GENERAL INTRODUCTION AND OUTLINE
BREAST CANCER OUTCOME – SOME FACTS AND FIGURES

Breast cancer is the most common cancer in women worldwide (Ferlay et al., 2010b; Ferlay et al., 2013). Although the incidence of breast cancer in Europe has increased over the years, the survival after breast cancer has improved (Bray et al., 2002; Ferlay et al., 2007; Ferlay et al., 2010a; Ferlay et al., 2013; Forouzanfar et al., 2011). In the Netherlands, woman diagnosed with breast cancer between 1975–1984 had a 5-year survival of 60-80%; this increased to over 80% for patients diagnosed between 1995-2002 (Louwman et al., 2008; Netherlands Comprehensive Cancer Organisation, 2016). Currently the 5-year breast cancer survival is over 90% (Drukker et al., 2013; Netherlands Comprehensive Cancer Organisation, 2016). Factors that caused this improved survival are, among others, early detection (Saadatmand et al., 2015) and improved (adjuvant) treatment (Berry et al., 2005).

Several biological features of breast tumours predict the prognosis of patients, and are therefore used in treatment decisions (Coates et al., 2015; Goldhirsch et al., 2011). Classical tumour features that lead to a worse prognosis are a large size, high histological grade, high number of affected lymph nodes and oestrogen receptor negativity. Thereafter biological subtypes, such as HER2-positive and triple negative, were discovered, and more often used for prognosis prediction. Also, genomic profiling by array-based approaches are getting more popular (e.g. Mamma print) (Goldhirsch et al., 2011; van de Vijver et al., 2002). Prognosis prediction is important to determine the best treatment options and optimal surveillance or screening modalities for specific patient groups (Baird & Caldas, 2013; Coates et al., 2015; Goldhirsch et al., 2011).

In The Netherlands, 20-25% of the breast cancer patients is diagnosed at an age below 50 (Netherlands., 2016). Available evidence strongly suggests that breast tumours are more aggressive in pre-menopausal than in post-menopausal women (Beadle et al., 2011; Brenner et al., 2016; Narod, 2012). This is partly due to the over-representation of aggressive biological features of breast tumours in young patients (Brenner et al., 2016; Liao et al., 2015; Narod, 2012). However, even after controlling for the known biological factors indicative of tumour aggressiveness, young age in itself remains an independent predictor of poor cancer-specific survival (Fredholm et al., 2009; Freedman & Partridge, 2013; Narod, 2012). The proportion of patients having a strong family history of cancer increase with younger ages of diagnosis: hereditary cancers typically present at a young age (Althuis et al., 2003; Narod, 2012). A part of these hereditary breast cancers is related to germline mutations in the BRCA1 or BRCA2 genes.
HIGH RISK BREAST CANCER GENES: **BRCA1 AND BRCA2**

In 1990 (Hall et al, 1990) and 1994 (Wooster et al, 1994) two breast cancer-associated genes were discovered using linkage analysis of a large group of families with cases of early-onset breast cancer. The genes were cloned and the first articles about the genes **BRCA1** and **BRCA2** were published in 1994 (Miki et al, 1994) and 1995 (Wooster et al, 1995), respectively.

*Function of the BRCA1 and BRCA2 genes*

The discovery that germline mutations in the **BRCA1** or **BRCA2** genes lead to a high inherited susceptibility for breast cancer triggered many investigations into mechanisms how both genes, and their protein products, work as tumour suppressors. Many functions of the **BRCA1** and **BRCA2** genes have been established after their discovery (Narod & Foulkes, 2004; Roy et al, 2012; Scully & Livingston, 2000; Venkitaraman, 2002; Venkitaraman, 2004; Venkitaraman, 2014). Both genes play an important role in the response to DNA damage. **BRCA1** and **BRCA2** initiate homologous recombination for the repair of double strand breaks. **BRCA2** binds to RAD51 and localizes to the damaged DNA; in the absence of functional **BRCA2**, homologous recombination is impaired. Although **BRCA1** is also involved in localization with RAD51, the role for **BRCA1** in the response to DNA damage seems to be broader, with for example controlling of the signal transduction process involved in homologous recombination. When homologous recombination is impaired, other repair pathways, such as non-homologous end joining, are used to repair DNA damage. These alternative repair pathways are more error-prone and generate chromosome deletions and translocations. Furthermore, additional functions of **BRCA1**, such as in chromatin remodelling, have also been revealed and may also contribute to the tumour suppression function of **BRCA1**. Taken together, it is argued that **BRCA1** and **BRCA2** work as custodians of chromosome integrity during the cell cycle. Impaired **BRCA1** or **BRCA2** protein functioning leads to genomic instability, which promotes carcinogenesis.

