group was 2.5 percent. The observed number of breast cancers in the surveillance group was consistent with the expected number (ratio of observed to expected cases, 1.2; 95 percent confidence interval, 0.4 to 3.7;  $P=0.80$ ).

**Conclusions** In women with a *BRCA1* or *BRCA2* mutation, prophylactic bilateral total mastectomy reduces the incidence of breast cancer at three years of follow-up.

## INTRODUCTION

The identification of the breast cancer susceptibility genes BRCA1 [1] and BRCA2 [2] evoked widespread interest in genetic testing among women at risk for a mutation in these genes [3-4]. We found that 57 percent of women without breast cancer who had a 50 percent chance of carrying a BRCA1 or BRCA2 mutation requested genetic testing [4]. This result indicates the need to determine the efficacy of the various options for reducing the risk of breast cancer and for early detection in women with a BRCA1 or BRCA2 mutation.

Women with a BRCA1 or BRCA2 mutation have a cumulative lifetime risk of invasive breast cancer (up to the age of 70 years) of 55 to 85 percent and of invasive epithelial ovarian cancer of 15 to 65 percent [5,6]. In these women the risk of breast cancer begins to increase near the age of 25 years, and their overall survival once breast cancer does develop is similar to that of age-matched patients with sporadic cases of breast cancer: in both, the 10-year survival rate is about 50 percent [7,8].

Current risk-reduction strategies for women with a BRCA1 or BRCA2 mutation include regular surveillance; prophylactic mastectomy and/or oophorectomy, and chemoprevention [9-11]. In our experience, 50 percent of the mutation carriers have chosen to undergo prophylactic bilateral mastectomy [4]. Until now, however, there have been only retrospective studies of the efficacy of the procedure in women with an increased risk of breast cancer on the basis of the family pedigree and not DNA testing [12].

We investigated the efficacy of prophylactic mastectomy in women with a proven pathogenic BRCA1 or BRCA2 mutation. Because a randomized trial is impossible for ethical reasons, we performed a prospective cohort study of women at a single institution who chose either prophylactic mastectomy or regular surveillance.

## METHODS

### *Study Subjects*

Beginning on January 1, 1992, we studied all women with a BRCA1 or BRCA2 mutation who were being monitored for breast cancer because of familial clustering of breast and/or ovarian cancer at the Daniel den Hoed Cancer Center in Rotterdam, the Netherlands. We included all women who had been given their DNA diagnosis before January 1, 2000. Mutation carriers who developed breast cancer before January 1, 1992 and one woman in whom breast cancer was detected at the first screening were excluded. The date January 1, 1992 was chosen because at that time, a multidisciplinary team at our family cancer clinic took over the care of women at high risk for breast cancer. A total of 139 women fulfilled the criteria. Eventually, 76 of these women chose to undergo prophylactic bilateral mastectomy

before the end of the follow-up period (March 1, 2001), whereas the other 63 women chose to remain under regular surveillance. In all but two women prophylactic mastectomy was performed after the DNA diagnosis was established.

#### *Data Collection and Follow-up*

Information on vital status and the occurrence of cancer was extracted from the women's medical files. All women were regularly monitored at our clinic until March 1, 2001, and were enrolled in clinical research programs approved by our medical ethics committee (protocol DDHK 91-17; updated in 1995). We obtained pathology reports of all mastectomy specimens and of all breast-biopsy specimens from the women who were being monitored. Information on oophorectomy performed for any reason (mostly at our clinic) was obtained from the women themselves and was verified by a review of all medical records. Premenopausal oophorectomy was defined as bilateral oophorectomy before the age of 56 years and was performed prophylactically in the case of 59 women, for benign disease in the case of 1 woman, for ovarian cancer in the case of 7 women, and for cervical cancer in the case of 1 woman (Table 1). No women were lost to follow-up after prophylactic mastectomy. Of the women in the surveillance group, 3 died of ovarian cancer and 2 chose to be monitored at another hospital for practical reasons.

#### *Surgical Techniques and Surveillance*

In all cases a standard, bilateral, simple total mastectomy (including the nipple) was performed by a surgical oncologist at the Daniel den Hoed Cancer Center. In 74 of 76 women, the breasts were reconstructed with silicone prostheses by a plastic surgeon in the same session, followed later by a nipple reconstruction.

According to national guidelines, regular surveillance for breast cancer consists of a monthly breast self-examination, a clinical breast examination every six months, and yearly mammography. Since 1995, magnetic resonance imaging (MRI) has been an option at our clinic for women with mammographically very dense tissue and those with a BRCA1 or BRCA2 mutation. When indicated, ultrasonography with or without fine-needle aspiration was also performed. The age at entry into the surveillance program was generally 25 years or younger in women with relatives in whom breast cancer had been diagnosed before the age of 30 years.

To rule out overt breast cancer at the time of prophylactic mastectomy, any or all of the following were performed no more than 3 months before surgery: a physical breast examination, mammography and/or MRI. After prophylactic mastectomy, the chest wall and regional lymph nodes were examined every 6 months. In most women, computed tomography was performed one year after prophylactic mastectomy.

**Table 1** Characteristics of the women\*

Characteristics	Mastectomy	Surveillance	P value
	Group (N=76)	Group (N=63)	
Age at entry†			0.42
Mean – yr	37.7 ± 7.7	39.5 ± 11.5	
Median – yr	35.8	39.9	
Range – yr	23-58	19-64	
<30 yr – no. (%)	11 (14)	17 (27)	
30-39 yr – no. (%)	39 (51)	17 (27)	
40-49 yr – no. (%)	18 (24)	16 (25)	
≥50 yr – no. (%)	8 (11)	13 (21)	
Premenopausal oophorectomy – no. (%)	44 (58)	24 (38)	0.03
For gynecologic cancer	2	6	
For benign gynecologic disease	1	0	
Prophylaxis	41	8	
Duration of follow-up after prophylactic mastectomy or start of surveillance			0.87
Mean – yr	1.3	–	
Median – yr	0.1-5.7	–	
Range – yr	128	–	
No. of woman-yr			
Mutations – no. (%)			0.42
<i>BRCA1</i>	64 (84)	56 (89)	
<i>BRCA2</i>	12 (16)	7 (11)	
No. of cases of breast cancer after study entry	0	8	

\*Plus-minus values are means ± SE. Premenopausal oophorectomy was defined as bilateral oophorectomy before the age of 56 years.

†The age at entry in the mastectomy group is based on the date of prophylactic mastectomy, and the age at entry in the surveillance group is based on the date on which surveillance was initiated.

### *Analysis of BRCA1 and BRCA2 Mutations and Histologic Examinations*

DNA analysis was performed according to standard procedures [13,15]. BRCA1 and BRCA2 linkage analysis was used until 1994 and 1995, respectively, to identify the presence of hereditary breast cancer; from 1994 to 2000 we used direct mutation analysis. All BRCA1 and BRCA2 mutations were pathogenic, since they resulted in a premature truncation of the BRCA1 or BRCA2 protein.

Mastectomy specimens were examined histologically to rule out the presence of occult breast cancer. From each quadrant of the specimen, microscopical sections from 3 random blocks were examined according to standard procedures.

### *Statistical Analysis*

We used a chi-square test and a t-test to compare the characteristics of the group of 76 women who chose to undergo mastectomy with those of the 63 women who opted to continue being monitored. We used a Cox proportional-hazards model to analyze the effect of prophylactic mastectomy on the incidence of breast cancer, with prophylactic mastectomy included as a time-dependent covariate. To adjust for the potential effect of change in menopausal status, either through premenopausal oophorectomy or through natural menopause (defined as occurring at the of 56 years), we included menopausal status in the model as time-dependent covariate. The women were followed from January 1, 1992, or from the time of the first visit after that date at our clinic until the occurrence of breast cancer or death, the end of follow-up at our clinic, or the end of the study (March 1, 2001). We determined the number of woman-years at risk for breast cancer in various age cohorts in the two groups; in this analysis we included in the surveillance-group data the number of years of surveillance in the women in the mastectomy group before prophylactic mastectomy was performed. The numbers of woman-years at risk were used to calculate the numbers of breast cancer expected on the basis of published estimates for women with a BRCA1 mutation [16]. We calculated 95% confidence intervals assuming a Poisson distribution. We used the method of Kaplan-Meier to calculate the actuarial probability of breast cancer during the surveillance period. We compared these probabilities with the cumulative incidence, assuming that the model was an exponential one with a constant hazard rate, in order to have more stable estimates with longer follow-up.

A two-sided P value of less than 0.05 was considered to indicate statistical significance. All analyses were performed using SPSS and STATA-software.

## RESULTS

### *Characteristics of the women*

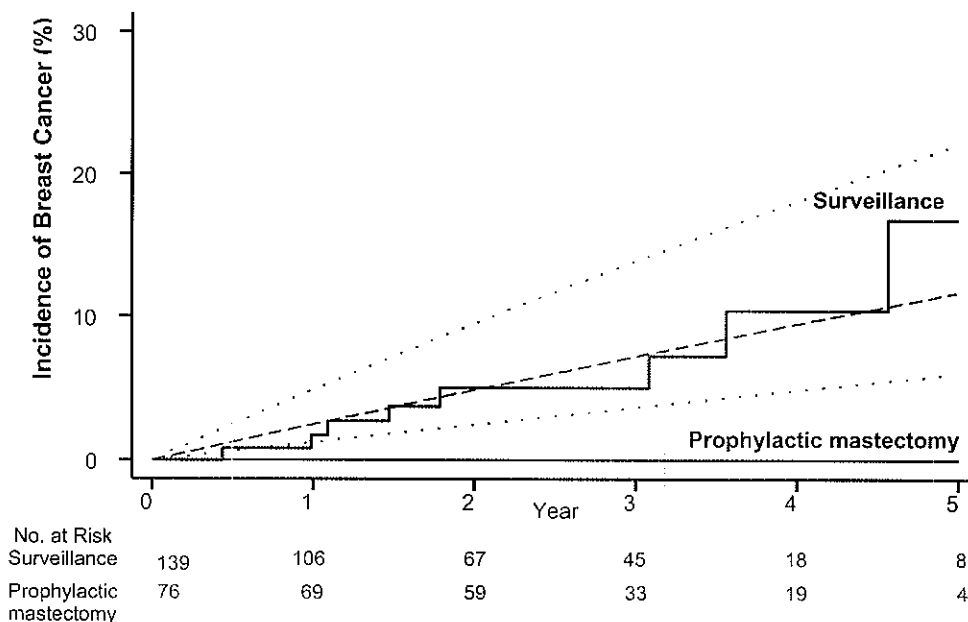
Table 1 lists the general characteristics of the women who chose to undergo prophylactic mastectomy and those who opted for surveillance. Significantly more women in the mastectomy group than in the surveillance group had undergone a premenopausal oophorectomy (44 vs. 24 [58 percent vs. 38 percent],  $P=0.03$ ). All gynecologic cancers occurred before the age of 56 years; the 2 such cases in the mastectomy group were ovarian cancer, stage Ic. There were no significant differences between the two groups with respect to age, average duration of follow-up after entry into the study, follow-up after premenopausal oophorectomy, and type of mutation. The 26 distinct mutations – 23 in BRCA1 and 3 in BRCA2 – were distributed in a similar fashion in the two groups. The 139 women were from a total of 70 families; the number of women from each family ranged from 1 to 5.

The mean ( $\pm$ SE) duration of follow-up was  $2.9\pm 1.4$  years (219 woman-years) in the mastectomy group and  $3.0\pm 1.5$  years (190 woman-years) in the surveillance group (Table 1). The total number of woman-years of surveillance increased from 190 to 318 when the 128 woman-years of surveillance years before prophylactic mastectomy was added.

### *Incidence of Breast Cancer*

After prophylactic mastectomy no case of invasive breast cancer was observed in any of the 76 women during 219 woman-years at risk (Fig. 1). In the surveillance group eight invasive breast cancers were detected during 318 woman-years at risk, for a yearly incidence of 2.5 percent. The observed/expected ratio was 1.2 (8 vs. 6.7; 95 percent confidence interval, 0.4 to 3.7;  $P=0.80$ ). All the affected women were from different families. The actuarial mean 5-year incidence of breast cancer in the women in the surveillance group (Fig. 1) was  $17\pm 7$  percent, but the number of women at risk at 5 years was only 8. To obtain a more stable estimate with longer periods of follow-up, we calculated cumulative incidence probabilities with the use of an exponential model in which the hazard rate was assumed to be constant. According to this model, the yearly incidence of breast cancer was 2.5 percent and the 5-year cumulative incidence was 12 percent (95 percent confidence interval, 6 to 23 percent) (Fig. 1). Disregarding the years of surveillance before prophylactic mastectomy and thus restricting the actuarial analysis to the 63 women in the surveillance group, we estimated that the 5-year risk of breast cancer was  $24\pm 9$  percent.

Cox proportional-hazard analysis showed that mastectomy significantly ( $P=0.003$ ) decreased the incidence of breast cancer (hazard ratio 0; 95 percent confidence interval, 0 to 0.36). After adjustment for the change in menopausal status, the protective effect of mastectomy remained statistically significant ( $P=0.01$ ).



**Figure 1** Actuarial incidence of breast cancer among women with a BRCA1 or BRCA2 mutation after prophylactic mastectomy or during surveillance.

The surveillance group includes data obtained before prophylactic mastectomy in 76 of the 139 women. The dashed line represents the probability of breast cancer during surveillance, and the dotted lines the 95 percent confidence interval. Values were calculated with the use of an exponential model in which the hazard rate was assumed to be constant.

#### *Outcome in the Women with Breast Cancer*

None of the 8 patients in the surveillance group in whom breast cancer developed had been scheduled to undergo prophylactic mastectomy at the time of the diagnosis. The characteristics of the women and the tumors are described in Table 2 and 3, respectively. Patients 7 and 8 underwent bilateral oophorectomy 14 and 12 months, respectively, before the diagnosis of breast cancer. Of the 8 cancers, 4 (in patients 1, 2, 4, and 6) were detected between screening sessions (so-called interval cancers. In these 4 patients the interval from screening to diagnosis was 2 to 5 months. The cancers in the other 4 patients (patients 3, 5, 7, and 8) were detected during a screening session. Patient 1 became symptomatic 8 weeks after her first clinical breast-cancer screening, the results of which were negative. In 4 of the 8 patients, breast cancer was detected before the DNA diagnosis was made.

*Histologic Findings in the Mastectomy Group*

Invasive cancer was not detected in any of the specimens obtained at the time of prophylactic mastectomy. One 44-year-old woman with a BRCA1 mutation had lobular carcinoma in situ.

**Table 2** Characteristics of the eight women in the surveillance group in whom breast cancer developed

Patient no.	Age at diagnosis	Mutation	Prior oophorectomy	Follow-up after diagnosis	Current status
	years			months	
1	23	4284delAG in BRCA1	No	15	NED
2	28	IVS12-1643del3835 in BRCA1	No	41	Deceased
3	39	4284delAG in BRCA1	No	18	NED
4	39	2804delAA in BRCA1	No	33	NED
5	43	IVS12-1643del3835 in BRCA1	No	97	NED
6	44	1129delA in BRCA1	No	25	NED
7	49	3668delA+G3669 in BRCA1	Yes	14	NED
8	53	IVS21-36del510 in BRCA1	Yes	19	NED

NED denotes no evidence of disease

**DISCUSSION**

In this prospective study we assessed the incidence of breast cancer in 139 women with a BRCA1 or BRCA2 mutation who chose to undergo either prophylactic mastectomy or regular surveillance. Whereas breast cancer developed in 8 of 63 women in the surveillance group, no cases of breast cancer occurred among the 76 women who underwent prophylactic mastectomy. The observed number of breast cancers in the group under surveillance is compatible with the reported incidence of breast cancers in women with a BRCA1 or BRCA2 mutation [16]. As compared with the incidence in the surveillance group, the incidence of breast cancer in the prophylactic mastectomy group was significantly reduced ( $P=0.003$ ), but the mean follow-up of three years calls for a cautious interpretation of our results.



