Introduction
1.1 The Clinical Problem

1.1.1 Breast cancer incidence and risk factors

Breast cancer is the most common cancer in women in the Western World with a prevalence of 1 in 8 (12% lifetime risk). Male breast cancer has a much lower prevalence and occurs in 1 in 1,000 men. Worldwide more than one million women are diagnosed with breast cancer yearly. In 2011, 13,987 women were diagnosed with invasive breast cancer in the Netherlands (The Netherlands Cancer Registry (IKNL), website www.cijfersoverkanker.nl). The majority of the women is postmenopausal at the time of diagnosis since annually approximately 3,000 of the Dutch women (21% of total) are diagnosed under the age of 50 years (premenopausal). In addition, approximately 2,000 women are annually identified in the Netherlands with precursor lesions of breast cancer, mostly Ductal Carcinoma in situ (DCIS) and Lobular Carcinoma in situ (LCIS). Breast cancer incidence has shown a dramatic increase by approximately 30% since 1989, mainly in Western countries. The rising incidence over a certain period of time is called a secular trend. The increase in breast cancer incidence is caused by many risk factors, including environmental factors, of which the majority remain to be unraveled.

The major risk factors for breast cancer are; female gender, age and a family history of breast cancer. Other risk factors are; demographic risk factors (Western world), endocrine risk factors (early menarche, no children or late age at birth of first child, late menopause, use of hormonal contraceptives), physical characteristics (high density breast tissue, atypical benign breast disease, high postmenopausal Body-Mass-Index (BMI)) and environmental risk factors (previously received (thoracic) radiation therapy, alcohol, smoking). Protective factors for breast cancer are; living in certain geographical regions, high parity, breast feeding, high premenopausal BMI, and physical activity. Most of these risk factors were determined in population-based studies on women from the general population.
1.1.2 Diagnosis and prognostic / predictive factors of outcome

For breast cancer diagnosis a multidisciplinary approach combining clinical aspects, breast imaging and pathological features is necessary. In the Netherlands an extensive (consensus based) guideline for breast cancer diagnosis and treatment has been made by a national multidisciplinary team (Nationaal Borstkanker Overleg Nederland (NABON) and is available online via www.oncoline.nl/mammacarcinoom.

Breast cancer, in Latin mamma carcinoma, originates in the mammary glands and is from epithelial origin. The mammary tissue consists of gland tissue to produce milk (lobules) and of ducts to drain the breast milk to the nipple in the reproductive period. Breast cancer usually develops in terminal ducts and lobules. Approximately 80% of the breast cancers is diagnosed as invasive ductal carcinoma; 55% is pure ductal from origin and 25% is mixed ductal carcinoma with another subtype. Invasive lobular cancer accounts for approximately 10% of breast cancers. Further less frequent histological subtypes are medullary breast cancer <5%, mucinous breast cancer 3% and some other rare subtypes (including tubular, papillary, metaplastic, malignant phyllodes, neuroendocrine carcinomas).

The precursor lesions of breast cancer, ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS), are not invasive and are confined to the intraductal (or intralobular) space. Over time these precursor lesions can develop into invasive lesions if left untreated (14-50%). Often DCIS lesions are detected by calcifications on mammograms and it is difficult to differentiate histological from invasive lesions. Aside from the histological classification according to the World Health Organization, breast cancer is also subdivided according to prognostic factors.

The TNM classification in which T stands for Tumor size, N for regional lymph Node involvement and M for distant Metastases, is widely used in prognosis of solid tumors in general. In breast cancer, T1 are breast tumors smaller than 2 cm, while T4 breast tumors invade the skin or thoracic wall. N0 stands for no regional lymph nodes affected with metastatic (breast) cancer cells, while N3 refers to more than 10 axillary lymph nodes or ipsilateral supraclavicular lymph nodes affected with breast tumor cells. Dissemination tests will determine if there is clinically or radiographic evidence for distant metastases (M1) (e.g. liver, bone) or no distant metastases (M0).