Why **BRCA1** and **BRCA2** mutation specifically lead to a high susceptibility for tumours in the breast, but also ovary and possible few other cancers, is not yet fully explained (Foulkes & Shuen, 2013; Roy et al, 2012; Venkitaraman, 2014). Suggested mechanisms are tissue-specific functions of the genes; the possibility that cells of specific tissues are more sensitive to mutagenesis; and that in some tissues cells lacking both of the **BRCA** alleles can survive longer and therefore can acquire more secondary mutations.
BRCA1 and BRCA2 germline mutations

Many germline mutations in BRCA1 and BRCA2 have been reported (Breast cancer Information Core; https://research.nhgri.nih.gov/bic/) since the year of the discovery of the genes. Germline mutations in the BRCA1 and BRCA2 genes are inherited in an autosomal-dominant way (Miki et al., 1994; Wooster et al., 1995). There are mutations in the forms of intronic changes, missense mutations, insertions, deletions and large genomic rearrangements (Couch et al., 2014). In BRCA1, missense mutations, that interfere with protein functioning and that lead to a high cancer susceptibility, are located in the domains that are critical for the DNA repair activity of BRCA1 (Millot et al., 2012). In BRCA2, missense mutations are mostly located in the DNA binding domain, with an important role for its functioning (Guidugli et al., 2014). Next to the pathogenic mutations that interfere with protein functioning and lead to a high cancer susceptibility, there are several mutations for which the effect on protein function is unknown, called Variants of Uncertain Significance, making it difficult to predict the consequences on risks of breast and ovarian cancers (Eccles et al., 2015; Guidugli et al., 2014; Millot et al., 2012; Moghadasi et al., 2016). The prevalence of women carrying a BRCA1/2 mutation in the general population is low (<0.01%) (Ford et al., 1995; Rahman & Stratton, 1998). However, in several populations founder mutations with a higher prevalence rate have been described. Best known are founder mutations in the Ashkenazi Jewish population (prevalence ~2.5%) (Oddoux et al., 1996; Rahman & Stratton, 1998; Roa et al., 1996; Warner et al., 1999), but also specific founder mutations in BRCA1 are seen in the Dutch population (Brohet et al., 2014; Papelard et al., 2000; Petrij-Bosch et al., 1997).

Cancer risks associated with BRCA1 and BRCA2 germline mutations

Carrying a germline mutation in the BRCA1 or BRCA2 gene that interferes with protein functioning, leads to a very high risk for breast cancer. The risk is estimated to be between 27% and 80% by age 70 years, with BRCA2 mutation carriers on the lower end of the range and BRCA1 mutation carriers on the higher end (Antoniou et al., 2003; Brohet et al., 2014; Chen & Parmigiani, 2007; King et al., 2003; Mavaddat et al., 2013). Also, the risk for ovarian cancer is substantially increased in BRCA1 or BRCA2 mutation carriers compared to woman not carrying a BRCA1 or BRCA2 germline mutation. The risk is estimated to be between 6% and 55% by age 70 years, with again BRCA2 mutation carriers on the lower end of the range and BRCA1 mutation carriers on the higher end (Antoniou et al., 2003; Brohet et al., 2014; Chen & Parmigiani, 2007; King et al., 2003; Mavaddat et al., 2013). A higher susceptibility
for other cancers, such as pancreatic cancer, is also reported but less pronounced (Breast Cancer Linkage, 1999; Iqbal et al, 2012; Thompson et al, 2002b; van Asperen et al, 2005).