**Table 3** Characteristics of the tumors in the eight women in the surveillance group in whom breast cancer developed

Patient No.	Tumor Size	No. of positive nodes/total no. assessed	Histologic type	Grade	Estrogen- and progesterone-receptor status	Interval from start of surveillance to diagnosis	Findings*			
							BSE	CBE	mammography MRI	
1	25, 13 mm	1/15	Ductal	III	Negative	2 months	SC	SC	PB	SC
2	40	2/13	Ductal	III	Negative	12	SC	SC	PB	ND
3	18	0/1 sentinel node	Ductal	III	Negative	31	NA	SC	NA	SC
4	7	3/21	Ductal	III	Negative	10	SC	SC	SC	ND
5	20	6/18	Ductal	III	Negative	23	NA	SC	PB	SC
6	12	0/19	Ductal	III	Negative	35	SC	SC	PB	SC
7	10	0/1 sentinel node	Ductal	I	Negative	42	NA	NA	NA	SC
8	15	0/1 sentinel node	Ductal	III	Positive	22	NA	NA	NA	SC

\*BSE denotes breast self-examination, CBE clinical breast examination, MRI magnetic resonance imaging, SC suspicion of cancer, PB high probability of a benign lesion, ND not done, and NA no abnormalities

Until now, only retrospective studies on the outcome of prophylactic mastectomy (mainly subcutaneous, and thus often incomplete) have been published [12]. Hartmann et al. [17] reported on the results of prophylactic bilateral mastectomy in 639 women with a family history of breast cancer: at least 12 of these women had a BRCA1 or BRCA2 mutation [18]. After a median follow-up of 14 years, there was an approximate 90 percent reduction in the risk of breast cancer; the risk of death was also reduced significantly. All seven breast cancers occurred after subcutaneous bilateral mastectomy; there were none after total mastectomy [17]. Moreover, breast cancer did not develop in any women with a confirmed BRCA1 or BRCA2 mutation after a median follow-up of 16 years [18], which leads us to anticipate that prophylactic mastectomy will reduce the long-term risk of breast cancer in the women with a BRCA1 or BRCA2 mutation whom we studied.

It is uncertain whether mammographic surveillance of premenopausal women with a BRCA1 or BRCA2 mutation contributes substantially to early detection of breast cancers [19]. Considering the women's young age in our study cohort and the stage and pathological characteristics of their breast cancers at diagnosis, we estimate that 35 to 50 percent of women under surveillance in whom primary breast cancer develops will die of distant metastasis within 10 to 15 years [7,8]. Assuming that within 10 years breast cancer will develop in approximately 25 percent of the women undergoing regular surveillance, we estimate that 10 to 20 percent of women who choose surveillance will die of breast cancer within 20 years. During the 3 years of follow-up in our study, there was one death due to breast cancer (Table 2).

Currently, several large, prospective studies are investigating whether MRI screening adds to the efficacy of mammographic screening in women at high risk for breast cancer [20,21]. In our study MRI was performed in 6 women at the time of diagnosis and detected all 6 cancers, but mammography was diagnostic in only 2 of the 8 women with breast cancer. In view of the high number of interval cancers (4 of 8), the use of high-resolution imaging and more frequent screening might be useful in women with a BRCA1 or BRCA2 mutation.

There is little in the literature on histologic findings in specimens obtained at the time of prophylactic mastectomy from women with a BRCA1 or BRCA2 mutation. In 2 studies, in about 25 percent of unaffected high-risk women, proliferative breast disease (marked or atypical hyperplasia) was found in the surgical specimens [22,23]. This abnormality was found in specimens from only 13 percent of women with an average risk of breast cancer [23]. In 2 women with a strong family history of breast cancer, microcalcifications and invasive breast cancer were detected within one year after the finding of proliferative disease [23]. In contralateral specimens obtained at the time of prophylactic mastectomy from women with prior breast cancer and either a genetic risk or a family history of breast cancer, a higher prevalence of malignant lesions was observed [9,22]. In our study, there was one carcinoma

in situ and several prophylactic-mastectomy specimens with various degrees of hyperplasia and atypia. However, we cannot exclude the possibility that small invasive tumors were overlooked. In our study all 8 breast cancers occurred in women with a BRCA1 mutation. This finding may be partly explained by the fact that only about 10 percent of the woman-years of surveillance were accounted for by women with BRCA2 mutations.

Apart from surveillance and prophylactic mastectomy, women with a BRCA1 or BRCA2 mutation may choose to undergo bilateral oophorectomy before menopause, and/or chemoprevention, to reduce the risk of breast cancer. Such interventions may reduce the risk of breast cancer by about 50 percent [24-26], but the use of tamoxifen as a preventive agent has been questioned in view of the long-term side effects [27].

Prophylactic mastectomy is a highly personal decision. In counseling high risk-women, the protective of prophylactic mastectomy must be weighed against possible surgical complications and psychological problems. Up to 30 percent of the women who undergo the procedure will have surgical complications, depending on the type of surgery and the length of follow-up [12,28]. A long-term study of prophylactic mastectomy reported unanticipated repeated operations in 49 percent of women [29], but these results may not be applicable to prophylactic mastectomies as they are currently performed. Psychological studies of women who had undergone a prophylactic mastectomy did not find that, overall, the procedure had detrimental effects on body image and sexuality [30,33].

In conclusion, our data and those of Hartmann et al [17,18] indicate that prophylactic bilateral total mastectomy substantially reduces the incidence of breast cancer among women with a BRCA1 or BRCA2 mutation. Nevertheless, longer follow-up and studies of more patients are required to establish the protective effect and determine the long-term complications of this procedure.

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## CHAPTER 6

### **Are ATM Mutations 7271T→G and IVS10-6T→G Really High-Risk Breast Cancer Susceptibility Alleles?**

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**Identification of high-risk breast cancer susceptibility alleles has significant clinical implications. Recently, the 7271T→G and IVS10-6T→G mutations of the ATM gene were suggested to confer breast cancer risks similar to mutations of BRCA1 or BRCA2. Here, we set out to confirm these findings in 961 non-BRCA1/BRCA2 breast cancer families from diverse geographical regions. We did not detect the ATM 7271T→G mutation in any family. The ATM IVS10-6T→G mutation was detected in 8 families. Mutation positive families all originated from the Netherlands or Austria. The frequency of ATM IVS10-6T→G among Dutch and Austrian non-BRCA1/BRCA2 families was similar to their population-matched control individuals (pooled Mantel-Haenszel odds ratio = 1.60; 95% confidence interval = 0.48 to 5.35;  $P = 0.44$ ). Bayesian analysis of linkage in the ATM IVS10-6T→G positive families showed an overall posterior probability of causality for this mutation of 0.008. We conclude that the ATM IVS10-6T→G mutation does not confer a significantly elevated breast cancer risk and that ATM 7271T→G is a rare event in familial breast cancer.**

Mutations of the high-risk breast cancer susceptibility genes BRCA1 and BRCA2 account for less than half of the breast cancer families with many cases of early onset breast cancer and only about one-quarter of clinically ascertained families [1,2]. It is thus likely that other breast cancer susceptibility genes exist. The ATM gene has been considered a candidate

breast cancer susceptibility gene since the observation of an increased breast cancer incidence in otherwise healthy female relatives of patients with the neurological disorder Ataxia Telangiectasia (A-T) [3]. Biallelic mutations of the ATM gene cause A-T. Notwithstanding a decade of intensive research, controversy still exists about ATM-related breast cancer risks, with estimates ranging from no increase to a 13-fold increase in risk [4-6]. A recent study of Australian breast cancer families suggesting that two A-T related mutations of the ATM gene [5-7] confer a high breast cancer risk [8] has renewed this debate. According to this study, the mutations ATM 7271T→G (also known as T7271G) and ATM IVS10-6T→G confer cumulative breast cancer risks of 55% and 78% by 70 years of age, equivalent to 14- and 26-fold increases in breast cancer risk, respectively [8]. The breast cancer risks of these two ATM mutations would thus compare with those of the high-risk breast cancer genes BRCA1 and BRCA2. We and others have shown that high-risk women often opt for genetic testing for BRCA1 and BRCA2 and, if a mutation is identified, proceed with risk reducing interventions including prophylactic bilateral mastectomy [9,10]. The clinical impact of the reported findings on the two ATM mutations [8] could thus be considerable. We therefore sought to replicate these findings.

We ascertained 961 breast cancer families without a pathogenic mutation of the BRCA1 or BRCA2 genes (further referred to as non-BRCA1/BRCA2 families) through an international collaborative effort by five centers (Table 1). All 961 families had at least two cases of invasive breast cancer in first or second-degree relatives, with at least one of them diagnosed under age 60 years. We also ascertained a series of 211 families in which a pathogenic mutation of the BRCA1 or BRCA2 gene had been identified (further referred to as BRCA1/BRCA2 families). Population controls were ascertained in the Netherlands [11,12] and Austria (Table 1). The ATM 7271T→G mutation was detected by a PCR-based allele-specific oligonucleotide hybridisation assay [11] (Rotterdam), DHPLC (Lyon and Vienna), DGGE (Amsterdam), or fluorescence-based direct sequencing (Québec). The ATM IVS10-6T→G mutation was detected by a mutation-specific *RsaI* restriction endonuclease assay [8] (Rotterdam, Lyon, Amsterdam), DHPLC (Vienna) or fluorescence-based direct sequencing (Québec). For each family, at least the index case was screened for the ATM mutations, defined as the youngest case with invasive breast cancer in the family from whom DNA was available. In non-BRCA1/BRCA2 families with a highly penetrant cancer predisposition pattern, additional breast cancer cases among first and second-degree relatives were also screened. All mutant samples were confirmed by direct sequencing of an independently amplified template. All index cases had been screened for mutations of the BRCA1 and BRCA2 genes by extensive analysis of the complete coding sequence and splice junctions of both genes, using a variety of techniques [11,13,14]. Informed consents to screen for breast cancer susceptibility genes were obtained from all individuals that participated in this study.



**Table 1** Frequencies of the ATM 7271T→G and ATM IVS10-6T→G mutations among controls and index cases of breast cancer families

Sample series	7271T→G	IVS10-6T→G
<b>Control populations</b>	<b>0/275 (0.0%)</b>	<b>4/543 (0.7%)</b>
Rotterdam group <sup>a</sup>	0/184 (0.0%)	1/184 (0.5%)
Amsterdam group <sup>b</sup>		2/268 (0.7%)
Vienna group <sup>c</sup>	0/91 (0.0%)	1/91 (1.1%)
<b>Non-BRCA1/BRCA2 families</b>	<b>0/840 (0.0%)</b>	<b>8/961 (0.8%)</b>
Rotterdam group	0/425 (0.0%)	3/425 (0.7%)
Lyon group	0/209 (0.0%)	0/209 (0.0%)
Amsterdam group	0/76 (0.0%)	3/196 (1.5%)
Vienna group	0/87 (0.0%)	2/87 (2.3%)
Québec group	0/43 (0.0%)	0/44 (0.0%)
Families with ≥ 3 BC cases below 60 years <sup>d,e</sup>	0/373 (0.0%)	2/426 (0.5%)
Families with 1 or 2 BC cases below 60 years <sup>d,f</sup>	0/377 (0.0%)	6/431 (1.4%)
Families with also OC and/or male BC <sup>g</sup>	0/90 (0.0%)	0/104 (0.0%)
<b>BRCA1/BRCA2 families</b>	<b>0/204 (0.0%)</b>	<b>1/211 (0.5%)</b>
Rotterdam group	0/153 (0.0%)	1/153 (0.7%)
Amsterdam group		0/7 (0.0%)
Vienna group	0/51 (0.0%)	0/51 (0.0%)

BC = breast cancer; OC = ovarian cancer

<sup>a</sup>184 healthy individuals (91 women, 93 men) from the Rotterdam area (11)

<sup>b</sup>268 healthy individuals (89 women and 179 men) from the Amsterdam area (12)

<sup>c</sup>91 healthy women over age 65 years from Austria.

<sup>d</sup>Excluded for the presence of ovarian cancer and male breast cancer.

<sup>e</sup>These 426 families included together 2008 breast cancer cases.

<sup>f</sup>These 431 families included together 1193 breast cancer cases.

<sup>g</sup>These 104 families included together 386 breast cancer cases.

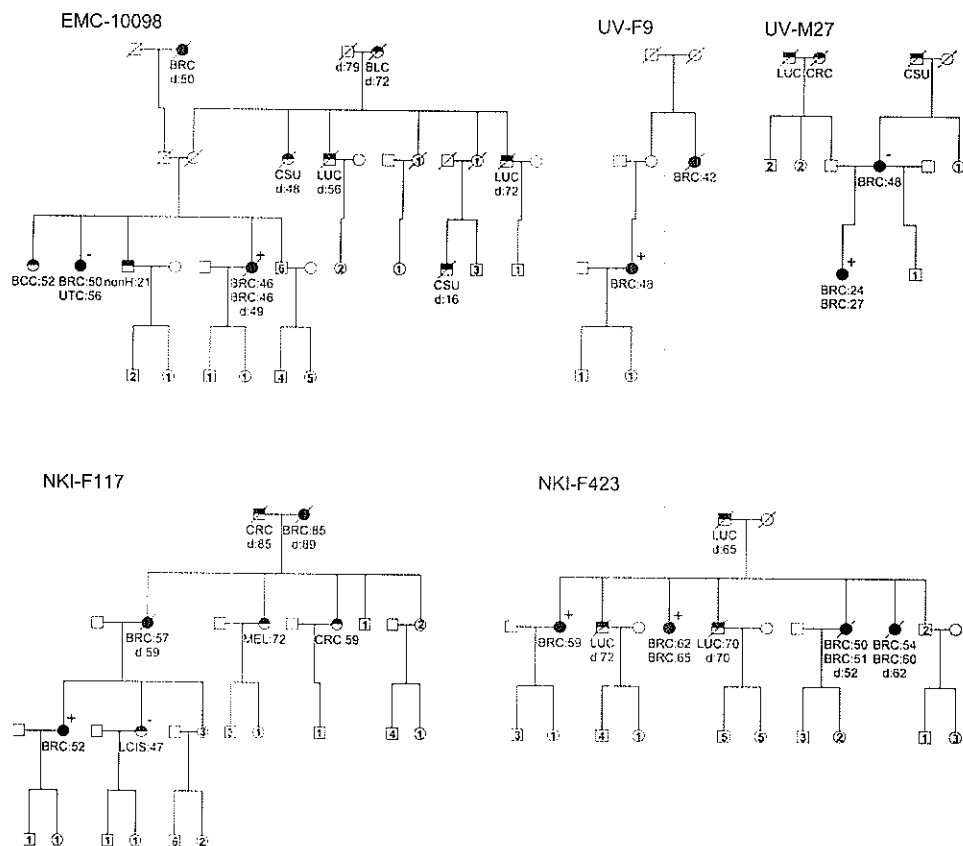
Descriptive statistics were used to determine the frequencies of index cases and control individuals that carried either ATM mutation. Population specific and pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated, using the Mantel-Haenszel estimator to allow for differences in population frequencies. For the ATM mutation positive non-BRCA1/BRCA2 families in which multiple individuals were tested, we calculated the

probability of causality using the Bayesian method of Petersen et al. [15], assuming a prior probability of causality of 0.5 to be comparable to the analysis of Chenevix-Trench et al. [8], and extended the method to incorporate general models of genotype, phenotype (including unaffected individuals), and pedigree structure using a modified version of the program LINKAGE [16]. All statistical tests were two-sided.

The ATM 7271T→G mutation was not detected in any of 1504 tested individuals from 840 non-BRCA1/BRCA2 families (1025 cases tested), 204 BRCA1/BRCA2 families, and 275 control individuals (Table 1), thus precluding any assessment of the breast cancer risk conferred by this mutation in our series.

We detected the ATM IVS10-6T→G mutation in eight of 961 tested non-BRCA1/BRCA2 families (1287 cases tested) and in one of the 211 BRCA1/BRCA2 families. Two of these nine families were ascertained through the University of Vienna and the remaining through Dutch centers (Table 1). The BRCA1 family included four ovarian but no breast cancer cases. The index case, diagnosed with ovarian cancer (age 62), also carried the BRCA1 2138delA mutation. The ATM IVS10-6T→G mutation frequency among Dutch and Austrian non-BRCA1/BRCA2 families was similar to its frequency among population-matched control series (Dutch series: 1.0% versus 0.7%; OR = 1.46; 95% CI = 0.36 to 5.87; Austrian series 2.3% versus 1.1%; OR = 2.12; 95% CI = 0.19 to 23.78) (Table 1). There was no significant frequency variation between the Dutch and Austrian series of non-BRCA1/BRCA2 families, nor between the respective control series. The pooled Mantel-Haenszel OR for the Dutch and Austrian series was 1.60 (95% CI = 0.48 to 5.35;  $P = 0.44$ ), thus providing no evidence for an increased breast cancer risk conferred by ATM IVS10-6T→G. Our findings are consistent with a German study [7], where no difference in carrier frequency of ATM IVS10-6T→G was observed between unselected breast cancer cases (3/500, 0.7%) and control individuals (7/1000, 0.7%).