In the Bloom and Richardson classification breast tumors are graded according to differentiation: well (grade I), moderately (grade II) or poorly differentiated (grade III) based on mitotic index (growth speed), degree of atypical cells and glandular differentiation. A grade III tumor is therefore considered the most aggressive breast cancer type. Treatment choice is determined on both the TNM and Bloom and Richardson classification, and on three immunohistochemical stainings of the tumor
for the expression of the estrogen receptor (ER), the progesterone receptor (PR) and the human epidermal growth factor receptor 2, known as HER2, ERBB2 or NEU. About 12-24% of all breast tumors have no expression of either three, and are indicated by 'triple negative' tumors. Triple negative tumors are associated with early recurrence of disease and poor outcome.

Tumor profiling refers to the measurement of all expressed genes and/or determination of genome wide genomic gains and losses in breast tumor cells. Tumor profiling divides breast tumors in different molecular subtypes; the Basal type, the Luminal A type, the Luminal B type and the HER2 positive type. Of note, the ER/PR-negative tumors mainly cluster in the Basal-group of breast tumors. Tumor profiling has recently been introduced as a new additional tool to predict prognosis and response to (chemo)therapy (e.g. Mammaprint).5

Disease related mortality of breast cancer has decreased over time, with an overall 5 and 10-year survival of 82% and 72% (period 1989-2010) dependent on breast cancer stage at diagnosis. Still over 3,200 women die of breast cancer in the Netherlands yearly. Breast cancer in women aged 35-50 years is the most common cause of death (www.cijfersoverkanker.nl).

1.1.3 Treatment

The national guidelines for breast cancer treatment are as mentioned above available at the website www.oncoline.nl. In short, breast cancer treatment is determined by many individual factors, such as age, TNM classification and the ER/PR/HER2 status. Surgery is the primary treatment of breast cancer; mastectomy or lumpectomy with excision of a sentinel node (hypothetical first lymph node to drain metastatic breast tumor/cancer cells) and/or axillary lymph node dissection. Depending on the above mentioned factors, surgery is preceded (neoadjuvant) or followed by chemotherapy. Lumpectomy is standard followed by radiation therapy. Depending on the above mentioned receptor status, women are treated with anti-hormones for years, or receive trastuzumab-infusions when HER2 positive (~15-20% of all breast cancer).

Several surgical methods are being used for mastectomies in combination with/without reconstruction by a plastic surgeon.
1.2 The Genetic Problem

1.2.1 Diagnosis

The diagnosis of hereditary breast cancer can be achieved clinically, or by molecular testing for mutations in causative genes. The high risk breast cancer susceptibility genes \textit{BRCA1} and \textit{BRCA2} are most frequently involved and are therefore routinely tested in clinical practice according to guidelines.

Clinical markers for hereditary breast cancer are; early age at onset of diagnosis, bilateral breast cancer, multiple primary tumors, tumor phenotype, familial clustering of breast and/or ovarian cancer, male breast cancer, and Ashkenazi Jewish ancestry. With respect to family history, 10-15% of women with breast cancer have one or more affected first degree relatives. The contribution of inherited susceptibility to all breast cancer is estimated to be up to 30%. The likelihood of finding a mutation in one of the high risk breast cancer susceptibility genes, \textit{BRCA1}/\textit{2}, is strongly dependent on family history, ancestry, and tumor characteristics. \textit{BRCA1}/\textit{2} germline mutations account for approximately 20% of hereditary breast cancer and less than 5% of breast cancer overall. In the Ashkenazi Jewish (AJ) population three common founder mutations in the \textit{BRCA1}/\textit{2}-genes account for 95% of the detectable \textit{BRCA1}/\textit{2}-mutations in dominantly inherited early onset breast and/or ovarian cancer families. Prevalence tables of \textit{BRCA1} and \textit{BRCA2} mutations based on ancestry and medical data of 10,000 individuals and their family members are available at www.myriad.com. To give an impression on the impact of personal and family history on finding a \textit{BRCA1}/\textit{2} mutation, a non-Ashkenazi Jewish woman with unilateral breast cancer age 48 without a family history of breast-and ovarian cancer has a likelihood of carrying a \textit{BRCA1}/\textit{2} mutation of 4.7%, while the same woman with one first or second degree relative with breast cancer below age 50 years and a relative with ovarian cancer at any age rises to 26.6%. Mutation prevalence in a woman with the same characteristics but of Ashkenazi Jewish ancestry is 7.9% and 33% respectively. Other available \textit{BRCA1}/\textit{2} mutation carrier probability tools are the Manchester scoring tables and carrier prediction software based on pedigree information including BRCAPRO and BOADICEA.