Several reports have indicated that specific regions and mutations in BRCA1 and BRCA2 determine a differential relative breast compared to ovarian cancer risk (Gayther et al, 1997; Rebbeck et al, 2015; Thompson et al, 2002a). The inter-individual variability in the risks of breast and ovarian cancer for BRCA1/2 mutation carriers is also determined by environmental factors (Friebel et al, 2014) and other common genetic variants identified through genome-wide association studies (Antoniou et al, 2012; Antoniou et al, 2008; Milne & Antoniou, 2011) are contributors.

**BRCA1/2-ASSOCIATED BREAST CANCER**

About 3-5% of all breast cancer cases are related to germline BRCA1 or BRCA2 mutations (Thompson & Easton, 2004). Breast tumours in BRCA1/2 mutation carriers arise at younger ages than tumours in women not known to carry a BRCA1 or BRCA2 mutation (also referred to as ‘sporadic’) (Breast Cancer Linkage, 1997; Foulkes et al, 1997; Rennert et al, 2007). The prevalence of BRCA1 and BRCA2 mutations in breast cancer patients diagnosed under age 50 is around 6%, while the prevalence in older patients is around 1% (John et al, 2007; Malone et al, 2006; Peto et al, 1999).

![Figure 1](image-url)

**Figure 1:** Prevalence (%) of BRCA1 and BRCA2 mutation carriers in different breast cancer age at diagnosis subgroups. Source - Malone et al. Cancer Research, 2006 - based on 1,628 breast cancer cases.

**BRCA1/2-associated biological tumour features**

Tumours in BRCA1 mutation carriers more often show aggressive biological features that are less common seen in tumours arising in sporadic patients. E.g. tumours in
BRCA1 mutation carriers are more often triple negative and of high grade (Honrado et al, 2006; Lakhani et al, 2002; Phillips, 2001). In contrast to the distinctive phenotype of BRCA1-associated tumours, it is less easy to define histopathological characteristics that distinguish tumours in BRCA2 mutation carriers from tumours in sporadic patients (Honrado et al, 2006; Lakhani et al, 2002; Phillips, 2001).

However, gene expression analyses showed that both BRCA1- and BRCA2-associated tumours exhibit a distinct pattern compared to tumours arising in sporadic patients (Hedenfalk et al, 2001; Honrado et al, 2006; Lakhani et al, 2001). Also, they both exhibit a higher frequency of somatic abnormalities in prognostically important genes such as P53 (Crook et al, 1998; Honrado et al, 2006; Phillips, 2001). Furthermore, comparative genomic hybridization, which investigates DNA copy-number changes and can identify genomic instability, has shown that gross chromosomal changes are more likely to occur in BRCA1- and BRCA2-associated tumours than in tumours arising in sporadic patients (Tirkkonen et al, 1997). Moreover, using these methods specific patterns of genomic changes have been identified that can very well differentiate BRCA1-associated tumours from sporadic cancers (Jonsson et al, 2005; van Beers et al, 2005; Vollebergh et al, 2011; Wessels et al, 2002). Although many of the tumours arising in BRCA1 and BRCA2 mutation carriers exhibit these specific features, there is still considerable histological and molecular heterogeneity between them, and not all tumours have this specific BRCA-related phenotype. Moreover, some of the tumours arising in patients not carrying a BRCA1 or BRCA2 mutation also exhibit this BRCA-related phenotype, also called “BRCA-ness” (Lord & Ashworth, 2016; Turner et al, 2004).

Figure 2: Percentage of tumors with a specific aspect per patient subgroup (BRCA1, BRCA2 vs sporadic). Source - Honrado et al. Modern Pathology, 2005 - based on data from several studies (review).
**BRCA1/2-associated breast cancer outcome**

The role of BRCA1/2 in the DNA damage response pathway, and the aggressive biological features of BRCA1/2-associated tumours, support the hypothesis that patients carrying a BRCA1 and/or BRCA2 mutation might have a worse breast cancer prognosis compared to patients that do not carry a mutation in one of the genes. Another important factor that might also influence the outcome for breast cancer patients carrying a BRCA1/2 mutation is the high risk to develop a second cancer, such as a ipsilateral or contralateral breast cancer, but also ovarian cancer (Chen & Parmigiani, 2007; Kirova et al, 2010; Mavaddat et al, 2013).

