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**Author:** Joosse, Simon Andreas  
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Summary

Chapter 1

One of the strongest risk factors for developing breast cancer is a family history of the disease. Although in many families the cause of the high breast cancer incidence can not be identified, most of the DNA mutations that are found reside within the genes BRCA1 and BRCA2. A pathogenic mutation in one of these genes increases the risk for developing breast or ovarian cancer substantially and it is therefore of high clinical importance to recognize the mutation. The gold standard to determine mutations is direct sequencing of genomic DNA. Because the prevalence of a mutation in BRCA1 or BRCA2 is in general low, pre-selection is performed based on family characteristics to determine the eligibility for genetic testing. Still, of all the breast cancer families that are eventually screened, it is estimated that approximately 15% of the BRCA1 and BRCA2 carriers are missed with standard DNA diagnostics. New techniques are being applied that are based on molecular characteristics of the tumor to identify the underlying genetic defects. Because BRCA1 and BRCA2 are both involved in DNA repair by homologous recombination, defects in one of these genes will be followed by chromosomal instability. Copy number alterations can be investigated by array comparative genomic hybridization (aCGH) which is the main technique used for studies described in this thesis.

Chapter 2

Usually, tumor material is formalin-fixed and paraffin-embedded (FFPE) after it has been surgically removed. This way, the malignant tumor can be preserved and even after long periods of time examined with newly developed techniques. However, fixation time and storage duration can affect the quality of the DNA negatively and the material might not always be suitable for array CGH. We have therefore designed a multiplex PCR that is able to produce amplification fragments of 100, 200, 300, and 400 bp depending on the quality of the DNA. Sample material, which allowed the amplification of at least the 100 and 200 bp fragments, showed to be suitable for array CGH.

Chapter 3

To standardize array CGH, and therefore be able to obtain reproducible results, we have developed an automated hybridization protocol optimized for FFPE material using a hybridization station. By studying the different hybridization durations, temperatures, washing conditions, and hybridization mixture contents, we were able to minimize noise, maximize the dynamic range, and obtain very reproducible array CGH data.

Chapter 4

To find markers specific for BRCA1-mutated breast cancer, we designed a classification method based on array CGH data. Chromosomal aberrations of 18 BRCA1-mutated and 32 sporadic breast tumors were obtained by array CGH and the two groups were compared. Gains on chromosome 3q and 6p and losses on chromosome 5p, 5q, 7p, 12q, and 20q were identified to be characteristic for BRCA1-mutated tumors. Based on the tumor group specific characteristics, a classifier was designed and 16 BRCA1-mutated and 16 sporadic breast tumors were tested in order to validate the classifier, resulting in a sensitivity and specificity of 88% and 94%,
respectively. Next, array CGH profiles of 48 non-BRCA1/2 tumors were tested using our classifier. We identified two cases to be BRCA1 like, of which we could identify BRCA1 deficiency through promoter hypermethylation in one of them.

Chapter 5

In order to find markers specific for BRCA2-mutated breast cancer, array CGH profiles of 28 BRCA2-mutated and 28 sporadic breast tumors were obtained. Chromosomal aberrations were compared and a classifier was constructed. Chromosomal regions that were characteristic for BRCA2-mutated breast tumors were gain on chromosome 17q and loss on chromosome 13q and 14q. Validation of the classifier was performed using 19 breast tumors of each of the two groups and resulted in a sensitivity and specificity of 89% and 84%, respectively. We tested the array CGH profiles of 89 non-BRCA1/2 breast tumor cases and found 17 cases to be BRCA2 like. Using additional analyses, clear indications were found that three of the BRCA2 like cases were indeed BRCA2 deficient.

Chapter 6

In order to investigate possible similarities between hereditary and sporadic BRCA1 deficient breast tumors, array CGH was performed on BRCA1-mutated and sporadic basal-like breast tumors. Within the sporadic basal-like tumor group, approximately half of the tumors were deficient for BRCA1 gene expression and presented array CGH profiles that were similar to that of true BRCA1-mutated breast tumors. Sporadic basal-like tumors proficient for BRCA1 gene expression exhibited array CGH profiles that were not similar to BRCA1-mutated breast cancer but rather resided to non-basal-like breast tumors using unsupervised hierarchical clustering.

Chapter 7

In more than half of the breast cancer families that are screened for mutations in BRCA1 or BRCA2, no mutation is found. These non-BRCA1/2 breast cancer patients form a large heterogeneous group. To be able to find breast cancer susceptibility genes through linking analysis, more homogenous groups are required. We have therefore investigated 58 tumors from non-BRCA1/2 (BRCAX) families and obtained the array CGH profiles of the genomic make-up of the tumors. The array CGH profiles of the BRCAX tumors were dissimilar to those of BRCA1- or BRCA2-mutated breast cancer. Within the families, a subgroup was identified that is characterized by gain of chromosome 22 that might be an interesting marker for further studies.

Chapter 8

In the last chapter of this thesis, the work that is presented here is discussed in more detail and compared to current literature; in addition, follow up studies are handled. After publication of our array CGH BRCA1 and BRCA2 classifiers, several studies applied our technique to indicate the pathogenicity of unclassified variants. Moreover, the classifiers have been used in order to find homologous recombination deficient tumors, that would respond differently on certain chemotherapy compared to tumors, in which homologous recombination is still intact.