Tumor characteristics may also be indicative for the presence of a germline mutation in \textit{BRCA1} and \textit{BRCA2} in a patient. Up to 15% of women with a triple negative breast cancer tumor carry a mutation in \textit{BRCA1} or \textit{BRCA2}. Of note, the number of triple negative breast tumors decreases with age of diagnosis in \textit{BRCA1} carriers and shows an opposite trend when compared to breast tumors in \textit{BRCA2} carriers.

In the Netherlands diagnostic molecular testing for \textit{BRCA1} and \textit{BRCA2} mutations are performed in the diagnostic laboratories of the departments of clinical genetics.
at the university medical centers. Sanger sequencing of blood-DNA is the commonly used technique. All coding exons and exon-intron boundaries of the large \textit{BRCA1/2} genes are sequenced. To detect larger rearrangements in the \textit{BRCA}-genes Multiplex Ligation-dependent Probe Amplification (MLPA) is used. High through-put techniques like massively parallel sequencing will replace Sanger sequencing as soon as these techniques are proven to be equally reliable.

In the Netherlands, women and/or families are eligible for \textit{BRCA1/2} testing, when the chance of finding a mutation is about 10\% or higher. All health insurance companies in the Netherlands cover the costs for diagnostic \textit{BRCA1/2} testing. Through 2012, more than 50,000 women underwent \textit{BRCA1/2} testing in the Netherlands, and approximately 2,000 \textit{BRCA1}- and 1,000 \textit{BRCA2}- positive families have been diagnosed (personal communication with F. Hogervorst, NKI/AVL). If a causative mutation in \textit{BRCA1} or \textit{BRCA2} is identified other relatives are invited to undergo predictive testing for the family-specific mutation. Molecular diagnostics in families where all affected individuals and putative obligate carriers are deceased, is challenging. Chapter 2 addresses this issue and demonstrates that molecular testing of formalin-fixed and paraffin-embedded (FFPE) material of deceased individuals may be an alternative.

\subsection*{1.2.2. Cancer risks, surveillance, interventions, treatment}

In the Netherlands population-based breast cancer surveillance for every woman is available, and encompasses bi-annual mammography, starting at age 50 until age 75.

Women without an identified \textit{BRCA1} or \textit{BRCA2} mutation but at increased risk of breast cancer, are eligible for additional breast surveillance according to national guidelines (available at www.oncoline.nl, based on the guideline for familial breast and ovarian cancer by the Dutch society of Clinical Genetics (VKGN)). To determine the increased family-based breast cancer risk of an individual woman, several instruments can be used. For instance the (extended) Claus formula and BOADICEA software.\textsuperscript{16}

The offered breast surveillance in the general population, and in the setting of familial breast cancer differ from country to country and is being subject to changes over time due to research outcomes. Several studies discuss the optimal age and method of surveillance, e.g. MRI’s versus mammography, and individual breast density.\textsuperscript{17,18}

In the Netherlands, women at increased risk of breast cancer are divided into three risk groups: a) with a relative breast cancer risk below 2; b) with a moderately increased relative risk (RR) between 2-3; and c) with a relatively high breast cancer
risk of 3-4. Each group is advised to undergo different surveillance schemes: a) RR<2 no surveillance besides population-based screening; b) RR2-3 yearly mammography between age 40-50; and c) RR 3-4 yearly mammography and breast examination between age 35-60.