A few small studies in the early years after discovery of the BRCA1/2 genes indicated that the outcome of BRCA1/2 mutation carriers may be worse compared to non-carriers (Ansquer et al, 1998; Robson et al, 1998). Whether this worse outcome could be attributed to a worse breast cancer-specific prognosis, or attributed to the higher prevalence of second tumours and their impact on overall survival, is unclear. The ongoing discussion about the impact of germline mutations in the BRCA1 and BRCA2 genes on breast cancer outcome is reflected by multiple reviews of mechanisms and prognosis studies (Bordeleau et al, 2010; Foulkes et al, 2001; Lee et al, 2010; Phillips et al, 1999; Robson, 2000; Roy et al, 2012; Shuen & Foulkes, 2011; Welch et al, 1998; Yoshida & Miki, 2004).

**BRCA1/2-associated breast cancer treatment**

It is suggested that mutations in BRCA1 or BRCA2 make tumours more sensitive to adjuvant chemotherapy. DNA cross-linking agents, such as platinum-containing chemotherapy, generate DNA damage that can only be repaired by BRCA1/2-dependent homologous recombination, and might therefore be more effective in tumours arising in BRCA1/2 mutation carriers (Arun et al, 2011; Huzarski et al, 2013; Robson, 2011; Robson et al, 2004).

More novel chemotherapeutic agents, the poly(ADP-ribose) polymerase (PARP) inhibitors, work by loss of function of PARP which results in accumulation of single strand breaks; these single strand breaks will be converted to double strand breaks. Without BRCA1/2-dependent homologous recombination, double strand breaks will accumulate and that will result in tumour cell death. Therefore, the PARP inhibitors are a promising treatment strategy for BRCA1/2-associated tumours, and research into the risks and benefits of the treatment is currently ongoing (Livraghi & Garber, 2015; Scott et al, 2015; Sonnenblick et al, 2015).
There have been speculations about increased sensitivity to radiotherapy for *BRCA1* mutation carriers, preventing local recurrences and ipsilateral breast cancers in patients who received radiotherapy (Kan & Zhang, 2015) but also increasing the risk for contralateral breast cancer (Bernstein *et al*, 2013; Broeks *et al*, 2007; Drooger *et al*, 2015). Moreover, there is ongoing discussion about the benefits and risks of radiotherapy in *BRCA1/2* mutation carriers (Drooger *et al*, 2015; Kan & Zhang, 2015).

**IDENTIFICATION OF BRCA1 AND BRCA2 GERMLINE MUTATION CARRIERS**

Identification of women with *BRCA1* and *BRCA2* mutations is important because it generates the possibility to offer them options to manage their high breast and ovarian cancer risks; e.g. more intensive screening or risk-reducing surgery (prophylactic mastectomy and/or salpingo-oophorectomy) (Bermejo-Perez *et al*, 2007; Hartmann & Lindor, 2016; Narod, 2010; Rebbeck *et al*, 2009). Additionally, it is important to identify breast cancer patients with a *BRCA1/2* mutation because their potentially worse breast cancer outcome, and the promising targeted treatment strategies, e.g. PARP inhibitors, that will be available for this patient group in the near future (Livraghi & Garber, 2015; Scott *et al*, 2015; Sonnenblick *et al*, 2015).

Women carrying a *BRCA1* and *BRCA2* mutation often have many relatives affected by breast and/or ovarian cancer. However, the prevalence of women carrying a *BRCA1/2* mutation in the general population is low (Rahman & Stratton, 1998), and within breast cancer patients it was estimated to be 1-2% for each gene (Peto *et al*, 1999; Thompson & Easton, 2004). Therefore, referral guidelines for testing are mainly based on family history of breast cancer and ovarian cancer of the woman (Gadzicki *et al*, 2011; NABON, 2012). Furthermore, for woman already diagnosed with breast cancer, age at diagnosis is also included in referral guidelines (Gadzicki *et al*, 2011; NABON, 2012); in young breast cancer populations a larger part of the patients is associated with *BRCA1/2* mutations (Peto *et al*, 1999).

