Chapter 9
Discussion
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The contribution of inherited factors to the etiology of overall breast cancer is estimated to be up to 30%. Until now family history has been an effective tool to identify women with a high risk of breast cancer due to genetic susceptibility.

In the Netherlands referral and genetic testing is currently offered in medical practice according to clinical guidelines (VGKN/NABON). In families with clustering of breast cancer only, or both breast cancer and ovarian cancer, the two known high risk breast cancer predisposing genes, BRCA1 and BRCA2 are presently screened for pathogenic mutations. In the Netherlands until 2012 more than 50,000 women underwent BRCA1/2 testing, and approximately 2,000 BRCA1- and 1,000 BRCA2-positive families have been diagnosed (personal communication with F. Hogervorst, NKI/AvL). BRCA1/2 mutations are identified in about 20% of the breast cancer families. This implies that in the larger majority of the breast cancer families seen at a family cancer clinic the genetic cause remains unresolved. Surveillance guidelines based on family history risk assessment models are used to advise unaffected women from these breast cancer families.

In this thesis we investigated several approaches to improve personalized genetic breast cancer risk assessment.

In Chapter 2 we show that testing of archival paraffin-derived DNA of women is accurate for specific BRCA1 and BRCA2 mutations, namely the Ashkenazi Jewish founder mutations, and can therefore establish a genetic diagnosis within a family. During the last years diagnostic genetic laboratories have been reluctant to perform mutational screens of BRCA1/2 on paraffin material due technical reproducibility issues. The current challenge is to develop reliable diagnostics of all coding regions of BRCA1/2 on formalin-fixed and paraffin-embedded (FFPE) material. New upcoming techniques such as massively parallel sequencing will probably be more suitable to perform such extensive DNA-tests on paraffin embedded tissue.

Testing of paraffin-embedded material of deceased women from high risk breast cancer families is clinically relevant as a genetic diagnosis in the family enables the diagnosis of unaffected female relatives as “true-negative” for the familial genetic cancer risk. Consequently these women, and their female offspring, can stop their intensive breast surveillance and do not have to consider risk-reducing surgery such as preventive bilateral salpingo-oophorectomy (pBSO).

Secondly, more knowledge of the BRCA1 and BRCA2 tumor phenotype may improve the selection of women / families for BRCA1/2 testing. Up till now Ductal Carcinoma in situ (DCIS) is not considered part of the clinical criteria to select cases.
for BRCA1/2 diagnostics. This is probably based on a misunderstanding that DCIS as precursor for invasive breast cancer is not part of the BRCA1/2 phenotype. However, pure DCIS was not included in the original reports on the histopathology of BRCA1-and BRCA2-related breast cancers. These studies only reported less DCIS surrounding invasive BRCA1/2-related breast cancers when compared to non-BRCA1/2 invasive breast cancer.

In this thesis we discuss if pure DCIS is likely to be associated with BRCA1/2 mutations when taken family history of ovarian cancer or (early onset) breast cancer into account (Chapter 3). Recent studies confirm our findings and show that (high risk) women with DCIS are appropriate candidates for BRCA1/2 testing and that testing can contribute to prevention of breast cancer and ovarian cancer. We therefore advocate to include DCIS in the clinical criteria for selection of women / families for BRCA1/2 testing.

Thirdly, the effectiveness of BRCA1/2 testing may further improve by gaining knowledge about the clinical impact of Variants of Unknown Significance (VUS). For example the R1699Q variant in BRCA1 was recently described by Spurdle et al. This VUS turned out to confer a moderate cumulative breast cancer risk and ovarian cancer risk to age 70, both of 24%. These risks estimates were only possible by pooling and analyzing 68 families with this specific variant in an international collaboration. An important tool to elucidate the clinical impact of recurrent VUS is data sharing on pedigree information and mutations, both at the national and international level. A problem herein remains the rarity of many VUS. Fortunately, in the Netherlands a Collaborative Group on Hereditary Breast Cancer, HEBON resource, initiated a nationwide database to gather information on genetic and non-genetic factors of BRCA1/2-mutation positive and mutation negative breast-and-ovarian cancer families (see www.hebon.nl).

For BRCA1/2-mutation carriers their precise and personalized risk assessment will importantly improve by determining influential modifiers in the nearby future. Multiple low-risk Single Nucleotide Polymorphism’s (SNP) will substantially alter (lower or heighten) the cumulative breast cancer and ovarian cancer risk in female carriers of a BRCA1/2-mutation. For instance recently Couch 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 female carriers. Cancer risk in BRCA1/2-mutation carriers is further influenced by their birth cohort (higher risk when born after 1940), possibly due to epigenetic factors and/or non-genetic environmental risk factors (Gail model). Currently these environmental
risk modifiers are translated into common advices like limited use or adjusted hormonal anti-conception, healthy life-style, non-smoking, sporting and preventing postmenopausal overweight. At present no all-inclusive risk model including all genetic and environmental factors is available for breast cancer risk assessment in clinical settings.