Often the gain of years of life and the clinical utility of national breast surveillance programs, but also breast surveillance programs for women at increased risk of breast cancer, have been debated. Apart from that, surveillance of the breasts may also have negative side-effects such as raising additional diagnostic procedures and anxiety in a substantial number of women. Several studies investigated quality of life and the level of psychological burden for women undergoing surveillance due to an inherited high risk to breast cancer. A recent review from these studies concluded that surveillance for most hereditary cancers was associated with normal levels of psychological distress and a normal quality of life.19

In case a mutation in BRCA1 or BRCA2 is identified in a family, more accurate individual cancer risks can be determined. Women with a BRCA1 or BRCA2 mutation are particularly at increased risk of breast cancer, bilateral breast cancer, and ovarian cancer. The cumulative breast cancer risk ranges from 45-65% at age 70 for women carrying a BRCA1 mutation and 27-84% for BRCA2.20-23 The overall 10- and 25-year contralateral breast cancer risk is approximately 20 and 44% for BRCA1 carriers and 13% and 33% for BRCA2 carriers.24 The lifetime risk for ovarian cancer by the age of 70 ranges from 18-59% for women with a BRCA1 mutation and 2.4-35% for BRCA2 mutation carriers.21,22,25 The lifetime risks of both breast cancer and ovarian cancer are higher in BRCA1 than in BRCA2 carriers.

The risks of cancers other than of the breasts and ovaries appear to be small in absolute terms, and only a few of them are beyond statistical doubt. BRCA1 mutations have been associated with an increased risk for cervical, uterine, pancreatic, pharyngeal, stomach, colorectal cancer and possibly prostate cancer.26,27 BRCA2 mutations are repeatedly associated with pancreatic cancer, male breast cancer and prostate cancer.28,29 The complete cancer phenotype for BRCA1/2 mutation families is still debated and needs to be unraveled further. Chapter 3 investigates if DCIS is associated with BRCA1/2 mutations.

Risk reducing interventions and surveillance programs are offered to women who carry a BRCA1 or BRCA2 mutation according to previously mentioned national guidelines.

Unaffected female carriers of a pathogenic mutation in BRCA1 or BRCA2 are advised to undergo an intensive breast surveillance program for early detection of breast tumors from age 25 onward, being annual breast MRI’s and physical
examination by a specialized surgeon. From age 30 onward carriers are also advised to have annual mammograms performed until age 60. Alternatively, women are offered to have preventive bilateral mastectomy. In the Netherlands, approximately 50% of unaffected female carriers from two studies from Rotterdam choose preventive bilateral mastectomy followed by direct reconstruction. In five other studies from the USA there was a much lower uptake for bilateral preventive mastectomy. In the UK approximately 40% opted for bilateral risk-reducing mastectomy. The wide range of 0-54% reflects local, national/center and international differences in risk perception/interpretation, both by physicians and female carriers. After a bilateral preventive mastectomy the remaining breast cancer risk is below 5%. Kurian et al. calculated best survival probabilities when prophylactic mastectomy was performed at age 25 years and prophylactic preventive bilateral salpingo-oophorectomy (pBSO) was performed at age 40 years.

Considering the high risk of ovarian cancer, annual gynecological surveillance through transvaginal ultrasound and CA125 measurement in blood for early detection of ovarian cancer was long advised. These methods were unable to reliably detect ovarian cancer at curable stages. Therefore female carriers are currently advised to undergo preventive bilateral salpingo-oophorectomy (pBSO) after fulfilling their desire to have children at the age of 35-40 years for BRCA1 carriers and 40-45 years for BRCA2 carriers. The remaining risk for peritoneal (coelomic) cancer is below 5% after pBSO. Performing pBSO in premenopausal women also reduces breast cancer risk.

Premenopausal carrier women are also confronted with the influence on reproductive choices. Pre-implantation-diagnostics (PGD) as part of an IVF procedure is possible for BRCA1/2 carriers in the Netherlands since 2008. Until 2011, 136 couples with a BRCA1/2 mutation as indication were referred for PGD intake (www.pgd.nl).

