Summary

Approximately 14,000 women are yearly diagnosed with breast cancer in the Netherlands. About 10-15% of women with breast cancer have an affected female relative with the disease. Current estimates indicate that about 20% familial breast cancer is due to a mutation in one of the high risk breast cancer susceptibility genes, BRCA1 or BRCA2. In those families often early onset breast cancer and/or ovarian cancer occur. In the majority of families with multiple cases of breast cancer without a mutation in BRCA1 or BRCA2, other genes or a combination of genetic and non-genetic factors are present. Fixed surveillance guidelines based on family history risk assessment models are used to advise unaffected women from these breast cancer families.

This thesis focuses on the clinical impact/utility of high and moderate breast cancer susceptibility genes, in order to improve personalized breast cancer risk assessment in women at inherited increased risk.

Part I (Chapters 2 and 3) of the results includes two BRCA1/2 related studies to explore a better diagnostic yield in BRCA1/2 testing. In these studies the three Ashkenazi Jewish (AJ) BRCA founder mutations, BRCA1*185delAG, BRCA1*5382insC, BRCA2*6174delT, were used as they account for 95% of the hereditary breast-and-ovarian cancer in this population.

In Chapter 2 we investigated the accuracy/concordance of AJ BRCA founder mutations genotyping (using multiplex PCR) in 160 blinded formalin-fixed and paraffin-embedded (FFPE) normal tissue derived DNA samples from women previously tested for the three mutations with lymphocyte-derived DNA. There was 100% concordance of 160 genotypes (120 mutation samples) derived from archival DNA compared to lymphocytes-derived DNA genotypes.

This study demonstrates the possibility of establishing a genetic diagnosis within a family even when affected women are deceased. Unaffected women from these families will be certain about the absence or presence of their personal increased cancer risk, and may act accordingly with respect to surveillance or risk-reducing interventions.

In Chapter 3 we investigated the association between Ductal Carcinoma in situ (DCIS) as a pre-stage for invasive breast cancer and BRCA mutations. AJ founder mutation frequency was compared in 3 groups of women with DCIS in comparison to controls with invasive breast cancer: 1) In the prevalent series of women with DCIS the frequency was 3/62 (4.8%) compared to 15/130 (11.5%) in breast cancer, 2) 0/58
In an incident series of women with DCIS compared to 6/116 (5.2%) controls with breast cancer and 3) in a clinic-based series of women with DCIS referred for hereditary cancer risk assessment the frequency was 10/79 (12.7%), similar to the frequency in IBC. In group 3, mutations were associated with family history of ovarian cancer (odds ratios (OR) 13.35, 95% confidence interval (CI): 2.48-71.94, \( p = 0.003 \)) or early onset breast cancer (OR 16.23, 95% CI: 1.68-157.01, \( p = 0.02 \)) but not with AJ ethnicity or age at diagnosis. Since women with DCIS and women with invasive breast cancer have a comparable BRCA1/2 mutation detection rate if family history of breast-and-ovarian cancer is taken into account, DCIS should therefore be considered as part of the BRCA1/2 tumor phenotype and part of the clinical criteria to select cases for BRCA1/2 diagnostics.

In Part II of the results of this thesis (Chapter 4-6) we focused on breast cancer genes related to the Fanconi Anemia (FA)-Breast Cancer (BRCA) pathway. As our group is reference center in the Netherlands for research and diagnostics on Fanconi Anemia, we were in the position to investigate the mutation prevalence of several known moderate risk Fanconi anemia breast cancer genes. We focused in particular on PALB2 (Partner and Localizer of BRCA2), FANCN, as this gene closely interacts with the BRCA2(FANCD1)-gene. These genes share clinical characteristics; mono-allelic germline mutations of BRCA2 and PALB2 are risk alleles of female breast cancer and have been reported in familial pancreatic cancer and bi-allelic mutations in PALB2/FANCN cause a severe form of Fanconi anemia (FA) with high risk to solid childhood cancers, such as Wilms tumors, just like BRCA2/FANCD1.