**RATIONALE AND OUTLINE OF THIS THESIS**

Although a lot of research in *BRCA1* and *BRCA2* mutation carriers has been performed, there is ongoing discussion about the outcome after breast cancer for these women.
Studies done so far on this subject have been challenged by the relatively low prevalence of BRCA1/2 mutations, and were limited due to methodological issues.

The main objective of this thesis is to: *Investigate with increased power and minimized bias whether germline mutations in the BRCA1 and BRCA2 genes affect the outcome (both survival and second tumours) of breast cancer patients.*

By answering this question, this thesis will contribute to improvement of personalized surveillance and treatment choices after breast cancer, guide re-evaluation of BRCA1/2 mutation screening and contribute to the overall understanding of the aetiology of breast cancer.

Chapter 2 describes a systematic review of the previously published literature about the impact of BRCA1 and BRCA2 mutations on the prognosis of breast cancer patients. Insight is given into limitations of previously performed studies, and the role of methodological quality in the inconsistencies of the published results. Evidence-based conclusions are described for recurrence-free survival, metastasis-free survival, breast cancer-specific survival and overall survival.

All results described in the remaining chapters of this thesis are based on the BOSOM (Breast Cancer Outcome Study Of Mutation Carriers) study, a retrospective cohort of young breast cancer patients diagnosed in 1970-2002 in the Netherlands. Chapter 3 explains the used methods and general characteristics of the BOSOM study. Different aspects of this consecutive patient series, such as the age distribution and tumour characteristics related to the BRCA1 and BRCA2 mutation status, are highlighted. Chapter 4 describes the results we found in the BOSOM study for the same survival outcomes we reported in the systematic review in chapter 2. In chapter 4 we also evaluate whether differential clinico-pathological factors influence and/or mediate the effect of BRCA1/2 germline mutations on breast cancer prognosis. Moreover, we explore whether the potential survival difference between BRCA1/2 mutation carriers and non-carriers can be explained by a differential chemotherapy response or increased risk of second primary tumours. In chapter 5 we evaluate again the prognosis of BRCA1/2 mutation carriers compared to non-carriers, but in this chapter, we explore whether breast conserving surgery, in which the affected breast is conserved and there remains a risk for in-breast recurrences, is a safe option for this group of patients. Randomized clinical trials investigating the outcome of breast conserving surgery versus mastectomy are not available for BRCA1/2 mutation carriers.
In the next two chapters we confirm the high susceptibility to cancer for \textit{BRCA1/2} mutation carriers, as defined by incidence the of second primary breast cancer and ovarian cancer. In \textbf{chapter 6} the risk estimates for contralateral breast cancer from the BOSOM study together with a summary of the previously published literature are described. An important risk modifier is identified. In \textbf{chapter 7} the risk estimates for contralateral breast cancer and ovarian cancer, related to the location of the \textit{BRCA1} mutations, are described.

Because of the potential differential outcomes and the high risks to develop second breast cancer and ovarian cancer (as described in the previous chapters), it is important to identify the \textit{BRCA1/2} mutation carriers among the breast cancer patients, in order to offer them surveillance and treatment options to reduce their risks. In \textbf{chapter 8} we evaluate the performance of the current Clinical Genetic Centre referral criteria to identify \textit{BRCA1/2} mutation carriers among breast cancer patients in the BOSOM study. Options to optimize the criteria are explored.

In the last part of this thesis, \textbf{chapter 9}, the main findings are described and placed into perspective. Strengths and weaknesses are discussed; conclusions are drawn and clinical implications are given.

Next to the high impact \textit{BRCA1/2} germline mutations, other low risk breast cancer germline variants exist in genes that are also involved in the DNA damage response pathway. These variants may also be of influence on the prognosis of patients (Guo et al, 2015; Pirie et al, 2015). Although other genetic variants are not the main focus of this thesis, one hypothesis-generating study is included in \textbf{Appendix 1}. Appendix 1 describes the effect of two variants, \textit{MDM2} SNP309 and \textit{TP53} R72P, on the survival of breast cancer patients. The interaction with specific tumour subtypes (based on gene expression profiling) is explored. The results described in appendix 1 are not based on data of the BOSOM study, but on another consecutive patient series with more extensive, i.e. gene expression data, available (van de Vijver et al, 2002).
REFERENCES


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