The treatment of breast cancer in women with a BRCA1/2 mutations does not substantially differ from women without such a mutation. However, newly diagnosed carrier women may want to consider bilateral mastectomy with reconstruction as primary treatment of their breast cancer instead of lumpectomy with subsequent radiotherapy. Here, the discussion raises concerns regarding quality of life since BRCA1/2 mutation carriers face a higher risk of a second primary breast cancer during their lifetime should they survive their first breast cancer. At present, the timing to raise all the issues related to BRCA1/2 related breast cancer in newly diagnosed women is under debate among physicians. Women may be overwhelmed and experience high psychological distress due to their recent cancer diagnosis. On the other hand, breast reconstruction after radiation therapy is suboptimal. Further, gene-tailored treatment
is possible in the near future through more favorable responses in treatment with for instance Poly-ADP-ribose polymerase (PARP)-inhibitors in mutation carriers of the breast cancer genes \textit{BRCA1} and \textit{BRCA2} with breast-and-ovarian cancer.\textsuperscript{35} PARP1 has an important function in the repair of single-strand DNA breaks. The overall survival of breast cancer in \textit{BRCA1} and \textit{BRCA2} mutation carriers is not substantially different from women without mutations in these genes.\textsuperscript{36}

1.3. Breast Cancer Susceptibility Genes

1.3.1. Risk conferred by breast cancer risk genes / alleles

Breast cancer susceptibility genes can be divided according to the breast cancer risk they confer: high risk genes with about 10-20 fold increased risk, moderate risk genes/alleles with about 2-4 fold increased risk, and common low risk loci (Single Nucleotide Polymorphisms (SNP’s)) with up to 1.4 fold increased risk. Figure 1 gives an overview of the presently reported breast cancer risk genes and alleles, according to their prevalence.

Mutations of the high risk breast cancer genes are relatively rare (0.1%) except for some founder mutations in specific populations. The over 65 SNP’s identified by Genome Wide Association Studies (GWAS) represent the other end with a population-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Breast cancer risk alleles and prevalence}
\end{figure}

With the courtesy of William Foulkes, Figure: Breast cancer susceptibility genes and loci from “Susceptibility to common cancers”, \textit{N Engl J Med} \textbf{2008} \textit{359}(20):2143-2153.
based prevalence of 30% or more.\textsuperscript{37} The moderate breast cancer susceptibility genes and alleles hold the position in between. Mutation in those genes are rare but may be relatively frequent in case of a founder mutation, e.g. CHEK2*1100delC mutation in the Dutch population.

1.3.2. Mode of inheritance of breast cancer risk genes / alleles

For many common diseases, including breast cancer, it has been shown that the increased risk is not solely caused by one specific mutation. Several genes may act together in an additive or multiplicative model (polygenic), and genetic modifiers and non-genetic modifiers may substantially alter the clinical effects of the risk alleles.\textsuperscript{38,39} This complex model not only applies to the moderate and low risk alleles but also to the high risk breast cancer genes with an autosomal dominant inheritance. Recently, it was shown that the risk conferred by BRCA1 and BRCA2 mutations are substantially influenced by other risk alleles.\textsuperscript{40,41}

For BRCA1, at least 10 common low risk loci are now known to influence breast cancer risk (1q32, 2q35, 19p13.1, 10q25.3, 6q25.1, 12p11, TCX3, RAD51L1, LSP1, TERT) and 7 loci (3q25, 4q32.3, 8q24, 9p22, 17q21, 17q21.31, 19p13) are known to be associated with the ovarian cancer risk in BRCA1 carriers.