No other high risk breast cancer genes (often named BRCA3 or BRCAX) have been found since 1995, and it seems unlikely that major high risk breast cancer susceptibility genes are still to be identified. However, several moderate risk breast cancer susceptibility genes and over 60 common low risk breast cancer susceptibility loci have been reported. In the second part of this thesis we focused on the Fanconi Anemia–Breast Cancer (FA-BRCA) pathway and its moderate risk breast cancer susceptibility genes. The PALB2/FANCN (PArtner and Localizer of BRCA2) gene and the BRIP1/FANCJ (BRCA1 interacting protein C-terminal helicase 1) gene are both examples of such moderate risk breast cancer susceptibility genes. PALB2 has a population-dependent relatively low prevalence of 0.5-0.9% in breast cancer cases and was estimated to be associated with a relative breast cancer risk of 2.3 and higher. PALB2 was also described to be an important pancreatic cancer susceptibility gene with a possible mutation prevalence of 3-4% in familial pancreatic cancer. BRIP1 was described to have a low prevalence of ~0.5% or less in familial breast cancer cases with an estimated relative risk for breast cancer of 2 in comparison to the population risk.

We investigated the prevalence and phenotype of PALB2 mutations in different cohorts of familial breast cancer. We selected 110 non-BRCA1/2 breast cancer families with male breast cancer, or ovarian cancer and/or pancreatic cancer. In only one male breast cancer patient a mutation was identified, the PALB2 frameshift mutation c.509_510delGA; p.Arg170X. Of note, the mutation did not segregate with breast cancer in the family (Chapter 4), confirming the moderate penetrance and heterogeneous risk profile of mutations in this gene.

A low PALB2 mutation prevalence in different cohorts of breast-and/or pancreatic cancer and no support for diagnostic testing in a breast-pancreatic cancer setting was endorsed by others. Further, in a large non-BRCA1/2 breast cancer cohort (n=734) no deletions were found in PALB2, BRIP1, and FANCD2 (Chapter 5). Later studies confirmed the lack of large rearrangements for these genes in breast cancer.

Bi-allelic mutations in the FA-genes BRCA2/FANCD1 and PALB2/FANCN cause a severe form of FA with a high risk to solid tumors such as Wilms tumor (WT) in
Therefore we tested a cohort of 47 sporadic Wilms tumor patients for bi-allelic BRCA2 and PALB2 mutations. No bi-allelic BRCA2 and PALB2 mutations were identified (Chapter 6).

We conclude that in the Netherlands PALB2/FANCN-gene deletions do not play a major role in non-BRCA1/2 breast cancer families, nor do PALB2/FANCN-gene mutations in breast cancer families with other BRCA2-associated tumors, namely pancreatic cancer, ovarian cancer, male breast cancer, and in Wilms tumors (Chapter 4-6).

In the third part of this thesis, we focus on another moderate risk breast cancer susceptibility gene, the CHEK2 gene, and in particular on the specific mutation CHEK2*1100delC. The population-based breast cancer risk for a female heterozygous CHEK2*1100delC carrier is associated with a 2-fold, corresponding to an estimated lifetime risk for breast cancer of approximately 20-25%. CHEK2*1100delC has a prevalence of 1.0% in the general population of Northern Europe and a prevalence of 2.5% in unselected breast cancer patients and 5% in familial non-BRCA1/2 breast cancer patients. In the Dutch population the prevalence of CHEK2 mutations other than the CHEK2*1100delC is negligible (unpublished data thesis M. Waselewski and own data). The CHEK2*1100delC mutation was calculated to confer a higher lifetime breast cancer risk in heterozygous female carriers of 34-44% till age 70 in a non-BRCA1/2 familial or bilateral breast cancer setting. Primarily the large CHEK2*1100delC meta-analysis based upon n=4,546 familial breast cancer cases by Weischer et al. initiated the international debate on the clinical utility of diagnostic CHEK2*1100delC testing. Differences in mutation rates in populations and the incomplete penetrance are important issues in the discussion.

In this thesis we describe a study in 2,554 breast cancer families from three Dutch family cancer clinics. In Chapter 8 we compared breast cancer incidence in first degree female relatives of non-BRCA1/2, CHEK2*1100delC positive breast cancer cases with the breast cancer incidence in first degree female relatives of non-BRCA1/2, non-CHEK2*1100delC breast cancer cases. We were the first to use this specific approach within a familial breast cancer setting. Strengths of our approach are that it is not prone to bias with respect to family/case selection, and that the results are applicable to the setting of our family cancer clinics.

