Search for new breast cancer susceptibility genes

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CHAPTER 1

AIMS AND OUTLINE OF THE THESIS

Worldwide, breast cancer is the most commonly occurring cancer among women. It accounts for 22% of all female cancers and the estimated annual incidence of breast cancer is about one million cases. Many risk factors have been identified but a positive family history remains among the most important ones established for breast cancer, with first-degree relatives of patients having an approximately two-fold elevated risk. It is currently estimated that approximately 20-25% of this risk is explained by known breast cancer susceptibility genes, mostly those conferring high risks, such as *BRCA1* and *BRCA2*.

However, these genes explain less than 5% of the total breast cancer incidence, even though several studies have suggested that the proportion of breast cancer that can be attributed to a genetic factor may be as high as 30%. It is thus likely that there are still breast cancer susceptibility genes to be found. It is presently not known how many such genes there still are, nor how many will fall into the class of rare high-risk (e.g. *BRCAX*) or of common low-risk susceptibility genes, nor if and how these factors interact with each other to cause susceptibility (a polygenic model). In general high-risk susceptibility genes will cause typical breast cancer families, which are characterized by breast cancer at an early age, bilateral breast cancer, the occurrence of other specific cancer types in the family (for example ovarian cancer or male breast cancer) and an autosomal dominant inheritance pattern.

On the other hand individual low-risk genes probably do not couse familial clustering of breast cancer. However it is possible that if there are many low-risk genes, different combinations of such genes could be involved in individual breast cancer susceptibility and familial clustering of breast cancer might occur. Early work of the Breast Cancer Linkage Consortium (BCLC) showed that respectively 52% and 32% of families with at least four cases of breast cancer diagnosed under 60 are caused by *BRCA1* and *BRCA2*. When selecting families with breast cancer and one or more cases with ovarian cancer 81% of the families is explained by *BRCA1* and 14% by *BRCA2*. However, when selecting families with four or more cases of breast cancer diagnosed under 60 and no cases of ovarian cancer or male breast cancer only 33% could be explained by *BRCA1* and *BRCA2* together. In some of these families the breast cancer will not be inherited, but on the whole this group is too big to be totally explained by coincidental clustering. More likely, most of these families are explained by mutations in other unknown genes.

The objective of this thesis is to describe our endeavours to localize new high-risk breast cancer susceptibility genes by genome wide linkage analysis and to set the first steps in isolating these genes. For this purpose we selected families which had to satisfy the following criteria: (1) at least three women diagnosed with breast cancer below age 60 years, (2) no case of ovarian cancer or male breast cancer in a blood relative (since these phenotypes are strongly predictive of the presence of *BRCA1* or *BRCA2* mutation), and (3) DNA samples available for genotyping from at least three women affected with breast cancer. In addition, to minimize the probability that the family segregated a *BRCA1* or *BRCA2* mutation, DNA from at least one affected individual was screened for mutations across both genes. Whenever possible a second affected individual was screened. Subsequently, we collected genotype data on at least three microsatellite markers flanking the *BRCA1* and *BRCA2* loci. Families with insufficient mutation screening or linkage data were not included in further analyses. Due to the excellent structure of the eight different departments of clinical genetics in the Netherlands and the willingness of the pathological departments to cooperate, it was relatively easy to collect data and tumor material from sufficient families.

One of the families we selected for the genome-wide linkage analysis harbours an extraordinarily high number of tumours, comprising, breast, lung, colon cancers, malignant melanoma and oral squamous cell carcinomas (OSCC). In this family a *p16-Leiden* germline mutation was found. Other researchers suggested a relationship between *p16* germline mutations and breast cancer. Therefore we studied the possibility of *p16* acting as a breast cancer susceptibility gene. See chapter 3.1. In the meanwhile Meijers-Heijboer et al.[175] identified *CHEK2* as a low-risk breast cancer susceptibility allele and Kainu et al.[228] suggested the 13q21 region as a candidate breast cancer susceptibility locus. Chapter 3.2 describes the role of the *CHEK2*1100delC* mutation in causing breast cancer in our group of families. As described in chapter 3.3 we could not confirm the claim by Kainu.

One of the biggest problems one might encounter in linkage analysis is the extent of genetic heterogeneity in the selected families. Chapter 4 describes attempts to subclassify the heterogenic group of families in more homogeneous groups of families by determining tumor characteristics.

Chapter 5.1 describes the results of the international genome wide linkage analysis conducted by the BCLC. Chapter 5.2 presents the genomewide linkage analysis in the Dutch population and in which suggestive linkage for a new breast cancer susceptibility locus at 9q was identified.