For BRCA2 these are partially overlapping loci and approximately 9 loci were associated with breast cancer (FGFR2, TOX3, MAP3K1, LSP1, 2q35, SLC4A7, 5p12, 6q25.1, 1p11.2).\textsuperscript{41,42} At least 4 loci (8q24, 3q25, 2q31, 17q21) are described to modify the ovarian cancer risk in BRCA2 carriers.\textsuperscript{43}

Recently Couch \textit{et al.} estimated a lifetime breast cancer risk for BRCA1 carriers at lowest and highest 5% risk limit to be 28%–50% compared to 81%–100% by carrying combinations of risk influencing SNP’s instead of the generally given lifetime breast cancer risk of 60-80% for BRCA1 and BRCA2 female carriers.\textsuperscript{40} Cumulative lifetime breast cancer risk therefore substantially differ (30-70% risk difference) for at least 20% of the BRCA1 carriers.

A similar risk prediction was made for BRCA2 carriers by Antoniou \textit{et al.} combining the above mentioned distribution of the 7 risk-associated SNP’s in BRCA2 mutation carriers. The 5% of BRCA2 carriers at highest risk were predicted to have a probability between 80% and 96% of developing breast cancer by age 80, compared with 42% to 50% for the 5% of carriers at lowest risk.\textsuperscript{41}

Recently an intermediate risk mutation in BRCA1 (R1699Q) formerly classified as a Variant of Unclassified Significance (VUS) category III was described. The variant was shown to confer a cumulative breast and/or ovarian cancer risk of approximately 24% by the age of 70.\textsuperscript{44}
Like risk increasing loci, breast cancer risk reducing (protective) factors have also been discovered. The CASP8 D302H variant for instance was described to be associated with a reduced risk of breast and ovarian cancer for BRCA1 mutation carriers.45

The involvement of more than one susceptibility gene or allele in the cause of familial breast cancer, may result in incomplete segregation of the individual risk genes or alleles with the disease. In particular when not all risk alleles are known and can be tested for, individual risk assessment of the family members remains inaccurate. A ‘hot’ example of such a situation is families with multiple cases of breast cancer, in which the moderate risk allele CHEK2*1100delC is present in some of the affected family members. Chapter 8 addresses this question in detail.

Nearly all individual breast cancer risk genes and alleles follow the dominant mode of inheritance. A recessive model of inheritance for risk factors for familial non-BRCA1/2 breast cancer has been suggested years ago, apart from a polygenic model.46,47 Interestingly, homozygosity for the moderate risk allele CHEK2*1100delC resembles the recessive mode of inheritance. Chapter 7 focus on this issue.

Human bi-allelic affected homozygous or compound heterozygous carriers of breast cancer susceptibility genes often give rise to a distinct autosomal recessive inherited disease, like Fanconi Anemia (see below) or are suggested not to be viable, like in TP53 or in BRCA1. Interestingly, recently Domchek et al. described a woman with two ‘deleterious’ BRCA1 mutations (c.2457delC (p.Asp821Ilefs*25 and c.5207T>C (p.Val1736Ala).48 She had ovarian cancer at age 28 and several congenital features fitting a Fanconi Anemia-like phenotype (microcephaly, short stature, chemotherapy toxicity). For bi-allelic BRCA2 affected patients, it has been suggested that at least one of the mutations must be hypomorphic due to potential lethality when two complete null mutations co-inherit.

Females with one BRCA1 and one co-inherited BRCA2 mutation have a phenotype with a possible earlier onset of disease.49,50

For many common low risk alleles / SNP’s associated with breast cancer, homozygous carriers have a higher risk of breast cancer – though in absolute terms still a small increased risk – when compared to heterozygous carriers.51

1.3.3 Individual breast cancer risk genes: high risk, moderate risk

In Table 1 the individual breast cancer risk genes are listed. Indicated are their prevalence, clinical phenotype if mono-and bi-allelic mutated, and their described lifetime risk to breast cancer.