To further investigate the causality associated with the ATM IVS10-6T→G mutation, we examined statistically its pattern of co-segregation in the five non-BRCA1/BRCA2 families for which multiple individuals were tested (EMC-10098, NKI-F117, NKI-F423, UV-F9, UV-M27, see Fig. 1). Three invasive breast cancer cases, a single case with lobular carcinoma *in situ* (LCIS), five unaffected women, and four men were tested in addition to the index cases. Of the three affected women, one carried the ATM IVS10-6T→G mutation (bilateral disease



**Figure 1.** Pedigrees of ATM IVS10-6T→G positive families in which multiple individuals were tested. Solid symbols = women with invasive breast cancer (BRC). Half-filled symbols = subjects with tumors other than breast cancer (BCC, basal cell carcinoma; BLC, bladder cancer; CSU, cancer site unknown; CRC, colorectal cancer; LCIS, lobular carcinoma *in situ* of the breast; LUC, lung cancer; MEL, melanoma; nonH, non-Hodgkin lymphoma; UTC, uterine cancer). The age at diagnosis follows the cancer type. Likewise, age at death (d) is indicated. (+) indicate carriers and (-) non-carriers of the ATM IVS10-6T→G mutation, respectively. Genetic test results from unaffected individuals are not shown to preserve confidentiality.

at 62 and 65 years; family NKI-F423), whereas the other two did not (diagnoses at 48 and 50 years, families UV-M27 and EMC-10098, respectively). The patient from family EMC-10098 was also diagnosed with uterine cancer at the age of 56 years. The index case of family NKI-F117, who was diagnosed at age 52 years with invasive lobular carcinoma with an LCIS

component, carried the ATM IVS10-6T→G mutation whereas her sister, who was diagnosed with LCIS at age 47 years, did not. Given the increased familial risk reported for LCIS, this observation argues against the ATM IVS10-6T→G mutation being causal in this family [17]. Of the five additionally typed unaffected women, two were shown to be carriers at ages 73 and 59 years, while the others were non-carriers at ages 62, 57 and 25 years. Using the Hazard Ratio of 26 estimated for ATM IVS10-6T→G [8], and age-specific incidence rates in the Netherlands and Austria, the overall evidence for or against this level of risk in these five families was assessed compared with the hypothesis that this mutation is not associated with breast cancer. Based on this analysis, the overall posterior probability of causality for these five families is 0.008 if the case of LCIS in family NKI-F117 is classified as unaffected, and 0.0004 if she is classified as affected with breast cancer. Thus, these five families are 125 to 2500 times more likely under the hypothesis of non-causality for this mutation, again suggesting that the ATM IVS10-6T→G mutation does not confer a significant breast cancer risk.

Our results thus refute those of Chenevix-Trench et al. [8] that the ATM IVS10-6T→G mutation confers a high breast cancer risk. Chenevix-Trench et al. based their estimates of the breast cancer risks conferred by the ATM IVS10-6T→G and ATM 7271T→G mutations on only three families (two and one, respectively), together including 14 breast cancer cases. The total LOD score for linkage of breast cancer to the ATM locus from these three families was 1.18 (odds of 15:1 in favour of linkage), which does not meet conventional criteria for significant linkage. Given the relatively little linkage information per family (LOD scores of 0.14, 0.64 and 0.40), precise estimates of the breast cancer risks conferred by the two mutations could not be derived from their dataset, and hence their Bayes factors should be viewed with caution. Combining the Bayes factors reported in the two Australian ATM IVS10-6T→G families [8] with those of the five families in this report, gives total Bayes factors of 0.04 (LCIS case considered as unaffected) and 0.0025 (LCIS case considered affected). These results imply overall odds of 25:1 and 400:1 against causality, respectively. Based on the published frequency data in breast cancer cases and controls, as well as our data reported here, a much lower prior probability of causality seems justified, resulting in even lower posterior probabilities of causality. The expectation that many of the breast cancer susceptibility alleles yet to be identified will confer low breast cancer risks [2], underlines the need for stringent thresholds of statistical significance, large sample sizes and independent replication before results can be considered convincing [18,19].

In summary, our results do not support an increased breast cancer risk for the ATM IVS10-6T→G mutation, though a slightly increased risk cannot be formally excluded. Neither the ATM IVS10-6T→G mutation nor the ATM 7271T→G mutation is likely to have a

substantial contribution to familial breast cancer. No evidence currently exists that any mutation of the ATM gene confers a high risk of breast cancer [3-8,12,20-25]. The final resolution of the role of ATM as a breast cancer susceptibility gene must await a more comprehensive analysis of the entire coding sequence of the gene in a large series of non-BRCA1/BRCA2 breast cancer families, and subsequent epidemiological evaluation of detected variants. In contrast to others [26,27], we believe that carrier screening in clinical settings for the purpose of breast cancer risk assessment is as yet not indicated for any ATM allele.

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## CHAPTER 7

### Low-Penetrance Susceptibility to Breast Cancer due to CHEK2\*1100delC in Noncarriers of BRCA1 or BRCA2 Mutations

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**Mutations in BRCA1 and BRCA2 confer a high risk of breast and ovarian cancer [1], but account for only a small fraction of breast cancer susceptibility [1,2]. To find additional genes conferring susceptibility to breast cancer, we analyzed CHEK2 (also known as CHK2), which encodes a cell-cycle checkpoint kinase that is implicated in DNA repair processes involving BRCA1 and p53 [refs 3-5]. We show that CHEK2\*1100delC, a truncating variant that abrogates the kinase activity [6], has a frequency of 1.1% in healthy individuals. However, this variant is present in 5.1% of individuals with breast cancer from 718 families that do not carry mutations in BRCA1 or BRCA2 ( $P=0.00000003$ , including 13.5% of individuals from families with male breast cancer ( $P=0.00015$ ). We estimate that the CHEK2\*1100delC variant results in an approximately twofold increase of breast cancer risk in women and a tenfold increase of risk in men. By contrast, the variant confers no increased cancer risk in carriers of BRCA1 or BRCA2 mutations. This suggests that the biological mechanisms underlying the elevated risk of breast cancer in CHEK2 mutation carriers are already subverted in carriers of BRCA1 or BRCA2 mutations, which is consistent with participation of the encoded proteins in the same pathway.**

To investigate breast cancer susceptibility that is not attributable to mutations in BRCA1 or BRCA2, we carried out a genome-wide linkage search in family EUR60, our largest family in which breast cancer susceptibility is not due to either gene. The highest lod score we obtained was 1.2 (maximum possible lod score = 4.7) on chromosome 22q between D22S1150 and D22S928. The haplotype linked to chromosome 22 showed partial segregation with breast cancer (Fig. 1).

The gene CHEK2 is located on chromosome 22q and encodes the human ortholog of yeast Cds1 and Rad53, which are G2 checkpoint kinases [7,8]. Activation of these proteins in response to DNA damage prevents cellular entry into mitosis. In mammalian cells, CHEK2 is activated, through phosphorylation by ATM [8-10], in response to DNA damage induced by ionizing radiation. CHEK2 phosphorylates p53, mediating activation and stabilization of p53 by ATM [3,4]. CHEK2 also phosphorylates Cdc25C, preventing entry into mitosis [7], and associates with, phosphorylates and activates functions of BRCA1 [5].

Germline CHEK2 sequence variants have been reported in families with Li-Fraumeni syndrome that do not carry TP53 mutations [11]. Screening for mutations in CHEK2 is complicated by the presence of many partial copies throughout the genome [12]. However, the mutation 1100delC clearly occurs in the functional copy of CHEK2 and abolishes the kinase activity of the protein [6,13]; thus CHEK2\*1100delC is a plausible candidate for causing cancer predisposition. Mutation screening of CHEK2 in family EUR60 revealed the 1100delC mutation in seven individuals with breast cancer (Fig. 1).





**Table 1** CHEK2\*1100delC in families with breast cancer, individuals with breast cancer unselected for family history and controls

	Positive for CHEK2*1100delC (%)	
	Index cases	All cases
<i>Controls</i>		
UK (UKCCS)	1/292 (0.3%)	
UK (RMHT/ICR)	3/288 (1.0%)	
UK (NWCCGP)	4/230 (1.7%)	
Netherlands (A)	3/184 (1.6%)	
Netherlands (B-ERGO)	6/460 (1.2%)	
North America (Philadelphia)	1/166 (0.6%)	
<b>Total</b>	<b>18/1620 (1.1%)</b>	
<i>Individuals with breast cancer unselected for family history</i>		
UK (UKCCS)	7/557 (1.3%)	
Netherlands (ERGO)	2/79 (2.5%)	
<b>Total</b>	<b>9/636 (1.4%)</b>	
<i>BRCA1/2-negative families with breast cancer<sup>a</sup></i>		
UK	12/211 (5.7%)	25/423 (5.9%)
Netherlands	11/226 (4.9%)	21/334 (6.3%)
North America	6/264 (2.3%)	8/273 (2.9%)
Germany	1/17 (5.9%)	1/41 (2.4%)
<b>Total</b>	<b>30/718 (4.2%)</b>	<b>55/1071 (5.1%)</b>
Families with at least one male with breast cancer <sup>b</sup>	4/33 (12.1%)	7/52 (13.5%)
Families with at least one female with ovarian cancer <sup>c</sup>	4/99 (4.0%)	5/117 (4.3%)
Families with 1 breast cancer case <60	2/93 (2.2%)	2/109 (1.8%)
Families with 2 breast cancer cases <60	7/192 (3.7%)	11/277 (4.0%)
Families with 3 breast cancer cases <60	6/175 (3.4%)	12/294 (4.1%)
Families with 4 breast cancer cases <60	5/84 (6.0%)	9/143 (6.3%)
Families with >4 breast cancer cases <60	3/49 (6.1%)	10/89 (11.2%)
<i>BRCA1/2-positive families with breast cancer<sup>d</sup></i>		
UK	0/52 (0.0%)	0/124 (0.0%)
Netherlands	1/141 (0.7%)	4/203 (2.0%)
North America	0/122 (0.0%)	1/187 (0.6%)
Germany	0/3 (0.0%)	0/6 (0.0%)
BRCA1 +ve	1/215 (0.5%)	5/352 (1.4%)
BRCA2 +ve	0/103 (0.0%)	0/168 (0.0%)
<b>Total</b>	<b>1/318 (0.3%)</b>	<b>5/520 (0.0%)</b>

<sup>a</sup>Refers to families with breast cancer that do not carry BRCA1 or BRCA2 mutations. <sup>b</sup>Eight males with breast cancer were tested, of whom two (from families harboring the CHEK2\*1100delC variant) carried the variant and six (from families without CHEK2\*1100delC) did not carry the variant. <sup>c</sup>Five women with ovarian cancer (from families without CHEK2\*1100delC) were tested in these families; none of these women harbored the variant allele. <sup>d</sup>Refers to families with breast cancer that carry BRCA1 or BRCA2 mutations.

To evaluate the significance of CHEK2\*1100delC in predisposition to breast cancer, we assessed its frequency in families with breast cancer, individuals with breast cancer unselected for family history, and controls. We detected CHEK2\*1100delC in 18 of 1,620 (1.1%) control individuals from the UK, the Netherlands and North America (including Canada) and found no significant frequency variation among the control groups (Table 1). By contrast, 55 of 1,071 (5.1%) individuals with breast cancer from 718 families without BRCA1 or BRCA2 mutations carry CHEK2\*1100delC (Table 1;  $P=0.00000003$ ).

The CHEK2\*1100delC variant is present in 7 of 52 (13.5%) individuals with breast cancer from families without BRCA1 or BRCA2 mutations who had one or more individuals with male breast cancer ( $P=0.00015$  compared with all controls combined,  $P=0.032$  compared with families without BRCA1 or BRCA2 mutations who did not have male breast cancer). The variant was found in 5 of 117 (4.3%) individuals from families without BRCA1 or BRCA2 mutations who had one or more individuals with ovarian cancer ( $P=0.016$  compared with all controls combined,  $P=0.97$  compared with families without BRCA1 or BRCA2 mutations who have female breast cancer only) and in 44 of 912 (4.8%) individuals from families without BRCA1 or BRCA2 mutations who have female breast cancer only ( $P=0.0000002$  compared with controls). Within the latter group, there was evidence of increasing prevalence of the variant, as increasing numbers of individuals were diagnosed with breast cancer before 60 years of age (Table 1;  $P_{\text{trend}}=0.003$ ). The mean age at diagnosis of individuals with breast cancer who harbored the CHEK2\*1100delC mutation (45.4 years) was not significantly different than that of affected individuals who did not carry the mutation (45.1 years).

We assessed linkage of CHEK2\*1100delC to breast cancer in 20 families without BRCA1 or BRCA2 mutations, in which the index case harbored the variant and at least one other individual with breast cancer had been typed. Of 27 additional individuals typed, 16 (59%) carried CHEK2\*1100delC, compared with the 41% that would be expected if the variant were unrelated to breast cancer (estimated relative risk 2.2,  $P=0.049$ ).

We then assessed the frequency of CHEK2\*1100delC in a population-based series of individuals with breast cancer (Table 1). Of 636 cases, 9 (1.4%, 95% CI=0.6-2.7%) carried CHEK2\*1100delC. This frequency did not differ significantly, either from the combined UK/Dutch control series (adjusted odds ratio 1.41, 95% CI=0.59-3.38) or from the control series directly matched to these individuals (odds ratio 2.52, 95% CI= 0.78-8.18). Finally, we assessed the frequency of CHEK2\*1100delC in families with breast cancer that carry BRCA1 or BRCA2 mutations. The frequency of the variant in individuals from families with BRCA1 or BRCA2 mutations (5/520, 1.0%) did not differ from that of control individuals, but was lower than in the families without BRCA1 or BRCA2 mutations (Table 1;  $P=0.002$ ).

We analyzed D22S275, a polymorphic marker within CHEK2, in individuals from 51 pedigrees containing CHEK2\*1100delC. All individuals harboring the variant carried the same allele of this marker, which we estimate has a frequency of 18% and 13%, respectively, in the UK and Dutch populations. This finding suggests that all CHEK2\*1100delC alleles are derived from a common founder.

That the CHEK2\*1100delC variant was found in 5.1% of individuals with breast cancer from families without BRCA1 or BRCA2 mutations, compared with its frequency of 1.1% in the healthy population, indicates that it confers an increased risk of breast cancer. However, the low frequency in the population-based series of individuals with breast cancer indicates that the risk of breast cancer conferred by CHEK2\*1100delC is modest (upper 95% confidence limit is 3.38). This is consistent with the limited segregation of the allele with breast cancer in families without BRCA1 or BRCA2 mutations. The high frequency of CHEK2\*1100delC in families without BRCA1 or BRCA2 mutations that include individuals with male breast cancer indicates that the variant confers a higher relative risk for male than female breast cancer. By contrast, there is no evidence that the frequency of CHEK2\*1100delC is elevated in families with breast and ovarian cancer compared with families having female breast cancer only (although the number studied is small).

The markedly higher frequency of CHEK2\*1100delC in affected families without BRCA1 or BRCA2 mutations, as compared with healthy controls, must in part result from a clustering of cases that is due to the variant conferring an elevated risk of breast cancer. However, it may also reflect an interaction between CHEK2\*1100delC and other (as-yet unidentified) breast cancer predisposition genes in these families. To evaluate this, we used segregation analysis to estimate the relative risk of breast cancer associated with CHEK2\*1100delC. Under a simple model in which the risks conferred by CHEK2\*1100delC and other genes combine multiplicatively, the estimated breast cancer risk ratio associated with CHEK2\*1100delC in families without BRCA1 or BRCA2 mutations was 1.70 (95% CI=1.32-2.20) in females and 10.28 (95% CI=3.54-29.87) in males. Although we did not observe a significant risk associated with CHEK2\*1100delC in the combined UK/Dutch population-based case-control studies, the estimated risk (OR 1.41, 95% CI=0.59-3.38) was of the same magnitude as that found in the family-based analysis, in agreement with a multiplicative model. On the assumption that estimates derived from the affected families without BRCA1 or BRCA2 mutations are applicable at the population level, approximately 1% of female breast cancer incidence, 9% of male breast cancer incidence and 0.5% of the excess breast cancer risk in first-degree relatives of affected individuals is attributable to CHEK2\*1100delC.

In contrast to families with breast cancer that do not carry BRCA1 or BRCA2 mutations, the frequency of CHEK2\*1100delC in affected families with BRCA1 or BRCA2 mutations is

not different from that of controls. Thus, although CHEK2\*1100delC seems to confer an increased risk of breast cancer on the background of some genotypes that show predisposition to breast cancer, the allele does not seem to confer an elevated breast cancer risk in carriers of BRCA1 or BRCA2 mutations. To our knowledge, this is the first example of genes that confer susceptibility to cancer interacting in a manner that is clearly demonstrable at an epidemiological level in humans. It is unlikely that this effect is simply attributable to the high risk of cancer in BRCA1 or BRCA2 mutation carriers leaving no potential for further increase. Most studies estimated the breast cancer risk by age 50 to be no more than 50% in BRCA1 mutation carriers and 30% in BRCA2 mutation carriers [1]. A twofold increase in risk conferred by CHEK2\*1100delC in BRCA1/2 mutation carriers is therefore theoretically possible and would have been detectable in our analyses, if present.