The CHEK2*1100delC mutation caused a significant excess of breast cancer risk of approximately 2-fold (HR 1.6-2.0) in a non-BRCA1/2 familial breast cancer setting when compared with the increased breast cancer risk related to the familial occurrence of breast cancer. The cumulative breast cancer incidence at age 75 was
53% (95% CI: 45 - 62%) in first degree female relatives (with a likelihood of at least 50% of being CHEK2*1100delC carrier) in the CHEK2*1100delC positive families.

Our data strongly support clinical routine testing of CHEK2*1100delC in non-BRCA1/2 breast cancer cases. First degree female relatives of CHEK2*1100delC positive breast cancer cases would be advised intensive breast surveillance in a specialist environment from age 35 onward concordant to current guidelines.

Future studies with the prospective assessment of the actual cumulative breast cancer risk for first degree relatives of non-BRCA1/2, CHEK2*1100delC positive breast cancer cases with known genotypes are needed to determine and differentiate breast surveillance, particularly in the carriers and non-carriers of the CHEK2*1100delC mutation. Till that time, testing for CHEK2*1100delC of the first degree female relatives of non-BRCA1/2, CHEK2*1100delC positive cases is not advocated.

Our study also confirmed a 2-fold increased (relative) risk for contralateral breast cancer in heterozygous CHEK2*1100delC mutation carriers as well as their sisters in comparison to non-BRCA1/2, non-CHEK2*1100delC breast cancer cases and their sisters. With reference to a large German study that estimated an overall 25-year cumulative contralateral breast cancer risk in familial non-BRCA1/2 breast cancer cases and their relatives of 17% (not differentiated to age of onset of primary breast cancer), our data would result in a cumulative contralateral breast cancer risk of about 34% in these CHEK2*1100delC positive breast cancer cases and their sisters. The increased risk of contralateral breast cancer additionally warrants CHEK2*1100delC testing of all breast cancer cases from familial settings. In case of carrier status, prolonged surveillance in breast cancer cases is indicated, aiming at early detection of a second primary breast cancer.

Although CHEK2 is well conserved in evolution and has been suggested to play an important role in cell cycle, apoptosis and DNA-repair processes, we surprisingly discovered the occurrence of germline homozygosity of CHEK2*1100delC in living humans. About 1:300 non-BRCA1/2 breast cancer cases from our cohort of 2,554 breast cancer cases turned out to be homozygous for the CHEK2*1100delC mutation. Of note, germline homozygosity of CHEK2*1100delC was earlier reported in a patient with colorectal cancer and was once mentioned in a bilateral breast cancer case.

In Chapter 7 we were the first to associate a severe cancer phenotype related to germline homozygosity of CHEK2*1100delC within nine families. Not only bilateral breast cancer, early onset breast cancer, but also colorectal cancer may be part of the phenotype. We identified in total 11 CHEK2*1100delC homozygous female carriers; all had developed breast cancer, and 8 out of 11 had multiple primary tumors, of which 5 bilateral breast cancer. Median age of diagnosis of (first) breast cancer for the
homozygous females was 47 years old (average age 44 years).
The severe cancer phenotype with early onset, bilateral breast cancer and multiple primary tumors related to homozygosity of \( \text{CHEK2*1100delC} \) was later confirmed by several other research groups in a small number of cases.\(^9\)

We investigated and confirmed the hypothesis that the breast cancer risk for homozygous \( \text{CHEK2*1100delC} \) female carriers is more than twice the risk of heterozygous carriers within a familial breast cancer cohort, and therefore likely to be more than 6-fold increased breast cancer risk. These data give, again, compelling evidence that \( \text{CHEK2*1100delC} \) should be tested in the setting of familial breast cancer, and even more so in the setting of a pattern of recessively inherited breast cancer within a family. Homozygous female carriers should be offered the same preventive options as are available for \( \text{BRCA1/2} \) carrier women.

Interestingly, functional assays to investigate homozygous \( \text{CHEK2*1100delC} \) cell sensitivity so far did not discover a particular cellular phenotype (unpublished data).

Currently tumor profiles of the tumors of homozygous \( \text{CHEK2*1100delC} \) carriers are being analyzed for specific characteristics.

Clinical geneticists and other specialists in this field are challenged in the upcoming years to incorporate the new genetic information on breast cancer risk alleles in clinical practice, resulting in more accurate and personalized risk figures. The rather straightforward model of dominant inheritance of \( \text{BRCA1/2} \) mutations is now past time and has moved to complex inheritance models. In the near future, women will benefit by incorporating the modifiers of \( \text{BRCA1/2} \), and \( \text{CHEK2*1100delC} \) mutation in routine molecular diagnostics for breast cancer susceptibility.

The integral translation into clinical practice of all identified high risk, moderate risk, and low risk breast cancer alleles together with established non-genetic risk factors, is challenging, and will take time.

The ultimate goal is not only to assess the breast cancer risk in an individual woman, but to prevent the disease and/or cure affected women without major side effects of the therapy. Knowledge of the breast cancer susceptibility genes, their biological function and their clinical impact on risk, may eventually help to achieve these goals.
References