In view of these phenotypical similarities, we performed PALB2 mutation analysis in several cohorts. In Chapter 4 we investigated the mutation prevalence of PALB2 in a cohort of 110 non-BRCA1/2 cancer patients from families with additional BRCA2-associated tumors: a) 53 ovarian cancer patients from female breast-and/or ovarian cancer families; b) 45 breast cancer patients with a first or second degree relative with pancreatic cancer; and c) 12 male breast cancer patients from female breast cancer families. One truncating PALB2 mutation was found in a male breast cancer patient. Despite our selection we did not find an enrichment for the prevalence of germline heterozygous PALB2 mutations. Based on the low yield we concluded that diagnostic testing of PALB2 in non-BRCA1/2 families does not seem to be worthwhile in the Dutch familial breast cancer setting. Latter studies confirmed 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.
In Chapter 5 we were the first to systematically investigate the occurrence of deletions in the FA-genes, \textit{PALB2/FANCN}, \textit{BRIP1/FANCJ} and \textit{FANCD2} by Multiplex Ligation-dependent Probe Amplification (MLPA) in a large non-\textit{BRCA1/2} familial breast cancer cohort. No aberrations were detected in a cohort of 734 Dutch breast cancer patients, suggesting large deletions within these genes do not significantly contribute to breast cancer susceptibility in familial cases.

Since FA is phenotypically variable we investigated in Chapter 6 the involvement of bi-allelic \textit{BRCA2} and \textit{PALB2} germline FA mutations in a Dutch unselected cohort of 47 Wilms tumor patients. This did not reveal bi-allelic pathogenic mutated cases, indicating that these genes do not seem to play a major role in sporadic Wilms tumor.

In Part III (Chapters 7 and 8) of the results of this thesis we investigated the clinical phenotype and impact of the highly prevalent and moderate breast cancer risk mutation, \textit{CHEK2*1100delC}, in a large multicenter cohort of 2,554 women with breast cancer from a Dutch familial breast cancer setting.

In Chapter 7 we describe nine families with recessively inherited homozygosity for the \textit{CHEK2*1100delC} mutation causative for a severe cancer phenotype. Not only bilateral breast cancer, early onset breast cancer, but also colorectal cancer may be part of the phenotype. We identified in total 11 \textit{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).

Homozygosity is calculated to happen much more frequent in a familial breast cancer setting (approximately 1:300 non-\textit{BRCA1/2} families) then expected purely based on population frequency. Homozygous women were calculated to have a more than 8-fold increased breast cancer risk. \textit{CHEK2*1100delC} homozygous women should be offered the same preventive options as are available for \textit{BRCA1/2} carrier women.

In Chapter 8 we separately investigated the cancer phenotype and risk associated to the \textit{CHEK2*1100delC} allele by estimating and comparing cancer incidence in first degree relatives of heterozygous \textit{CHEK2*1100delC} mutation positive familial non-\textit{BRCA1/2} breast cancer cases with first degree relatives of non-\textit{BRCA1/2} familial breast cancer cases without the \textit{CHEK2*1100delC} mutation. The \textit{CHEK2*1100delC} mutation caused a significant excess of breast cancer risk of approximately 2-fold (HR 1.6-2.0) in a non-\textit{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 study also confirmed a 2-fold increased (relative) risk for contralateral breast cancer in heterozygous CHEK2*1100delC mutation carriers as well as their sisters. Therefore it is important to keep breast cancer patients who are carrier under prolonged surveillance for early detection of a second primary breast tumor or offer them preventive operational options.

The incidence of other cancers did not significantly differ between relatives of non-BRCA1/2, CHEK2*1100delC positive versus relatives of non-BRCA1/2, non-CHEK2*1100delC familial breast cancer cases.

CHEK2*1100delC is an excellent example of a moderate breast cancer risk allele for which time has come to implement in genetic counselling. Due to the high prevalence of the CHEK2*1100delC mutation in a Dutch breast cancer setting and risks as described in our studies, we should test for the CHEK2*1100delC mutation in addition to mutations of BRCA1/2 in our population and integrate this in risk prediction and individualized preventive strategies.

Chapter 9 discusses the topics in this thesis and how these publications (may) contribute to optimizing breast cancer risk assessment and advices in women from breast cancer families in the Netherlands. In the nearby future we will be able to improve BRCA1/2 testing by incorporating paraffin-derived DNA testing, include DCIS in cases/families in our clinical ascertainment for BRCA1/2 diagnostics and to include important BRCA1/2 risk modifiers.

Testing the CHEK2*1100delC mutation in a familial non-BRCA1/2 setting in the Netherlands is clinically relevant for improving contralateral breast cancer risk determination and for optimizing breast cancer preventive strategies in female relatives.