The genetic interaction between CHEK2 and BRCA1 or BRCA2 mutations probably reflects functional interactions among BRCA1, BRCA2, and CHEK2. CHEK2 is regulated by ATM (as is BRCA1) and itself phosphorylates and regulates BRCA1. It is thus plausible that CHEK2 and BRCA1 are components of the same biological pathway. If this pathway is already subverted by inactivating mutations in BRCA1, then abolition of CHEK2 function may confer no demonstrable additional risk of disease (an additive, rather than multiplicative, effect of CHEK2\*1100delC and BRCA1 or BRCA2 mutations, which might be predicted by this model, would not be excluded by our data because it would result in a very small relative risk). The low frequency of CHEK2\*1100delC in families with BRCA2 mutations suggests that a similar functional interaction also exists between BRCA2 and CHEK2.

We have shown that CHEK2\*1100delC is a low-penetrance allele conferring susceptibility to breast cancer. Although many such alleles have previously been suggested [14], this is the first to be confirmed to a high degree of statistical significance. Moreover, our data indicate that CHEK2\*1100delC cannot be a high-penetrance-allele for Li-Fraumeni susceptibility [11,15], as the population prevalence of the variant is approximately 1%, but Li-Fraumeni syndrome is very rare. Our results provide a scientific basis for management of breast cancer susceptibility related to CHEK2\*1100delC in clinical practice. However, the demand for clinical testing of an allele that confers an approximately twofold risk of female breast cancer is unknown. Moreover, the utility of such testing and the contexts in which it is undertaken are currently unclear and will require careful consideration.

## **METHODS**

### *Affected families and individuals, and controls*

We ascertained families with breast cancer through several clinical genetics centers in the UK, the Netherlands, North America (including Canada) and Germany. All families include

at least two cases of female breast cancer in first- or second-degree relatives, or at least one case of female breast cancer and a case of ovarian cancer or male breast cancer in first- or second-degree relatives. We tested two series of individuals with breast cancer unselected for family history: (i) a population-based series of 557 affected individuals diagnosed under age 45, ascertained through the UK Case Control Study of Breast Cancer (UKCCS) as described previously [2] and (ii) a population-based series of 79 affected individuals diagnosed at ages 55 and older, ascertained through the Erasmus Rotterdam Health and the Elderly Study (ERGO). We used six groups of healthy control individuals. Three of these were from the UK: (i) controls from the UKCCS, chosen as age-matched healthy women from the same general practice as the affected individual (n=292); (ii) spouses of siblings of individuals with cancer attending the Royal Marsden Hospital National Health Service Trust (n=288) and (iii) children from the North Cumbria Community Genetics Project from the northwest UK control (n=230), from whom umbilical cord blood was obtained. Two series of control individuals were from the Netherlands: 184 (91 female, 93 male) spouses of individuals heterozygous with respect to cystic fibrosis from the southwest Netherlands; and 460 age-matched controls from the ERGO study. The North American control individuals (n=166) were individuals from the same neighborhood from a breast cancer case-control study in the Philadelphia area, or spouses marrying into families with breast cancer ascertained for linkage analysis from the same area. All studies were approved by local ethical committees or institutional review boards, and all individuals (or, in the case of the cord-blood samples from newborns, their parents) gave full informed consent.

#### *Mutation screening of BRCA1, BRCA2 and CHEK2*

We screened the full coding sequence and splice junctions of BRCA1 and BRCA2 for mutations in at least one individual from every family, either by using heteroduplex analysis (conformation-sensitive gel electrophoresis) or the protein truncation tests for exons 10 and 11 of BRCA2 and exon 11 of BRCA1 and heteroduplex analysis for the remainder of the coding sequence, or by direct sequencing. In addition, we screened families from the Netherlands for the large genomic rearrangements known to be present in this population [16]. We defined families as noncarriers of BRCA1 or BRCA2 mutations if they did not have a mutation clearly associated with breast cancer (such as a truncating mutation or one of the previously described pathogenic missense variants). One family without a detectable BRCA1 or BRCA2 mutation (CRC114) was classified as a BRCA2 carrier family because we found evidence of linkage to chromosome 13q markers flanking the gene (lod score greater than 3). In EUR60, we fully screened individuals 214, 224, 226, 309, 336, 345, 353, 355, 356, 359, 403 and 405 and offspring of 315, 318, 334 and 350 (Fig. 1) for mutations in both genes and for the known Dutch genomic rearrangements. Moreover, analyses of microsatellite markers

flanking BRCA1 and BRCA2 in this family provide evidence against linkage to both loci (lod scores: BRCA1, -1.75; BRCA2, -2.22). We also screened the full coding sequence of CHEK2 for mutations using heteroduplex analysis, first amplifying exons 10-14 in a long-distance PCR to avoid genomic copies of CHEK2 [17].

We detected the 1100delC mutation in CHEK2 of family EUR60 by PCR amplification of exon 10, application of PCR products to nylon filters and hybridization under high stringency of [<sup>32</sup>P] oligonucleotides complementary to CHEK2\*1100delC and the wildtype sequence. Oligonucleotides used for amplification of exon 10 were designed so that the reverse primer had a base mismatch in the most 3' nucleotide compared with sequences from nonfunctional copies; the primers thus preferentially amplified the functional CHEK2 on chromosome 22 rather than nonfunctional copies elsewhere in the genome [17]. PCR primers are available upon request. Every filter contained samples with (positive) and without (negative) CHEK2\*1100delC and was scored independently by at least three individuals. We confirmed all instances of the 1100delC mutation by PCR re-amplification from genomic DNA and direct forward and reverse sequencing of PCR products.

To validate the oligohybridization assay, we analyzed 209 samples by this assay and independently by heteroduplex analysis of a nested PCR product from a chromosome 22-specific template generated by long-distance PCR. Both methods identified 204 negatives and 5 positives (which were separately confirmed for each method by sequencing of newly amplified templates).

#### *Analysis of microsatellite markers*

For the genome-wide linkage search in EUR60, we amplified fluorescently labeled polymorphic microsatellite markers and electrophoresed the products on ABI377 DNA sequencers (Applied Biosystems). Gels were analyzed using the ABI Genescan and Genotyper software. In regions generating lod scores greater than -1, additional markers were end-labeled with [ $\gamma$ -<sup>32</sup>P]ATP, electrophoresed on denaturing polyacrylamide gels and exposed to X-ray film. We analyzed more than 500 markers across the genome and calculated lod scores on the same basis as our previous breast cancer linkage analyses [1] using Vitesse. For analyses of D22S275, we typed individuals with CHEK2\*1100delC from families with and without BRCA1 or BRCA2 mutations, population-based breast cancer cases and controls. To assess the population frequency of the D22S275 allele found in individuals with CHEK2\*1100delC, we typed 360 chromosomes of control individuals from the UK and 54 chromosomes from Dutch controls.

### *Statistical Method*

We evaluated differences in the prevalence of CHEK2\*1100delC in individuals with breast cancer by family type, adjusting for possible differences in population prevalence, using logistic regression with population-specific strata (UK, Netherlands, Germany, North America). As several affected individuals were tested in some families, we used a robust variance approach, implemented in Stata software (v. 7), to account for the dependence between individuals in the same family. We also carried out separate analyses of the prevalence among the index cases (one per family). For those families in whom several individuals had been tested, we defined the index case as the youngest individual with breast cancer who had been tested for both CHEK2 and BRCA1 or BRCA2 mutations. In comparing families with and without BRCA1 or BRCA2 mutations, we excluded individuals in carrier families who did not have the disease-associated mutation. To assess the linkage of CHEK2\*1100delC with disease within families of variant-positive index cases, we computed the probability of each secondary case carrying the variant according to the formula  $\psi/(\psi+2^{r-1})$ , where  $\psi$  is the risk ratio associated with the disease and  $r$  is the degree of relationship. We then constructed a test of the hypothesis that the segregation differed from chance ( $\psi=1$ ) using a pseudo-likelihood approach, using a robust variance estimation to allow for dependence among relative pairs.

To estimate the risk of breast cancer associated with CHEK2\*1100delC, we carried out segregation analysis using the package MENDEL [18]. Parameters estimated were the CHEK2 allele frequency in each population and the breast cancer risk ratio for CHEK2 carriers relative to noncarriers. We computed risks to noncarriers of CHEK2 mutations so that the total risk averaged across all genotypes agreed with national age- and population-specific breast cancer incidence rates, as described in previous segregation analyses [19]. We carried out ascertainment correction by conditioning on the phenotypic and BRCA1 or BRCA2 genotypic data available for each pedigree. Because this model does not explicitly incorporate the effects of other susceptibility genes, it assumes implicitly that the effects of CHEK2 and other genes conferring susceptibility can be regarded as independent, as in a multiplicative model. (For simplicity, we ignored the effect of BRCA1 or BRCA2 mutations in noncarrier families that were missed in the mutation screen. Under the assumption that CHEK2\*1100delC confers no risk in carriers of BRCA1 or BRCA2 mutations, this simplification would imply that our estimate of relative risk in noncarrier individuals is slightly biased towards one). We evaluated goodness of fit of the models by computing the predicted CHEK2 carrier probability for each tested individual, and thus comparing predicted frequency in different categories of family with the observed frequency. All analysis excluded family EUR60, in which the association was initially observed.



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## CHAPTER 8

### The CHEK2 1100delC Mutation Identifies Families with a Hereditary Breast and Colorectal Cancer Phenotype

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**Because of genetic heterogeneity, the identification of breast cancer-susceptibility genes has proven to be exceedingly difficult. Here, we define a new subset of families with breast cancer characterized by the presence of colorectal cancer cases. The 1100delC variant of the cell cycle checkpoint kinase *CHEK2* gene was present in 18% of 55 families with hereditary breast *and* colorectal cancer (HBCC) as compared with 4% of 380 families with non-HBCC ( $P < 0.001$ ), thus providing genetic evidence for the HBCC phenotype. The *CHEK2* 1100delC mutation was, however, not the major predisposing factor for the HBCC phenotype, but appeared to act in synergy with another, as-yet-unknown susceptibility gene(s). The unequivocal definition of the HBCC phenotype opens new avenues to search for this putative HBCC susceptibility gene.**

Cell cycle checkpoint kinase 2 (CHEK2, also known as “CHK2” [MIM 604373], and as “Cds1” in *Schizosaccharomyces pombe* and RAD53 in *Saccharomyces cerevisiae*) is a key mediator in DNA damage-response pathways [Zhou and Elledge 2000; Bartek et al. 2001; Myung and Kolodner 2002; Rouse and Jackson 2002]. In the course of our search for new breast cancer genes, we recently identified the kinase-deficient 1100delC variant of *CHEK2* as a low-penetrance breast cancer-susceptibility allele [Meijers-Heijboer et al. 2002]. The prevalence of the *CHEK2* 1100delC mutation among families with non-*BRCA1/BRCA2* breast

cancer was 4.2% as compared with 1.1% among healthy individuals, implying an estimated twofold increased risk to develop breast cancer for women carrying the mutant allele. Similar results were then reported for Finnish families with breast cancer, thus independently confirming our observations [Vahteristo et al. 2002]. We had noted that several of the families with *CHEK2* 1100delC breast cancer also included colorectal cancer cases, but its significance had been unclear (H.M.-H. & M.S.; unpublished observations). Family EUR60, for example, encompassed six colorectal cancer cases, four of which had been diagnosed before age 50 years, and none could be explained by mutations of the *APC* (MIM 175100), *MLH1* (MIM 120436), *MSH2* (MIM 120435), or *MSH6* (MIM 600678) genes (Figure 1). A subtype of familial breast cancer that includes colorectal cancer had already been recognized by one of us in the early 1970s [Lynch et al. 1972], but evidence for such a phenotype has never been provided. Here, we have evaluated the involvement of the *CHEK2* 1100delC mutation in colorectal-cancer susceptibility.

Families with colorectal cancer were collected through the International Concerted Action Polyp Prevention (CAPP) and the Dutch Foundation for Detection of Hereditary Tumors (STOET). Families with colorectal cancer were classified by clinical and genetic criteria, resulting in two main groups of (i) families with familial adenomatous polyposis (FAP [MIM 175100]), characterized by >100 adenomatous polyps in the colorectum ( $n=91$ ) or >20 polyps in case of attenuated FAP ( $n=4$ ), and (ii) families with hereditary nonpolyposis colorectal cancer (HNPCC [MIM 114500]) or with a phenotype reminiscent of HNPCC ( $n=234$ ), defined by at least two patients with colorectal cancer who were first-degree relatives, of whom at least one had been diagnosed before age 50 years. Pathogenic mutations of the *APC* gene were identified in 61 of 95 families with FAP and of the *MLH1*, *MSH2*, or *MSH6* genes in 127 families with HNPCC (Table 1). Extensive mutational analyses had failed to identify mutations of these genes in the index cases of the remaining 34 families with FAP and 107 families with HNPCC and HNPCC-like disease. Of the 107 mutation-negative families with HNPCC and HNPCC-like disease, 70 met the Amsterdam criteria for HNPCC. Mutational analyses included the complete coding sequences of the *APC*, *MLH1*, *MSH2* and *MSH6* genes, as well as all known Dutch founder mutations and deletions, as described elsewhere [van der Luijt et al. 1997; Wijnen et al. 1997, 1998, 1999]. All families with breast cancer had been clinically ascertained through the Rotterdam family cancer clinic. Families with breast cancer were defined by at least two patients with breast cancer who were first- or second-degree relatives, of whom at least one had been diagnosed before age 60 years. A first cohort of families with non-*BRCA1/BRCA2* breast cancer ( $n=188$ ) was described elsewhere, as part of a study by the International *CHEK2*-Breast Cancer Consortium [Meijers-Heijboer et al. 2002]. Note that we used more stringent inclusion criteria for the current study, resulting in minor differences between the data sets. A second cohort of families with non-

*BRCA1/BRCA2* breast cancer ( $n=247$ ) did not overlap with the first cohort, and the *CHEK2* 1100delC mutation status was unknown prior to this study. Both cohorts of families with breast cancer were excluded for pathogenic mutations of the *BRCA1* (MIM 113705) or *BRCA2* (MIM 600185) genes by mutational analyses of the complete coding sequences of both genes, as well as screening for all known Dutch founder mutations and deletions, as described elsewhere [Petrij-Bosch et al. 1997; Meijers-Heijboer et al. 2002]. Informed consents to search for the cancer-susceptibility genes have been obtained for all families, and all studies have been approved by local medical ethical committees.

**Table 1** Prevalence of the *CHEK2* 1100delC mutation among families with colorectal cancer and families with breast cancer

Cohorts and subgroups	<i>CHEK2</i> 1100delC+/Total tested
<i>Controls<sup>a</sup></i>	18/1620 (1.1%)
<i>Families with colorectal cancer</i>	
Families with FAP	0/95 (0.0%)
Families with HNPCC and HNPCC-like disease	6/234 (2.6%)
<i>MLH1</i> -positive	1/61
<i>MSH2</i> -positive	1/58
<i>MSH6</i> -positive	1/8
non- <i>MLH1/MSH2/MSH6</i>	3/107
<i>Families with non-BRCA1/BRCA2 breast cancer</i>	
Families with HBCC	10/55 (18.2%)
Cohort 1	4/30
Cohort 2	6/25
Families with non-HBCCs	15/380 (4.0%)
Cohort 1	8/158
Cohort 2	7/222

<sup>a</sup>Chapter 7

We determined the prevalence of the *CHEK2* 1100delC mutation in a cohort of 329 families with colorectal cancer (Table 1). DNA from a blood sample of the index case of each family was screened for the *CHEK2* 1100delC mutation by an allele-specific oligo-hybridization assay [Meijers-Heijboer et al. 2002], and all positive samples were confirmed

by direct sequencing of independently amplified templates [Sodha et al 2002]. The *CHEK2* 1100delC mutation was not identified in any of 95 families with FAP. Of the 234 families with HNPCC or HNPCC-like disease, 6 (2.6%) carried the *CHEK2* 1100delC mutation. Mutational analysis of the three main mismatch-repair genes had previously detected a germline mutation of *MLH1*, *MSH2*, or *MSH6* in three of the six families with *CHEK2* 1100delC colorectal cancer but had failed to identify pathogenic sequence variants in the other three families (Table 1). Although the prevalence of the *CHEK2* 1100delC mutation among the families with HNPCC and HNPCC-like disease was somewhat higher than among control subjects, this difference was not significant (2.6% vs. 1.1%; odds ratio (OR) = 2.34; 95% CI = 0.95 - 5.79;  $P = 0.07$ ).