Of note, mutations in breast cancer risk genes may also predispose to specific
Table 1 Breast cancer risk genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Prevalence</th>
<th>Phenotype mono-allelic mutation</th>
<th>Breast cancer lifetime risk</th>
<th>Phenotype bi-allelic mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>0.1%</td>
<td>HBOC</td>
<td>45 - 85%</td>
<td>FA-like</td>
</tr>
<tr>
<td>BRCA2</td>
<td>0.1%</td>
<td>HBOC</td>
<td>27 - 84%</td>
<td>FA subtype - D1</td>
</tr>
<tr>
<td>TP53</td>
<td>&lt;0.1%</td>
<td>Li-Fraumeni*</td>
<td>28 - 56%</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>&lt;0.1%</td>
<td>Cowden*</td>
<td>25 - 50%**</td>
<td></td>
</tr>
<tr>
<td>LKB1/STK11</td>
<td>&lt;0.1%</td>
<td>Peutz-Jeghers*</td>
<td>29 - 54%</td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td>&lt;0.1%</td>
<td>Gastric cancer</td>
<td>20 - 40%</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>0.5%</td>
<td>BC</td>
<td>15-20%</td>
<td>Ataxia-telangiectasia</td>
</tr>
<tr>
<td>CHEK2</td>
<td>1.0-4.9%</td>
<td>BC</td>
<td>20 - 25% pop. 34 - 44% fam.</td>
<td>Bilateral BC/ Multiple cancers</td>
</tr>
<tr>
<td>PALB2</td>
<td>0.5-0.9%</td>
<td>BC</td>
<td>20-30%</td>
<td>FA subtype - N</td>
</tr>
<tr>
<td>BRIP1</td>
<td>&lt;0.5%</td>
<td>BC</td>
<td>~20%</td>
<td>FA subtype - J</td>
</tr>
<tr>
<td>RAD51C</td>
<td>0.3%</td>
<td>Ovarian</td>
<td>?</td>
<td>FA subtype - O</td>
</tr>
<tr>
<td>RAD51D</td>
<td>?</td>
<td>Ovarian</td>
<td>RR1.3</td>
<td>FA</td>
</tr>
<tr>
<td>XRCC2</td>
<td>?</td>
<td>BC</td>
<td>?</td>
<td>FA</td>
</tr>
<tr>
<td>SLX4</td>
<td>0.1%</td>
<td>BC</td>
<td>?</td>
<td>FA subtype - P</td>
</tr>
</tbody>
</table>

HBOC=Hereditary Breast and Ovarian Cancer, BC=Breast cancer, FA=Fanconi Anemia, pop = population-based, fam = family-based, FA is a rare heterogeneous autosomal recessive inherited disease with variable clinical features, including short stature, radial hypoplasia, thumb anomalies, hyper-and hypo-pigmentation, bone marrow failure and predisposition to cancers at young age. *refers to the gene-related autosomal dominant cancer syndromes. Cowden syndrome is part of the PTEN Hamartoma Tumor syndrome. **Recently a much higher lifetime risk for breast cancer (85%) was estimated in female PTEN mutation carriers. Li-Fraumeni syndrome gives rise to a high cancer risk at child-age and has a broad spectrum of associated tumors. CDH1/E-cadherin)-gene mutations confer to the diffuse gastric cancer syndrome with (mainly) lobular breast cancer risk in female mutation carriers. For further information about the specific syndrome features and cancer risk you are referred to www.erfelijkheid.nl or to the GeneReviews website of www.ncbi.nlm.nih.gov.
benign features and/or an increased risk of cancers other than breast cancer. As a consequence the medical history of an individual and/or her/his family members is often helpful in pinpointing the causative gene/syndrome in that person or family (see below).

1.3.4. CHEK2 and CHEK2*1100delC

The multifunctional cell cycle checkpoint kinase 2, CHEK2, is involved in DNA-repair, (G1) cell-cycle arrest and (radio-induced) apoptosis. In the Netherlands the prevalence of the CHEK2*1100delC mutation in the general population is 1.1%, 2.5% in unselected breast cancer cases and up to 4.9% in familial breast cancer cases. In the Dutch population the prevalence of CHEK2 mutations other than the frameshift mutation CHEK2*1100delC is negligible.