The presence of colorectal cancer cases in some of the families with *CHEK2* 1100delC breast cancer prompted us to further analyze our original cohort of families with breast cancer from the Rotterdam family cancer clinic [Meijers-Heijboer et al., 2002]. In this cohort, the prevalence of the *CHEK2* 1100delC mutation was 6.4% among the families with non-*BRCA1/BRCA2* breast cancer (12 of 188 families; Table 1). We then set to classify the families with breast cancer within this cohort by more stringent clinical criteria that defined a putative hereditary breast *and* colorectal cancer phenotype (HBCC). We define a “family with the HBCC phenotype” as a family with breast cancer characterized by the presence of at least two patients with breast cancer who were first- or second-degree relatives and of whom at least one is diagnosed before age 60 years *and*

1. at least one patient with breast cancer and colorectal cancer diagnosed at any age; or
2. at least one patient with colorectal cancer diagnosed before age 50 years who was a first- or second-degree relative of a patient with breast cancer; or
3. at least two patients with colorectal cancer diagnosed at any age of whom at least one was a first- or second-degree relative of a patient with breast cancer.

(An anamnestic report of colorectal cancer was considered reliable only when the diagnosis had been made after 1960). Of the 188 families with breast cancer, 30 met our clinical criteria for HBCC (Table 1). Four of these 30 (13.3%) families with HBCC carried the *CHEK2* 1100delC mutation, suggesting that the mutant allele indeed identified an HBCC phenotype. Such retrospectively defined criteria are, however, inherently subjective. We therefore applied the HBCC criteria prospectively to another cohort of 247 families with non-*BRCA1/BRCA2* breast cancer from the Rotterdam family cancer clinic. This second cohort of families with breast cancer did not overlap with the first cohort, and the *CHEK2* 1100delC mutation status of the families was unknown. Of the 247 families with breast cancer from this second cohort, 25 met our clinical criteria for HBCC (Table 1). Of these 25 families with HBCC, 6 (24.0%) carried the *CHEK2* 1100delC mutation, as compared with 7 of 222 (3.2%) families with non-

HBCC from this cohort, thereby confirming the strong association of the HBCC phenotype with the *CHEK2* 1100delC mutation.

Identification of a similar phenotype with an increased risk of breast cancer among families with colorectal cancer was not unequivocal. When 'mirror' HBCC criteria were applied to our cohort of families with colorectal cancer, comparable with the HBCC criteria for families with breast cancer families (see list above), 44 of the 234 families with HNPCC and HNPCC-like disease met these criteria. Of these 44 families with HBCC-like colorectal cancer, 2 (4.5%) carried the *CHEK2* 1100delC mutation, as compared with 4 of the 190 (2.1%) remaining families with HNPCC and HNPCC-like disease. Although these data may suggest an 'HBCC-like' phenotype for families with colorectal cancer similar to that of families with HBCC breast cancer, the evidence is circumstantial and awaits further evaluation. We anticipate that the *CHEK2* 1100delC mutation does confer a colorectal cancer risk but that this risk is even lower than its rather modest breast cancer risk of twofold. Substantially larger series of families with HNPCC and HNPCC-like disease would thus be required to reach sufficient statistical power to identify such a low-penetrance colorectal cancer risk.

Altogether, we identified the *CHEK2* 1100delC mutation in 10 of 55 (18.2%) families with HBCC compared with 15 of 380 (4.0%) families with non-HBCC breast cancer (OR = 5.41, 95% CI = 2.29 - 12.8,  $P < 0.001$ ). To evaluate the influence of other parameters thought to associate with the *CHEK2* 1100delC mutation, we performed univariate and multivariate analyses on all 435 families with non-*BRCA1/BRCA2* breast cancer from the two Rotterdam family cohorts (Table 2). Consistent with our previous report elsewhere [Meijers-Heijboer et al. 2002], but in contrast with the Finnish report [Vahteristo et al. 2002], the prevalence of the *CHEK2* 1100delC mutation was increased among families with more than three members with breast cancer diagnosed before age 60 years (11% versus 5%; OR 2.36, 95% CI 0.94 - 5.90,  $P = 0.07$ ). Consistent with both reports [Meijers-Heijboer et al. 2002; Vahteristo et al. 2002], there was no difference in the age at breast cancer diagnosis for the index cases of the families with *CHEK2* 1100delC breast cancer, as compared with the index cases of families without the mutant allele (45.5 vs 45.8 years). The prevalence of the *CHEK2* 1100delC mutation was similar among families with and without patients with bilateral breast cancer (4.4% versus 6.2%). Cases of male breast cancer were not observed in any of the families with *CHEK2* 1100delC breast cancer [Meijers-Heijboer et al. 2002]. No significant differences between the families with HBCC and the non-HBCC breast cancer were observed for any of the parameters, except for the prevalence of the *CHEK2* 1100delC mutation (Table 2). The association of the *CHEK2* 1100delC mutation with the HBCC phenotype remained strong after correction for the number of breast cancer cases diagnosed before age 60 years

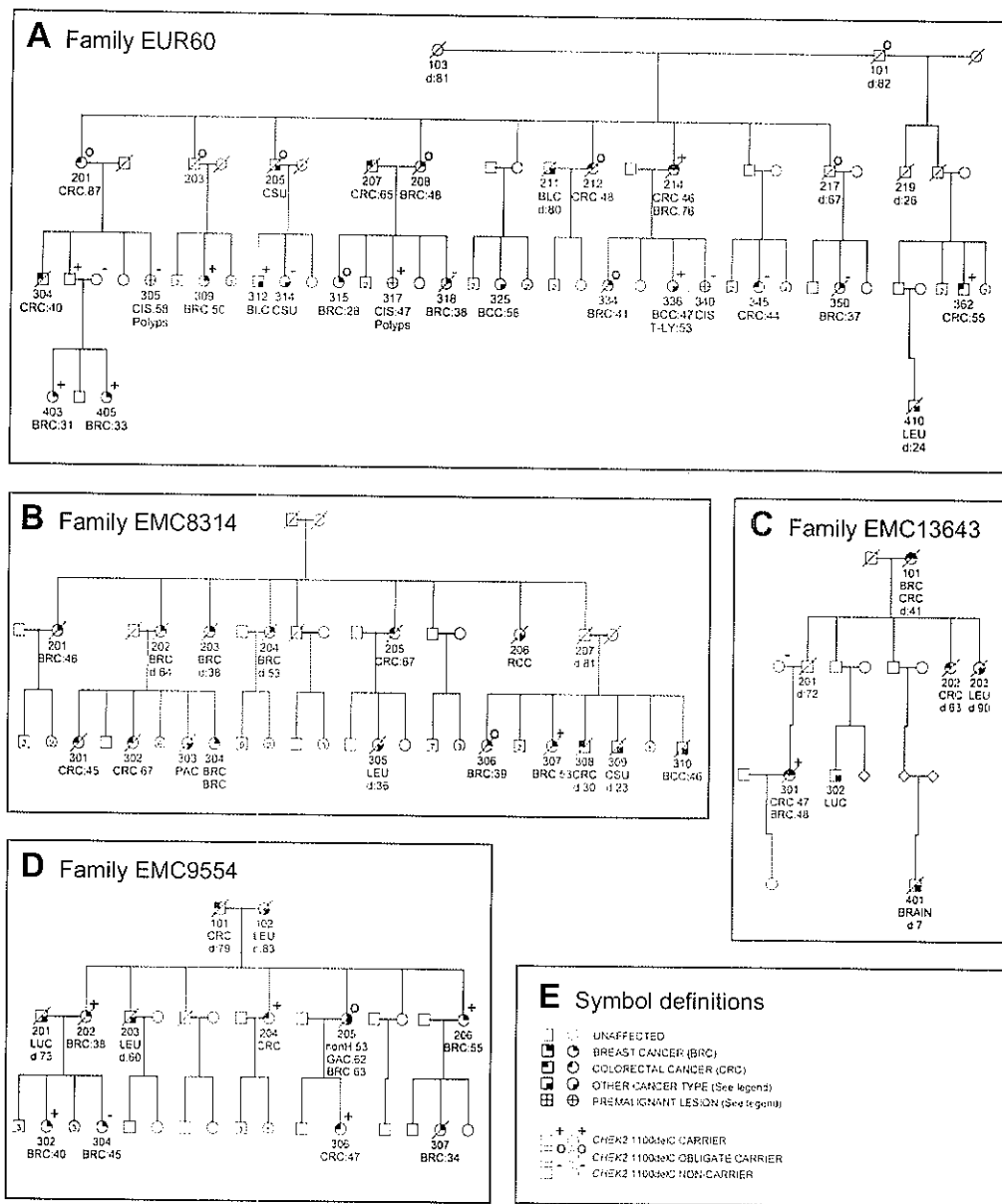
**Table 2** Particulars of the families with non-*BRCA1/BRCA2* breast cancer

	Finding in families with breast cancer						<i>P</i>
	With <i>CHEK2</i> 1100delC (%)			Without <i>CHEK2</i> 1100delC (%)			
	HBCC	non-HBCC	All	HBCC	non-HBCC	All	
Average age at diagnosis of index cases	43.8	46.6	45.5	46.7	45.8	45.8	0.87 <sup>a</sup>
Families including bilateral BRC cases	3 (30)	2 (13)	5 (20)	13 (29)	96 (26)	109 (27)	0.45 <sup>a</sup>
Number of BRC cases in the family							
1 case diagnosed <60y	1 (10)	5 (33)	6 (24)	14 (31)	84 (23)	98 (24)	
2 cases diagnosed <60y	3 (30)	5 (33)	8 (32)	17 (38)	151 (41)	168 (41)	
3 cases diagnosed <60y	2 (20)	2 (13)	4 (16)	6 (13)	80 (22)	86 (21)	
>3 cases diagnosed <60y	4 (40)	3 (20)	7 (28)	8 (18)	50 (14)	58 (14)	0.29 <sup>a</sup>
Total number of breast cancer families	10 (40)	15	25	45 (11)	365	410	<0.001 <sup>b</sup>

<sup>a</sup> *P* value for the difference between all families with *CHEK2* 1100delC-positive and *CHEK2* 1100delC-negative breast cancer.

<sup>b</sup> *P* value for the difference between all families with *CHEK2* 1100delC-positive and *CHEK2* 1100delC-negative HBCC.





**Figure 1** Abridged pedigrees of families with HBOC breast cancer who carry the *CHEK2* 1100delC mutation. Tumor type and age at diagnosis of the tumors are indicated below the individual identifiers. When known, the age of death (d:) is indicated below the tumor type for those cases where the age at diagnosis was unknown. Data from unaffected individuals who are not obligate *CHEK2* 1100delC mutation carriers are omitted to preserve confidentiality. For simplicity, unaffected family members of the youngest generations are also omitted. Abbreviations for the various tumor types: BCC = basal cell carcinoma; BLC = bladder cancer; BRAIN = brain cancer, BRC = breast cancer; CIS = carcinoma *in situ* of the breast; CRC = colorectal cancer; CSU = cancer site unknown; GAC =

gastric cancer; LEU = leukemia; LUC = lung cancer; nonH = non-Hodgkin lymphoma; PAC = pancreatic cancer; Polyps = adenomatous polyps in the colorectum; RCC = renal cell carcinoma; T-LY = T-cell lymphoma. Note that several individuals affected with early-onset cancer are noncarriers of the *CHEK2* 1100delC mutation, illustrating incomplete cosegregation (A and C). The high penetrant cancer- predisposition pattern among all four families contrasts the estimated twofold breast cancer risk associated with the *CHEK2* 1100delC mutation, supporting synergism of *CHEK2* with the putative HBCC-susceptibility gene(s).

and the presence of bilateral breast cancer cases in the family (multivariate OR = 5.19, 95% CI 2.17-12.4,  $P < 0.001$ ). The *CHEK2* 1100delC mutation thus provided conclusive genetic evidence for the existence of an HBCC subtype of familial breast cancer.

We identified 55 families with HBCC in a series of 435 families with non-*BRCA1/BRCA2* breast cancer from the Rotterdam family cancer clinic, representing 13% of the total (Table 1). Examples of pedigrees with HBCC are shown in figure 1. Of the 55 families with HBCC, 17 (31%) had been included by the first HBCC criterion, 7 (13%) by the second criterion, and 21 (38%) by the third criterion (see list above). Ten (18%) families met multiple HBCC criteria, and five of these carried the *CHEK2* 1100delC mutation. Forty-five families with HBCC also included cancers from other anatomical sites than the mammary glands or colorectum, with an average of almost three cases per family (altogether 129 other cancers, excluding basal cell carcinomas). None of these 45 families with HBCC had a cancer pattern reminiscent of the Li-Fraumeni syndrome (LFS [MIM 151623]) [Bell et al. 1999].

We assessed cosegregation of the *CHEK2* 1100delC genotype with the disease phenotype for nine informative families with breast cancer from the two Rotterdam family cohorts. Cosegregation was incomplete for five of these nine families. Among first- and second-degree relatives of the index patients, only 7 of 13 (54%) typed patients with breast cancer carried the *CHEK2* 1100delC mutation. The age at breast cancer diagnosis was similar for the mutation carriers and the noncarriers (52.7 vs 56.8 years;  $P = 0.63$ ), and double tumors were not observed among these 13 additionally typed patients with breast cancer. When colorectal cancer was considered to be part of the phenotype, 9 of 16 (56%) patients carried the mutant allele. For comparison, we observed cosegregation of the family-specific mutation with the disease phenotype for 86% of additionally typed patients with breast and ovarian cancer from families with *BRCA1* and *BRCA2* mutations [Meijers-Heijboer et al., in press], indicating that the incomplete cosegregation of the *CHEK2* 1100delC mutation could not be explained just by the presence of sporadic breast or colorectal cancer cases in the families. Cosegregation was also incomplete for all three informative families with *CHEK2* 1100delC colorectal cancer, where none of five additionally typed patients with colorectal cancer carried the

mutant allele. Three of the six families with *CHEK2* 1100delC colorectal cancer also carried a pathogenic mutation of a mismatch-repair gene. In the family with *MLH1* HNPCC, three patients with colorectal cancer were carrier of the *MLH1* mutation, two were obligate carriers, and none were known to be noncarrier of the *MLH1* mutation. Two patients with *MLH1* colorectal cancer were available for typing. One of these also carried the *CHEK2* 1100delC mutation and was diagnosed with colorectal cancer at age 34 years and with endometrial cancer at age 55 years. The other patient was a noncarrier of the *CHEK2* 1100delC mutation and was diagnosed with colorectal cancer at age 52 years. In the family with *MSH2* HNPCC, one patient with colorectal cancer was diagnosed at age 30 years and was carrier of both the *MSH2* mutation and the *CHEK2* 1100delC mutation. Another patient with colorectal cancer from this family was diagnosed at age 37 years and was a noncarrier of the *MSH2* mutation but was not available for *CHEK2* 1100delC typing. In the family with *MSH6* HNPCC, two patients with colorectal cancer were carriers of the *MSH6* mutation, one was an obligate carrier, and none were known to be noncarriers of the *MSH6* mutation. Of the two *MSH6* mutation carriers, one was diagnosed with colorectal cancer at age 65 years and also carried the *CHEK2* 1100delC mutation. The other *MSH6* mutation carrier was diagnosed with colorectal cancer at age 45 years and with endometrial cancer at age 54 years, but did not carry the *CHEK2* 1100delC mutation.

The *CHEK2* 1100delC mutation is an unusual cancer susceptibility allele, in that not all patients with breast or colorectal cancer from the families with the *CHEK2* 1100delC mutation carry the mutant allele, even though the mutant allele was significantly associated with their familial clustering of breast and colorectal cancer [Meijers-Heijboer et al. 2002; Vahteristo et al. 2002; the present study. We hypothesize that the *CHEK2* 1100delC mutation acts in synergy with another, as yet unknown, cancer susceptibility gene or genes. Thus, the estimated twofold increase in breast cancer risk for *CHEK2* 1100delC mutation carriers [Meijers-Heijboer et al. 2002] represents a *surplus* to the cancer risk among the families with *CHEK2* 1100delC that is due to the unknown susceptibility gene. Considering the generally high-penetrant cancer-predisposition pattern among the families with the *CHEK2* 1100delC mutation (Figure 1), the unknown susceptibility gene would appear to be at least moderately penetrant, or low penetrant in a more complex polygenic model. If this is true, one may comprehend that the *CHEK2* 1100delC mutation tends to associate with the more severely affected families with breast cancer [Meijers-Heijboer et al. 2002; the present study], even though the increased breast cancer risk conferred by the *CHEK2* 1100delC mutation is estimated to be only a modest twofold. Also, the *CHEK2* 1100delC mutation would not completely cosegregate but merely associate with the cancer phenotype, since it confers only a *surplus* of cancer risk. Two recent reports suggested a synergistic role of *CHEK2* at the intra-S phase checkpoint of the cell division cycle. Using a variety of human cells defective in

DNA damage-response proteins, it was shown that ATM-dependent radio-sensitive DNA synthesis (RDS) diverges via the CHEK2-CDC25A-CDK2 pathway and the MRE11-RAD50-NBS1 pathway. Whereas each of these pathways induced a partial replication block upon ionizing radiation, complete inhibition of RDS was achieved only by concerted action of both pathways [Falck et al. 2002] (ATM [MIM 208900], CDC25A [MIM 116947], CDK2 [MIM 116953], MRE11 [MIM 600814], RAD50 [MIM 604040], and NBS1 [MIM 602667]). In *S. cerevisiae*, mutation of the *CHEK2* homologue *RAD53* caused a modest increase in the rate of spontaneous gross chromosomal rearrangements (GCR), whereas double mutants of *RAD53* and *TEL1* (*hATM*) had a highly synergistic effect on the GCR rate [Myung et al. 2001]. Perhaps the putative HBCC-susceptibility gene should be looked for among candidates that are known to function in suppression of genome instability at the intra-S phase checkpoint of the cell cycle.

## ELECTRONIC DATABASE INFORMATION

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for APC [MIM 175100], ATM [MIM 208900], BRCA1 [MIM 113705], BRCA2 [MIM 600185], CDC25A [MIM 116947], CDK2 [MIM 116953], CHEK2 [MIM 604373], FAP [MIM 175100], HNPCC [MIM 114500], LFS [MIM 151623], MLH1 [MIM 120436], MRE11 [MIM 600814], MSH2 [MIM 120435], MSH6 [MIM 600678], NBS1 [MIM 602667], and RAD50 [MIM 604040]).

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## CHAPTER 9

### GENERAL DISCUSSION

In Chapters 2 through 5 we provided evidence that high-risk breast cancer families appreciate the opportunity of genetic testing and the possible preventive measures. In comparison with a presymptomatic DNA test for an incurable late onset disease such as Huntington's chorea, the uptake of genetic testing is much higher for breast cancer susceptibility genes (60% versus 15-20%)<sup>375</sup> but lower than for the inherited disease hypercholesterolemia which is easier amenable (90%).<sup>376</sup> Apparently the options for intervention and their acceptability are the main determinants for the choice for presymptomatic genetic testing.

Some healthcare professionals still consider prophylactic mastectomy controversial though the efficacy of the procedure has now been established (Chapter 5).<sup>15,20,21</sup> Mastectomy is an irreversible and mutilating intervention affecting body image and sexual relations. Still, I believe that this procedure should be offered to all women at very high risk of breast cancer in a non-directive way in view of the current lack of reliable and equally effective alternatives. Personal opinions of individual doctors should not obstruct this. Reversely, doctors should not put pressure towards prophylactic mastectomy as the procedure may cause psychological harm in women who proceed with the procedure under these circumstances.<sup>367</sup> It is reassuring that women who had the procedure after adequate counseling showed no significant psychological damage but instead showed often psychological benefit.<sup>366,367,369,371</sup> It should be realized that a large proportion of women at increased genetic risk are highly motivated to prevention by sad experiences with close relatives, sometimes in different generations (Chapters 3 and 4). The demand for prophylactic mastectomy will continue to emerge as long as no better alternatives are developed. Prophylactic bilateral salpingo-oophorectomy can be offered to BRCA1 and BRCA2 mutation carriers more directive, particularly at postmenopausal ages in view of its medical benefit and limited side effects.<sup>17,18</sup>

Family history is extremely helpful in selecting women at high risk of breast cancer (Chapter 2). At present the standard of breast cancer related care should include taking family history. According to Federal and State rulings in the United States, failure to inquire constitutes 'negligence, an assignable fault, and not just an error judgment or mistake', and may result in assignment of liability to clinicians.<sup>377</sup>

Currently DNA testing reveals a gene mutation in a quarter of clinically ascertained breast cancer families (Chapter 2). Though the exclusion of mutations of BRCA1 and BRCA2 within a family is of clinical and psychological importance, questions about the cause of the familial cancer then remain. Apart from finding new breast cancer susceptibility genes it is important to establish the pathogenic potential of sequence variants of BRCA1 and BRCA2 of

unknown significance. A significant fraction of the unresolved breast cancer families show such unclassified variants, and the effect of some of them might equal that of protein disrupting mutations of these genes. Segregation analyses, functional assays, and/or molecular analyses of tumors will hopefully provide answers regarding the effect of unclassified variants of BRCA1 and BRCA2 in the near future.

Knowledge of the BRCA1 and BRCA2 mutation status is of importance for the choice of treatment in newly diagnosed breast cancer patients (Chapter 4). A 'signature' with regard to both prognosis and BRCA1 and BRCA2 mutation status can now rapidly be obtained using expression profiling of small amounts of tumor tissue.<sup>40,259,260</sup> At present this type of diagnostics costs about € 1500 per profile, but costs will diminish over time. We showed that mutation carriers with a newly diagnosed breast cancer prefer bilateral mastectomy with simultaneous breast reconstruction as primary breast cancer treatment instead of lumpectomy with subsequent radiotherapy in view of their increased risks of ipsilateral and contralateral breast cancer (Chapter 4). In the near future I therefore recommend to offer routinely to every newly diagnosed woman below age 50 years with a close relative with breast cancer or ovarian cancer this expression profiling diagnostics before decisions are made about treatment. Since this type of diagnostics involves information about the hereditary nature of the disease and hence information about close relatives, the ethical standard of informed consent and pre-test genetic counseling must be upheld. For the Netherlands this would imply that annually about an additional thousand breast cancer patients need to be counseled. If all these women would opt for expression profiling of their tumor, about 150 of them would be detected as a BRCA1 or BRCA2 associated tumor. Genetic services should be adapted accordingly.

We describe the discovery of a low-risk breast cancer predisposing gene in Chapter 7. The CHEK2 1100delC allele only confers a twofold increase in breast cancer risk per se, but the cancer risk associated with the mutation appears to multiply in the presence of other hitherto unknown risk factors (Chapters 7 and 8). This finding fits with a polygenic model of breast cancer susceptibility.<sup>116,117</sup> We further showed that CHEK2 1100delC associates with families with a complex pattern of breast and colorectal cancer (HBC: Chapter 8). But how do these discoveries help breast cancer families? At present the clinical implications of the identification of the low-risk CHEK2 1100delC mutation are small. This may change however when CHEK2 1100delC appears to have prognostic and/or predictive significance in cancer patients, and/or when the accuracy of cancer risk estimates in carriers and noncarriers of the mutation improves due to the identification of the additional risk factors thought to be present within these families. Presymptomatic genetic testing for CHEK2 1100delC is at present not justified in view of the lack of certainty about cancer risks in carriers and noncarriers of the mutation. Importantly, our identification of a combined hereditary breast



and colorectal cancer phenotype should alert doctors for a possible common hereditary cause of these cancers within families with both tumor types and clinical surveillance should be adapted accordingly.

Genetic testing for low-risk alleles such as CHEK2 1100delC might become useful once a significant number of such alleles will be identified and their effects on risk will be established. Such information may then be valuable not only for members of breast cancer families but also for the general population. It has been estimated that when half of the genes involved in breast cancer susceptibility will be typed, useful discrimination of risk groups might be possible among the general population.<sup>122</sup> Then half of the population at highest risk would account for 80% of all breast cancer patients. It is conceivable that breast cancer risk assessment by such genetic risk profiling will become standard medical care for all women from the age of 40 years onward. About 3 million of a total of 18 million inhabitants in the Netherlands are women aged 40 to 75 years.<sup>32</sup> Selection of the half at highest risk would not only allow for their optimal surveillance and/or other risk-reducing interventions but would also relieve a large group of women from unwarranted screening programs for the rest of their lives. Such an approach would likely improve the benefit-harm and cost-effectiveness ratios of breast cancer screening programs. At present it is unclear whether genetic risk profiling for breast cancer risk assessment in the general population would be socially acceptable. In fact, risk assessment by genetic risk profiling may become feasible for several common late-onset diseases, such as cardiovascular disease and diabetes and cancers at other sites. Some have applauded an era of population genetic testing<sup>378</sup> but others are skeptical about its application in medicine<sup>379</sup> or in breast cancer specifically.<sup>136</sup> If indeed the goal of identifying the majority of breast cancer susceptibility alleles will be achieved, major medical, social and ethical issues will have to be addressed before genetic risk profiling at the scale of populations can be introduced.

Finding new breast cancer susceptibility genes has been proven to be difficult. The identification of new genes may be facilitated by unraveling the genetic heterogeneity among breast cancer families. This may be achieved by selecting the families under study for specific familial, patient or tumor characteristics. We found that the CHEK2 1100delC mutation identifies families with a complex pattern of both breast cancer and colorectal cancer (HBCC; Chapter 8). Selecting for HBCC and/or CHEK2 1100delC may assist the identification of additional cancer susceptibility genes that are likely present within these families. Also, searching for associations of breast cancer with cancer at other sites than the ovaries and colorectum in the hitherto unresolved breast cancer families is worthwhile as they may represent single gene disorders.

Considering the wide implications of the identification of a high-risk allele for an individual, new high-risk breast cancer alleles should only be presented in the scientific

literature as *beyond doubt* in case of robust statistics; in addition independent replication of results is required. Two mutations of the ATM gene were recently presented as unambiguous breast cancer alleles with associated risks comparable to BRCA1 and BRCA2 mutations.<sup>3</sup> We demonstrated that one of these mutations impossibly confers a significant breast cancer risk per se, whereas the other mutation had a prevalence of less than 0.1% among breast cancer families and thus in any case has a limited clinical impact (Chapter 6). The original erroneous findings<sup>3</sup> likely resulted from a too small data.

Genetic mutations in cancer cells may provide new targets for therapy. A proportion of breast cancer patients, for example, are currently treated with Herceptin. Herceptin is a blocking antibody directed at HER2/neu, a transmembrane tyrosine kinase growth factor receptor that is amplified in approximately 30% of breast cancers.<sup>380</sup> Another example of molecular targeting is ST1571, an Abl kinase inhibitor that targets the fusion protein that is aberrantly formed by a t(9;22) chromosome translocation in bone-marrow cells of patients with chronic myelocytic leukemia.<sup>381,382</sup> ST1571 is also a potent inhibitor of the Kit receptor tyrosine kinase, and has demonstrated therapeutic value in patients with the Kit-driven gastrointestinal stromal tumor.<sup>383</sup> For BRCA1 and BRCA2, it has been shown that tumor cells deficient of these proteins are markedly sensitive to agents inducing DNA damage *in vitro*.<sup>283</sup> Indeed, some evidence exists that patients with BRCA1 or BRCA2 mutations have a better outcome upon chemotherapeutical treatment that often induces double-strand DNA breaks when compared to noncarriers.<sup>282,284</sup> Noteworthy, CHEK2 is also implicated in DNA damage response pathways. Studies on the prognosis of patients with CHEK2 1100delC associated breast cancer and the outcome upon chemotherapeutical treatment specifically are under way.

The identification of genes predisposing to breast cancer is an essential step towards understanding the molecular events underlying tumorigenesis and is critical for the clinical management of affected families. The development of high throughput molecular and biological technologies and the increasing knowledge about the human genome sequence<sup>139,384-386</sup> suggest that the upcoming decade is likely to surpass the last decade with respect to breakthroughs in breast cancer genetics. Members of breast cancer families undoubtedly will be among the first to benefit from such progress.

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## SUMMARY

*Chapter 1* is a general introduction to the research described. Breast cancer is a major health problem in women in Europe and the United States. In 1998 10317 new invasive breast cancers were diagnosed in the Netherlands. One-third of these patients will die of metastases of the primary tumor within 10-15 years from diagnosis (3542 breast cancer deaths in 1998 in the Netherlands).

Breasts are specialized sweat glands. They represent the classifying organ for mammalian vertebrates. A breast consists of about 15 duct systems that drain separately to the nipple. Each duct system is subdivided into lobules, the functional units of the gland. Breast cancers usually arise in the glandular epithelium of the terminal duct and units. Therefore cancers that arise in the breast are mostly adenocarcinomas. The carcinomas are graded according the criteria of Bloom and Richardson (a) degree of glandular differentiation, b) degree of nuclear atypia and c) mitotic index). Breast cancer patients are classified in 4 stages according the TNM classification (Tumor size, metastases in regional lymph Node and distant Metastases).

Treatment modalities offered to a newly diagnosed breast cancer patient are determined by the expected prognosis and treatment outcome. The tumors are generally surgically removed, followed by radiation therapy in case of breast conserving therapy. For clinical staging surgery of the tumors is usually accompanied by removal of the sentinel node or all the lymph nodes in the ipsilateral axillary fat tissue. Patients receive adjuvant endocrine and/or chemotherapy depending on age, tumor grade, stage and hormone receptor status.

The cumulative lifetime risk of breast cancer for Dutch women is 9%. One of the major risk factors for breast cancer is a positive family history. The increase in breast cancer risk for female first-degree relatives of breast cancer patients is about two-fold averaged across all ages. During the nineties of the 20<sup>th</sup> century two major breast cancer susceptibility genes, BRCA1 and BRCA2, were identified. Mutations of BRCA1 and BRCA2 account for about 16% of breast cancer susceptibility. Until now intensive large scale international efforts did not lead to the identification of additional high-risk breast cancer susceptibility genes. Recently a polygenic model for breast cancer susceptibility was introduced. This model implies that the remaining of breast cancer susceptibility results from mutations in a number of common, low-risk genes with additive and/or multiplicative effects on risk. In agreement with this Peto and Mack suggested that the majority of breast cancers arise in only a susceptible minority of women.<sup>120</sup>

The high-risk breast cancer susceptibility genes BRCA1 and BRCA2 encode nuclear proteins that are involved in DNA repair and recombination, regulation of gene transcription, chromatin remodeling, cell cycle checkpoint control and centrosome amplification. It is unclear why mutations of these ubiquitously expressed genes are associated with cancers of

the breast or ovary. Thus far 1200 distinct germline variants have been reported for both BRCA1 and BRCA2. It is sometimes difficult to discriminate pathogenic sequence variants and neutral polymorphisms. The frequency of BRCA1 and BRCA2 mutations is 0.23% in the general population. However this frequency is 3% in breast cancer patients, and 6% in breast cancer patients under age 50 years. Although the inheritance pattern of BRCA1 and BRCA2 mutations is autosomal dominant, the gene mutations are functionally recessive. In BRCA1 and BRCA2 associated tumors one mutant allele stems from the germline. The inactivation of the other allele is acquired at the somatic cellular level during life.

Female BRCA1 and BRCA2 mutation carriers have an increased risk of breast and ovarian cancer from the age of 25 years onwards. In BRCA1 mutation carriers the cumulative lifetime risk at age 70 years for breast cancer is 65-85% and for ovarian cancer 39-63%. In BRCA2 mutation carriers these risks are for breast cancer 45-84%, and for ovarian cancer 11-27%, respectively. The cancer risks depend on the position of the mutation within the coding sequence. For example mutations in the OCCR region of BRCA2 are associated with a high risk of ovarian cancer.

Breast cancers associated with BRCA1 or BRCA2 mutations are usually poorly differentiated (Bloom and Richardson grade III). Interestingly, the prognosis of BRCA1 or BRCA2 associated breast cancer is similar to that of sporadic patients.

With the advent of the identification of cancer susceptibility genes the practice of clinical genetics changed from mainly the diagnostics and counseling of rare and usually incurable diseases towards also diagnostics and counseling within a multidisciplinary team involved in the prevention and early detection of common diseases (breast cancer [BRCA1 and BRCA2] and colorectal cancer [MSH2, MLH1 and MSH6]). Men and women over 18 years are eligible for genetic counseling and testing for breast cancer susceptibility. The aspects of the counseling process are explained. The main reason for genetic testing is to define accurately cancer risks. This is of importance for decisions on surveillance and preventive strategies. In the absence of genetic testing breast cancer risk assessments are aided by usage of either the Gail or the Claus model. Since the diagnosis of genetic breast cancer susceptibility has life-long implications, the decision for genetic testing should follow after thorough reflection.

Women with BRCA1 and BRCA2 mutations with high cumulative lifetime risks of breast and ovarian cancer have access to the following risk reducing interventions 1) breast self-examination, 2) clinical breast examination, 3) regular mammography, 4) regular magnetic resonance imaging (MRI), 5) bilateral/contralateral mastectomy, 6) regular ultrasonography of the ovaries and determination of serum CA125 levels, 7) bilateral oophorectomy and 8) chemoprevention. The pros and cons of the different measures are discussed.



*Chapter 2* is a manuscript published in the *European Journal of Cancer* (37:2082-90) in 2001. It entails a study regarding the prevalence of BRCA1 or BRCA2 mutations in 517 breast and/or ovarian cancer families at the Rotterdam Family Cancer Clinic. BRCA1 or BRCA2 mutations were found in 23% of these families. The detection rate of mutations was highest in families with one or more patients with ovarian cancer and in families with two or more breast cancer patients diagnosed below age 40 years. BRCA1 IVS12-1643del13835 and BRCA2 5579insA were frequently found among breast cancer patients from two distinct small geographical regions (West-Brabant and Zuid-Beveland, respectively). Eight recurrent (> 5 times encountered) mutations represented 61% of all mutations found.