The lifetime risk of breast cancer in a CHEK2*1100delC mutation carrier from the general population is 20-25% however, carriers from a familial breast cancer setting have a 34-44% lifetime risks. See also Table 1. Female CHEK2*1100delC mutation carriers are described to have an increased risk to develop contralateral breast cancer (bilateral breast cancer risk up to 30-40%). Radiation therapy was suggested to increase the risk of a second primary/contralateral breast cancer. Some studies also showed that carrier women have a worse recurrence–free and breast cancer specific survival.

Breast tumors from CHEK2 mutation female carriers have a higher frequency (80-90%) of estrogen and progesterone (ER/PR) hormone expression in comparison to the general population (luminal).

Besides breast cancer, CHEK2 and the CHEK2*1100delC mutation have been associated with other tumors, mainly colorectal cancer, and prostate cancer. The cancer type of the CHEK2 carrier index seems to determine the risk for first degree relatives for that specific tumor type. This is suggested to be due to the additive effect of the CHEK2*1100delC mutation in a polygenic model.

Since the prevalence of the CHEK2*1100delC allele is high in the Netherlands we addressed the cancer risk and cancer phenotype associated with the CHEK2*1100delC allele in a familial breast cancer setting, see Chapters 7 and 8.

Clinical utility of CHEK2*1100delC mutation testing is currently under debate. Challenges, such as differences in mutation rates in different populations, incomplete penetrance of the mutation in families, and the relative low lifetime risks in carriers, are still subject to discussion.
1.4 Pathways

Intriguingly most known high and moderate breast cancer genes function in two parallel functioning major DNA-repair /signaling pathways; the Fanconi Anemia/Breast cancer (FA/BRCA) pathway and the ATM-CHEK2 pathway (See Figure 2).

Upon DNA-damage both pathways are activated and proteins are recruited for DNA-repair, cell cycle arrest and apoptosis. The proteins in blue are encoded by genes for which germline mutations have been associated with increased breast cancer risk. The P indicates phosphorylation sites and Ub indicate ubiquitination sites by which proteins are activated.

Understanding the genes and their function in DNA-repair processes is valuable in searching other new breast cancer genes and will importantly influence targeted and personalized treatment in the future.

Figure 2 DNA-damage response pathways
With the courtesy of Antoinette Hollestelle and Marijke Wasielewski, Figure; DNA-damage response pathways involved in breast cancer susceptibility from “Discovering moderate-risk breast cancer susceptibility genes”, Current Opinion in Genetics & Development. 2010 20(3):268–276.
Aims and outline of thesis

Mutations in the high risk BRCA1 and BRCA2 breast cancer susceptibility genes are causative in approximately 20% of familial breast cancer. The majority, up to 80% of breast cancer families have an unknown genetic cause of breast cancer. In this thesis we investigate aspects of clinical testing for high and moderate breast cancer risk alleles in the, mainly Dutch, familial breast cancer setting.

Part I concerns BRCA1 and BRCA2 diagnostic testing. A method is evaluated to cost-effectively produce true negative BRCA1/2 test results in women from breast- and-ovarian cancer families in which only paraffin-derived DNA is available when all affected women are deceased. Our method enables to discharge safely a subgroup of unaffected women from these families from cancer risk reducing strategies. The second study investigated if Ductal Carcinoma in situ (DCIS) is part of the BRCA1/2 cancer phenotype. We discuss whether DCIS should be taken into account during risk assessment for finding a BRCA1/2 mutation in a particular patient or family.

In Part II the mutation prevalence and the cancer phenotype of several Fanconi Anemia breast cancer risk genes (BRCA2/FANCD1, PALB2/FANCN, BRIP1/FANCI, FANCD2) is evaluated by investigating families/individuals selected for specific cancer phenotypes.

In Part III the cancer risk and cancer phenotype associated with the moderate breast cancer risk allele CHEK2*1100delC, both in homozygous and heterozygous state, is explored in a large multicenter study. We address the question whether diagnostic testing for the CHEK2*1100delC mutation in a familial non-BRCA1/2 setting is indicated in the Dutch population.
References


References


References