*Chapter 3* is a manuscript published in the *Lancet* (355:2015-20) in 2000. It entails a study of 682 unaffected individuals from 53 consecutive families with a BRCA1 or BRCA2 mutation at the Rotterdam Family Cancer Clinic regarding the demand for presymptomatic DNA testing and the request for prophylactic bilateral mastectomy and oophorectomy (in case a mutation was present). 57% and 29% of unaffected women with a pre-test genetic risk of a mutation of 50% and 25%, respectively, opted for a DNA test. The choice towards DNA testing correlated positively with young age and having children. 35 of the 68 unaffected mutation carriers (51%) opted for bilateral mastectomy and 29 of 45 eligible unaffected mutation carriers (64%) for bilateral oophorectomy. Women with children more often opted for bilateral mastectomy.

*Chapter 4* is a manuscript published in the *Journal of Clinical Oncology* (21:1675-81) in 2003. It entails a study of 220 breast and/or ovarian patients from 112 high-risk breast cancer families in which eventually a BRCA1 or BRCA2 mutation was identified. The demand for genetic testing and prophylactic bilateral/contralateral mastectomy and prophylactic bilateral oophorectomy is described. 192 of these 220 breast and/or ovarian cancer patients (87%) underwent DNA testing, of whom 11 (6%) were shown not to carry the BRCA1 or BRCA2 mutation that was known in their families. Young age and the presence of multiple primary tumors in a patient correlated positively with the decision for a DNA test. 35 of 101 eligible patients with a mutation (35%) requested bilateral/contralateral mastectomy, and 47 of 95 eligible patients with a mutation (49%) requested bilateral oophorectomy.

*Chapter 5* is a manuscript that was published the *New England Journal of Medicine* (345:159-64) in 2001. It entails a prospective study of the efficacy of bilateral mastectomy in 139 unaffected BRCA1 and BRCA2 mutation carriers from the Rotterdam Family Cancer Clinic. 76 of these 139 high-risk women eventually underwent bilateral mastectomy, while the other 63 women remained under surveillance. No breast cancers were observed in the 76 women

who underwent mastectomy after a mean follow-up of 3 years. In contrast, breast cancer was diagnosed in 8 of the 63 women under surveillance. The number of breast cancers in the surveillance group was in line with the expected number based on risk estimates in mutation carriers. Bilateral mastectomy in BRCA1 and BRCA2 mutation carriers had a significant risk reducing effect ( $P=0.003$ ). At the time of diagnoses, 6 of 8 breast cancers were larger than 10 mm; 4 of 8 patients had positive axillary lymph nodes; 6 of 8 breast cancers were missed by mammography; and MRI identified 6 of 6 tumors (two patients were not investigated using MRI).

*Chapter 6* is a manuscript *submitted for publication* (2003). It entails a confirmational study on the status of the ATM gene as a high-risk breast cancer susceptibility gene. Recently the ATM mutations 7271→G and IVS10-6T→G were proposed to confer breast cancer risks as high as mutations of BRCA1 and BRCA2.<sup>3</sup> These risk estimates were based on findings within three families with a total LOD score of 1.18 for linkage of breast cancer to the mutations. We report on the prevalence of these two ATM mutations in 961 breast cancer families without BRCA1 or BRCA2 mutations and in 543 population-matched controls. The 7271→G mutation was not detected in any sample indicating that this mutation is a rare event and does not contribute substantially to breast cancer susceptibility. The prevalence of the IVS10-6T→G mutation was similar in the breast cancer cases and controls (0.8% vs. 0.7%;  $P=0.44$ ). Bayesian analysis of linkage in the IVS10-6T→G mutation positive families showed an overall posterior probability of causality for this mutation of 0.8%. Thus our study refuted ATM IVS10-6T→G as a high-risk breast cancer allele.

*Chapter 7* is a manuscript that was published in *Nature Genetics* (31:55-9) in 2002. It entails a collaborative study towards the identification of CHEK2 1100delC as the first low-risk allele that contributes to the development of breast cancer. A genome-wide linkage search in a Dutch breast cancer family with 17 breast cancer patients yielded suggestive but not conclusive LOD scores for a susceptibility locus at chromosome 22q11. Shortly after our linkage analysis it was published that germline mutations of the CHEK2 gene, located on chromosome 22q11, cause the Li-Fraumeni syndrome. The search for mutations of CHEK2 was complicated by duplications of DNA fragments containing exons 10 through 14. The truncating mutation 1100delC (exon 10) of CHEK2 was eventually identified in several members of the above mentioned Dutch breast cancer family, in 4.2% of 718 breast cancer families without BRCA1 and BRCA2 mutations and in 1.1% of the general population ( $P=0.00000003$ ). CHEK2 1100delC conferred only a twofold increase in breast cancer risk in women. There was incomplete co-segregation of the mutation and breast cancer within the

CHEK2 1100delC families. Analyses within these families pointed to other hitherto unknown risk factors with a multiplicative effect on risk.

*Chapter 8* is a manuscript that was published in the *American Journal of Human Genetics* (72:1308-14) in 2003. In several breast cancer families with CHEK2 1100delC we observed also patients with colorectal cancer. We therefore studied the association of CHEK2 1100delC with colorectal cancer. We identified CHEK2 1100delC in 2.6% of 234 families with Hereditary Non-Polyposis Colorectal Cancer syndrome or a phenotype reminiscent of this syndrome ( $P$  for difference with general population =0.07), but in none of 95 Familial Adenomatous Polyposis families. 18.2% of 55 families with a hereditary pattern of breast *and* colorectal cancer carried CHEK2 1100delC but only 4% of 380 breast cancer families without a hereditary pattern of colorectal cancer ( $P<0.001$ ). CHEK2 1100delC thus identified preferentially families with a hereditary pattern of both breast and colorectal cancer. Again, within the CHEK2 1100delC families there was incomplete co-segregation of the mutation and breast and colorectal cancer.

*Chapter 9* is a general discussion on the findings. We provided evidence that high-risk breast cancer families appreciate the opportunity of genetic testing and subsequent surgical prevention and/or surveillance. The option of prophylactic mastectomy should be offered to women at high risk of breast cancer in a non-directive way. Family history is extremely helpful in selecting women at high risk of breast cancer and should be taken as part of standard medical care. Elucidation of the pathogenic potential of unclassified variants of BRCA1 and BRCA2 is at present of major clinical concern. Knowledge on BRCA1 and BRCA2 mutation status influences decisions on primary breast cancer treatment (mastectomy versus breast conserving therapy) in newly diagnosed patients. As expression profiling of breast tumors now enables rapid identification of BRCA1 and BRCA2 associated tumors and this type of diagnostics will be available in short time, we advocate to offer this new diagnostics routinely in the near future to a subset of women with newly diagnosed breast cancers before decisions are made on primary treatment. The clinical implications of the identification of the low-risk CHEK2 1100delC mutation are at present small. This may change however when CHEK2 1100delC appears to have prognostic significance in cancer patients and/or when the accuracy of cancer risk estimates in carriers and noncarriers of the mutation improves due to the isolation of the additional risk factors thought to be present within these families. Genetic testing of low-risk alleles like CHEK2 1100delC may also become useful within breast cancer families when a significant fraction of the remaining of low-risk alleles has been identified and simultaneous testing of them becomes feasible. It is also conceivable that this type of genetic risk profiling will be useful at the population level

for selecting a proportion of the female population for breast cancer screening programs. Our finding of the association of CHEK2 1100delC with families with the combined phenotype of breast and colorectal cancer implies that doctors should be alerted for a possible common genetic cause of these forms of cancer within families. Surveillance within families with the combined cancer phenotype should be adapted accordingly. Further, the identification of CHEK2 1100delC and of the hereditary breast and colorectal cancer phenotype open new avenues to search for additional breast cancer genes. Our knowledge on breast cancer susceptibility genes and their function will increase in the future, resulting in improvements in risk estimates, therapies and risk reduction interventions. Members of breast cancer families undoubtedly will be among the first to benefit from such progress.

## SAMENVATTING

*Hoofdstuk 1* bevat een algemene inleiding van het verrichte onderzoek. Borstkanker is een belangrijk gezondheidsprobleem voor vrouwen in Europa en de Verenigde Staten. In 1998 werden 10317 nieuwe invasieve borsttumoren gediagnosticeerd in Nederland. Een derde van deze patiënten zal binnen 10 tot 15 jaar na het stellen van de diagnose overlijden aan de gevolgen van uitzaaiingen van de primaire tumor (in 1998 waren er in Nederland 3542 sterfgevallen als gevolg van borstkanker).

Borsten zijn eigenlijk gespecialiseerde zweetklieren. Zij vormen het orgaan waarmee zoogdieren worden geclassificeerd. Een borstklier bestaat uit ongeveer 15 klierbuizen die afzonderlijk op de tepel uitkomen. Borstkanker ontstaat meestal in het epitheel dat de klierbuizen en secretie-units bekleedt. De meeste vormen van borstkanker zijn daarom adenocarcinomen. Borstkanker wordt gegradeerd volgens criteria van Bloom and Richardson (a) de mate van differentiatie van de klierbuizen; b) de mate van kernatypie; en c) de mitotische activiteit). Borstkanker patiënten worden geclassificeerd in 4 stadia volgens de TNM classificatie (T staat voor tumor grootte; N staat voor metastasen in regionale lymfklieren; M staat voor metastasen op afstand).

De behandeling van nieuw gediagnosticeerde borstkanker patiënten wordt bepaald door de prognose en het te verwachten behandelingsresultaat. Over het algemeen worden de tumoren chirurgisch verwijderd, gevolgd door radiotherapie in geval van borstsparende behandeling. Voor de klinische staging worden de schildwachtklier of multipele okselklieren uit de ipsilaterale oksel verwijderd. Patiënten krijgen adjuvante endocriene en/of chemotherapie afhankelijk van de leeftijd, tumor graad, het klinische stadium en de hormoon receptor status.

Het cumulatieve lifetime risico op borstkanker voor Nederlandse vrouwen is 9%. Eén van de belangrijkste risicofactoren voor het ontstaan van borstkanker is een belaste familie-anamnese. Gedurende de negentiger jaren van de twintigste eeuw werden twee belangrijke borstkanker predispositie genen, BRCA1 en BRCA2, geïdentificeerd. Mutaties in deze genen veroorzaken ongeveer 16% van het totaal aan borstkanker predispositie. Tot nu hebben grote internationale samenwerkingsverbanden niet geleid tot de identificatie van additionele hoog risico borstkanker predispositie genen. Recent werd een polygeen model voor borstkanker predispositie geïntroduceerd. Dit model houdt in dat borstkanker predispositie het gevolg is van frequent voorkomende mutaties in meerdere laag risico genen. Bij mutatiedraagsters van meerdere van deze laag risico genen leidt dit tot additieve en/of multiplicatieve toename van het risico op borstkanker. In overeenstemming hiermee suggereerde Peto en Mack dat de meerderheid van borsttumoren ontstaat in vatbare vrouwen, die slechts een minderheid vormen van de totale populatie.<sup>120</sup>

De twee belangrijkste hoog risico predispositie genen voor borstkanker, BRCA1 en BRCA2, coderen voor nucleaire eiwitten die betrokken zijn bij DNA herstel en recombinitie, de regulatie van gen transcriptie, modelleren van chromatine, controle van de celcyclus en de amplificatie van centrosomen. Het is onduidelijk waarom mutaties in deze genen, die vrijwel in elk weefsel tot expressie komen, leiden tot borstkanker en/of ovariumcarcinoom. Tot op heden zijn er meer dan 1200 kiembaan varianten in beide genen gerapporteerd. Soms is het moeilijk om een onderscheid te maken tussen pathogene varianten in de sequentie en neutrale DNA polymorfismen. Mutaties in BRCA1 en BRCA2 komen bij 0.23% van de algemene populatie voor. De frequentie van deze mutaties in borstkanker patiënten is echter 3%, en in patiënten met borstkanker ontstaan onder de leeftijd van 50 jaar 6%. De erfgang van mutaties in beide genen geschiedt op een klassieke autosomaal dominante wijze. Functioneel zijn de mutaties echter recessief. In BRCA1 en BRCA2 geassocieerde tumoren is één mutant allel via de kiembaan geërfd. Inactivatie van het andere allel wordt op somatisch niveau (in het epitheel van de borstklier) verkregen gedurende het leven.

Draagsters van een BRCA1 of BRCA2 mutatie hebben een verhoogd risico op borst- en ovariumcarcinoom vanaf de leeftijd van 25 jaar. Het cumulatieve risico tot aan de leeftijd van 70 jaar voor vrouwen met een BRCA1 mutatie bedraagt 65-85% op het krijgen van borstkanker en 39-63% op het krijgen van ovariumcarcinoom. Voor vrouwen met een BRCA2 mutatie bedragen deze risico's 45-84% voor borstkanker en 11-27% voor ovariumcarcinoom. De risico's op kanker zijn gerelateerd aan de positie van de mutatie in het gen. Mutaties in de OCCR regio van BRCA2 bijvoorbeeld zijn geassocieerd met een hoger risico op het krijgen ovariumcarcinoom.

BRCA1 en BRCA2 gerelateerde borstkankers zijn veelal slecht gedifferentieerd (Bloom en Richardson graad III). Interessant is dat de prognose van BRCA1 en BRCA2 geassocieerde borsttumoren niet verschilt van sporadische (niet BRCA-gerelateerde) tumoren.

De dagelijkse praktijk van de klinische genetica is veranderd door de ontdekking van kanker predispositie genen bij veel voorkomende tumoren zoals borstkanker en dikke darmkanker. Uit een medisch specialisme dat zich hoofdzakelijk bezighield met de diagnostiek van en adviezen bij (zeer) zeldzame en meestal ongeneeslijke ziekten en aandoeningen, ontstond een medisch specialisme dat binnen een multidisciplinair team verantwoordelijk is voor genetische diagnostiek van en advies bij frequent voorkomende aandoeningen waarvoor preventie en vroege diagnostiek veelal mogelijk is (borstkanker en dikke darmkanker). Mannen en vrouwen boven de 18 jaar komen in aanmerking voor erfelijkheidsadvies en -onderzoek aangaande borstkanker predispositie. De verschillende aspecten van het counselingsproces worden besproken. De belangrijkste reden voor een genetische test is het nauwkeurig definiëren van het risico op kanker. Dit risico is van belang voor verdere besluitvorming aangaande surveillance en preventieve strategieën. Bij

afwezigheid van genetische testuitslagen wordt voor het schatten van het risico op borstkanker gebruik gemaakt van het “Gail” model ofwel het “Claus” model. Het vaststellen van een hoog risico mutatie voor borstkanker heeft levenslange implicaties. Daarom moet genetisch onderzoek plaats vinden na zorgvuldige reflectie.

BRCA1 and BRCA2 mutatie-draagsters met hoge risico's op borst- en ovariumcarcinoom hebben toegang tot de volgende risico-reducerende interventies: 1) zelfonderzoek van de borsten; 2) onderzoek van de borsten door een arts; 3) mammografie; 4) MRI; 5) bilaterale/contralaterale mastectomie; 6) echografisch onderzoek van de ovaria en bepaling van serum CA125 spiegels; 7) bilaterale ovariectomie met meenemen van de tubae; en 8) chemopreventie. De voor- en nadelen van de verschillende interventies worden bediscussieerd.

*Hoofdstuk 2* werd gepubliceerd in *European Journal of Cancer* (37:2082-90) in 2001. Het beschrijft een onderzoek naar het voorkomen van mutaties in BRCA1 en BRCA2 in 517 families met erfelijk borst- en/of ovariumcarcinoom in de Rotterdamse Family Cancer Clinic. Mutaties in BRCA1 en BRCA2 werden in 23% van deze families gevonden. De meeste mutaties werden gedetecteerd in families met ovariumcarcinoom en in families met twee of meer familieleden met borstkanker onder de leeftijd van 40 jaar. De BRCA1 IVS12-1643del3855 en BRCA2 5579insA mutaties bleken frequent aanwezig in borstkanker patiënten afkomstig uit twee onderscheiden kleine geografische regio's (West-Brabant en Zuid-Beveland respectievelijk). Acht mutaties die herhaaldelijk (meer dan 5 maal) werden gevonden vertegenwoordigden 61% van alle gevonden mutaties.

*Hoofdstuk 3* werd gepubliceerd in de *Lancet* (355:2015-20) in 2000. Het betreft een onderzoek in 682 gezonde personen uit 53 opeenvolgende BRCA1 of BRCA2 mutatie-positieve families in de Rotterdamse Family Cancer Clinic. De vraag naar presymptomatische DNA diagnostiek en het verzoek om preventieve dubbelzijdige mastectomie en/of ovariectomie in geval van de aanwezigheid van een mutatie in BRCA1 of BRCA2 worden beschreven. 57% en 29% van de vrouwen met pre-test genetische risico's van respectievelijk 50% en 25% op een mutatie ondergingen DNA diagnostiek. Jonge vrouwen en vrouwen met kinderen lieten vaker DNA diagnostiek verrichten. 35 van de 68 gezonde mutatie-draagsters (51%) kozen voor dubbelzijdige mastectomie en 29 van de 45 in aanmerking komende gezonde mutatie-draagsters kozen voor dubbelzijdige ovariectomie (64%). Vrouwen met kinderen lieten vaker een dubbelzijdige mastectomie verrichten.

*Hoofdstuk 4* werd gepubliceerd in de *Journal of Clinical Oncology* (21:1675-81) in 2003. Het beschrijft een onderzoek aangaande de vraag naar genetische testen en preventieve mastectomie en ovariectomie van borst- en/of ovariumcarcinoom patiënten uit 112 families waarin uiteindelijk een BRCA1 of BRCA2 mutatie werd gevonden. 192 van de 220 borst- en/of ovariumcarcinoom patiënten uit deze families (87%) lieten zich onderzoeken op mutaties in BRCA1 en BRCA2. 11 patiënten bleken geen draagster van de in de familie voorkomende BRCA1 of BRCA2 mutatie (6%). Jonge leeftijd en het voorkomen van multipale primaire tumoren in een patiënt waren geassocieerd met de keuze voor een DNA test. 35 van de 101 in aanmerking komende patiënten met een mutatie (35%) kozen voor bilaterale/contralaterale mastectomie. 47 van de 95 in aanmerking komende patiënten met een mutatie (49%) kozen voor bilaterale ovariectomie.

*Hoofdstuk 5* werd gepubliceerd in de *New England Journal of Medicine* (345:159-64) in 2001. Het beschrijft een prospectief onderzoek naar de effectiviteit van bilaterale mastectomie bij draagsters van BRCA1 of BRCA2 mutaties, die voorafgaand en tijdens het uitvoeren van de ingreep geen tekenen van borstkanker vertoonden. Van 139 vrouwen met een BRCA1 of BRCA2 mutatie kozen na verloop van tijd 79 vrouwen voor een preventieve bilaterale mastectomie. De resterende 63 mutatiedraagsters bleven onder regelmatige borstcontrole. Bij de vrouwen die kozen voor mastectomie waren er na 3 jaar follow-up geen tekenen van borstkanker. Borstkanker werd echter wel geconstateerd gedurende dezelfde follow-up periode bij 8 van de 63 vrouwen die hadden gekozen voor continuering van de borstcontrole. Het aantal borsttumoren dat ontstond in de borstcontrolegroep kwam overeen met het aantal dat werd verwacht op basis van risicoschattingen bij mutatiedraagsters. Bilaterale mastectomie bij draagsters van BRCA1 of BRCA2 mutaties had een significant risico-reducerend effect ( $P=0.003$ ). Ten tijde van de diagnose waren 6 van de 8 borsttumoren groter dan 10 mm; 4 van de 8 patiënten hadden metastasen in de okselklieren; 6 van de 8 tumoren waren niet op het mammogram te zien; MRI visualiseerde echter 6 van de 6 tumoren (twee patiënten ondergingen geen MRI).

*Hoofdstuk 6* is een manuscript dat is aangeboden voor publicatie. Het betreft een confirmatiestudie naar de status van het ATM gen als zijnde een hoog risico predispositie gen voor borstkanker. Recent werd gepubliceerd dat de ATM mutaties 7271→G en IVS10-6T→G een vergelijkbaar risico geven op borstkanker als mutaties in BRCA1 en BRCA2. Deze risicoschattingen werden gebaseerd op drie families met een totale LOD score van 1.18 voor koppeling van borstkanker met deze mutaties. De frequentie van deze twee ATM mutaties in 961 borstkanker families zonder BRCA1 en BRCA2 mutaties en in 543 controle-individen



uit dezelfde populaties wordt beschreven. ATM 7271→G werd in geen enkel onderzocht individu gevonden. Dit wijst erop dat deze mutatie zeldzaam is en geen wezenlijke bijdrage levert aan borstkanker predispositie. ATM IVS10-6T→G was even frequent in borstkanker families als in controle individuen (0.8% versus 0.7%;  $p=0.44$ ). Bayesiaans koppelingsonderzoek in de ATM IVS10-6T→G families gaf een kans van 0.8% op een causale relatie van deze mutatie met borstkanker. Onze studie verwierp dus dat ATM IVS10-6T→G een hoog risico op borstkanker veroorzaakt.

*Hoofdstuk 7* is een manuscript dat werd gepubliceerd in *Nature Genetics* (31:55-9) in 2002. Het beschrijft de identificatie van de CHEK2 1100delC mutatie als het eerste laag risico allel dat bijdraagt aan het ontstaan van borstkanker. Koppelingsonderzoek met markers in het gehele genoom in een Nederlandse familie met 17 borstkanker patiënten leverde aanwijzingen op voor een oorzakelijk borstkanker locus op chromosoom 22q11. De LOD score was echter te laag voor een definitief bewijs. Kort na deze bevinding werd gepubliceerd dat mutaties in het CHEK2 gen, gelegen op chromosoom 22q11, het Li-Fraumeni syndroom veroorzaken. CHEK2 mutatie-onderzoek werd bemoeilijkt door het feit dat er van exon 10 tot en met 14 meerdere kopieën voorkomen in het menselijke genoom. Uiteindelijk werd de truncerende mutatie CHEK2 1100delC (exon 10) geïdentificeerd in verschillende leden van de bovengenoemde Nederlandse borstkanker familie, in 4.2% van 718 borstkanker families zonder BRCA1 of BRCA2 mutaties en in 1.1% van de algemene populatie ( $P=0.00000003$ ). CHEK2 1100delC veroorzaakte bij vrouwen slechts een tweemaal zo hoog risico op borstkanker. Binnen de CHEK2 1100delC families was er incomplete co-segregatie van de mutatie en borstkanker. In deze families werden aanwijzingen verkregen voor andere tot dusver onbekende risicofactoren die een multiplicatief effect hebben op het borstkanker risico.

*Hoofdstuk 8* is een manuscript dat werd gepubliceerd in *American Journal of Human Genetics* (72:1308-14) in 2003. In verschillende borstkanker families met de CHEK2 1100delC mutatie viel op dat ook dikke darmkanker voorkwam. Om deze reden werd een mogelijke associatie van CHEK2 1100delC met deze tumor vorm onderzocht. CHEK2 1100delC werd gevonden in 2.6% van 234 families met het HNPCC (Heredair Non-Polyposis Colorectaal Carcinoom) syndroom of hiermee vergelijkbare klinische uitingsvormen ( $P$  voor verschil met de normale bevolking =0.07), maar in geen van de 95 families met FAP (Familiaire Adenomateuze Polyposis). CHEK2 1100delC kwam voor in 18.2% van 55 families met een erfelijk patroon van zowel borstkanker als dikke darmkanker, doch slechts in 4% van 380 borstkanker families waarin geen erfelijk patroon van dikke darmkanker voorkwam ( $p<0.001$ ). CHEK2

1100delC identificeerde dus preferentieel families met een erfelijk patroon van zowel borstkanker als dikke darmkanker. Zoals ook boven beschreven was er in de families met CHEK2 1100delC incomplete co-segregatie van de mutatie en borstkanker en dikke darmkanker.

*Hoofdstuk 9* bevat een samenvattende discussie over de bevindingen. Families met erfelijk borstkanker waarderen de mogelijkheid van DNA onderzoek en daaruit voortvloeiende risico-reducerende interventies. De optie van preventieve mastectomie moet worden aangeboden aan vrouwen met een hoog risico op borstkanker op een non-directieve wijze. De familie-anamnese is zeer behulpzaam bij het identificeren van vrouwen met een hoog risico op borstkanker en moet daarom standaard worden afgenomen in de dagelijkse medische praktijk van de zorg rond borstkanker. Het ophelderen van de ziekteveroorzakende potentie van ongeclassificeerde varianten in de sequentie van BRCA1 en BRCA2 is dringend nodig. Kennis ten aanzien van BRCA1 en BRCA2 mutatie status beïnvloedt beslissingen aangaande de primaire behandeling van nieuw gediagnosticeerde borstkanker patiënten (mastectomie versus borstsparende behandeling). Expressieprofilering van borsttumoren maakt snelle identificatie van BRCA1 en BRCA2 geassocieerde borsttumoren op korte termijn mogelijk. Wij stellen daarom voor deze diagnostiek in de nabije toekomst standaard aan te bieden aan een subgroep van patiënten voordat beslissingen worden genomen aangaande hun primaire borstkanker behandeling. De klinische implicaties van het vinden van de laag risico mutatie CHEK2 1100delC is op dit moment gering. Dit kan echter veranderen wanneer CHEK2 1100delC prognostische/predictieve betekenis blijkt te hebben voor borstkanker patiënten, of wanneer nauwkeurigere risico schattingen mogelijk worden in dragers en niet-dragers van de mutatie door het vinden van de additionele predispositie factoren die waarschijnlijk aanwezig zijn in families met deze mutatie. DNA diagnostiek in borstkanker families naar laag risico allelen zoals CHEK2 1100delC kan tevens zinvol worden wanneer een belangrijk deel van de nog te vinden laag risico allelen ontdekt wordt en een gemeenschappelijke test hiervoor beschikbaar komt. Het is denkbaar dat dergelijke genetische risico-profilering ook toepassing kan vinden op populatie niveau, bijvoorbeeld voor het selecteren van een deel van de vrouwelijke bevolking voor borstscreening programma's. Onze bevinding van de associatie van CHEK2 1100delC met families met een gecombineerd patroon van erfelijk borstkanker en dikke darmkanker impliceert dat artsen alert moeten zijn op een mogelijke gemeenschappelijke erfelijke oorzaak van deze tumor typen binnen families. Controle-schema's binnen deze families dienen hieraan aangepast te worden. De identificatie van CHEK2 1100delC en het erfelijke patroon van borstkanker en dikke darmkanker geven nieuwe ingangen voor het vinden van additionele predispositie genen voor borstkanker. Onze kennis over predispositie genen voor borstkanker en hun functie zal met de tijd toenemen, en

dit zal in de kliniek resulteren in verbeteringen in risicoschattingen, behandelingen en risico-reducerende interventies. Leden van borstkanker families zullen hiervan ongetwijfeld als eersten profiteren.



## LIST OF TABLES AND FIGURES

*summarizing the following items:*

<i>Tables</i>	page
Risk factors for breast cancer	13
Clinical features of breast cancer susceptibility genes	16
Basic elements of informed consent for germline DNA testing for cancer susceptibility	31
Minimal criteria for BRCA1/BRCA2 mutation analysis	44
Frequency of BRCA 1 and BRCA2 mutations depending on familial cancer phenotype	46/48
Presymptomatic BRCA1 and BRCA2 test uptake in unaffected individuals	64
Choices towards prophylactic bilateral mastectomy and oophorectomy of unaffected BRCA1 and BRCA2 mutation carriers	67
BRCA1 and BRCA2 test uptake in women with breast and/or ovarian cancer	80
Choices towards prophylactic mastectomy of affected BRCA1 or BRCA2 mutation carriers	81
Choices towards prophylactic oophorectomy of affected BRCA1 or BRCA2 mutation carriers	82
Characteristics of breast cancers diagnosed under surveillance in carriers of BRCA1 or BRCA2 mutations	96/97
Frequencies of ATM 7271T→G and ATM IVS10-6T→G in breast cancer	105
Frequency of CHEK2 1100delC in breast cancer	114
Frequency of CHEK2 1100delC in colorectal cancer and breast cancer	125
 <i>Figures</i>	
Schematic presentation of BRCA1 and BRCA2	22
Cumulative risks of breast cancer and ovarian cancer in female BRCA1 and BRCA2 carriers	27
Organizational impact of the identification of BRCA1 and BRCA2 on clinical genetic services	40
Breast cancer among women with a BRCA1 or BRCA2 mutation after prophylactic mastectomy and under surveillance	95
Pedigrees of breast cancer families with the ATM IVS10-6T→G mutation	107
Pedigree of family EUR60 with the CHEK2 1100delC mutation	113
Pedigrees of families with breast and colorectal cancer and the CHEK2 1100delC mutation	129



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Tenslotte, lieve Carel, Gratia, Eva. **Grazie Mille.** Voor alles.



## CURRICULUM VITAE

The author of this thesis was born on 24<sup>th</sup> of June, 1961 in Pijnacker, the Netherlands, as the third daughter of Gerrigje Vonk and Johannis Heijboer. She passed her gymnasium B exam at the 'Christelijke Scholen Gemeenschap voor Delft en Rijswijk' in 1979 and obtained her MD degree from the Erasmus University Rotterdam in 1986. As a student she participated in a research project on the identification and cloning of DNA repair genes at the Institute of Genetics (head Prof. Dr. D. Bootsma). She started her training as a clinical geneticist at the department of Clinical Genetics at the Erasmus University Rotterdam (head Prof. Dr. H. Galjaard; instructor Prof. Dr. M.F. Niermeijer) after rotations in gynaecology and obstetrics, and cardiology. During her residency, she was a fellow at the Institut für Anthropologie und Humangenetik, Karls Ruprechts Universität, Heidelberg, Germany and investigated genetic aspects of aniridia and Wilms' tumour (group leader Dr. B. Royer-Pokora; head Prof. Dr. F. Vogel). In 1992 she was registered as a clinical geneticist and received a permanent position at the Department of Clinical Genetics, Erasmus University Rotterdam. Oncogenetics has been her main focus since 1994. In 2002 she was appointed head of the section Genetic Counselling of the Department of Clinical Genetics, Erasmus MC, and became responsible for the training of residents in clinical genetics. She was awarded with the "Henny C. Dirven" price at the Leiden University in 2002.

She married Carel Meijers in 1983. They have two daughters Gratia (1991) and Eva (1993).







**STELLINGEN**  
behorend bij het proefschrift

**BREAST CANCER**  
Predisposing Genes and their Clinical Implications

1. De kans op het vinden van een BRCA1 of BRCA2 mutatie wordt in grote mate bepaald door de familiegeschiedenis, met name ten aanzien van het voorkomen van borstkanker op jonge leeftijd en eierstokkanker.  
*Dit proefschrift.*
2. Vrouwen uit families met erfelijk borstkanker willen vaak weten of zij de aanleg hebben geërfd.  
*Dit proefschrift.*
3. De kans op borstkanker is erg klein na profylactische bilaterale mastectomie.  
*Dit proefschrift.*
4. Mastectomie wordt ook in Nederland beschouwd als een mutilerende ingreep, in tegenstelling tot hetgeen Franse collega's (*Julian-Reynier et al., Lancet 356, 1687, 2000*) suggereren.
5. De kiembaanmutatie CHEK2\*1100delC is in vrouwen geassocieerd met slechts een tweemaal verhoogd risico op borstkanker.  
*Dit proefschrift.*
6. Het feit dat 1% van de gewone bevolking drager is van de CHEK2\*1100delC mutatie sluit uit dat deze mutatie het uiterst zeldzame Li-Fraumeni syndroom veroorzaakt (*Bell et al., Science 286, 2528-31, 1999*).  
*Dit proefschrift.*
7. Vrouwen met de kiembaanmutatie ATM\*IVS10-6T→G hebben geen hoog risico op borstkanker in tegenstelling tot hetgeen Chenevix-Trench et al. (*J Natl Cancer Inst 94, 205-15, 2002*) concluderen.  
*Dit proefschrift.*
8. Er zijn veel minder grote verschillen tussen de resultaten van populatiestudies en familiestudies met betrekking tot kankerrisicoschattingen dan tot dusver gesuggereerd.  
*Antoniou et al., Am J Hum Genet 72, 1117-30, 2003; Begg, J Natl Cancer Inst 94, 1221-6, 2002; Burke and Austin, J Natl Cancer Inst 95, 78-9, 2003.*
9. Moleculair genetische diagnostiek naar een laag-risico ziekte-allel is alleen zinvol indien er klinische consequenties zijn.
10. Ondanks het feit dat borstkanker grote invloed heeft op het leven van een vrouw en de behandeling vaak ingrijpend is, is het de vraag of borstkanker tot de categorie van aandoeningen behoort waarvoor prenatale diagnostiek met de mogelijkheid van abortus bedoeld is.
11. Door het toenemende belang van de genetica in diverse klinische specialismen is het essentieel dat een afdeling klinische genetica gesitueerd is binnen de muren van een academisch medisch centrum.
12. Van je familie moet je het hebben.

