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Identification of genetic risk factors associated with familial breast cancer

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Breast cancer is the most frequent cancer among women. The cumulative risk for the disease is 10% up to the age of 80 years. A familial history of breast cancer is a significant risk factor. Some 5-10% of all cases of breast cancer and 25-40% of cases in patients under the age of 35 years have a hereditary origin. BRCA1/2 mutations are responsible for about 2% of all cases of breast cancer and 20%-25% of familial cases. The identification of further susceptibility genes and gene variants associated with disease risk is essential for the understanding of the pathogenesis of disease, the development of medical diagnostics, prevention and therapeutic strategies. Genetic association studies, case-control studies, provide an efficient design for the identification of common genetic variants that confer modest disease risks. The analyses of this thesis mainly focus on candidate single nucleotide polymorphisms (SNPs) that may be functionally relevant. Only familial index patients were chosen for our study, because it has been shown that the use of familial cases in case-control studies significantly increases the power to detect alleles contributing to breast cancer risk. In addition, only BRCA1/2 mutation-negative familial breast cancer cases were included in the study to avoid all effects derived from mutations in these two high-penetrance susceptibility genes.

We investigated whether the Aurora genes, implicated in the regulation of vital mitotic events, including G2/M transition, centrosome duplication, chromosome condensation, bipolar spindle-kinetochore attachment, chromosome segregation and cytokinesis, may be associated with breast cancer risk. To test this hypothesis, we performed a case-control study on a German study population examining SNPs in *AURKA* and *AURKB*. The synonymous Ser295Ser (885A>G) in *AURKB* revealed a significant association with familial breast cancer risk, resulting in an increased breast cancer risk for carriers of the homozygous 885G

genotype (OR = 1.45, 95 % C.I. = 1.05-2.00, *P* = 0.024). Additional studies are necessary to investigate if this observation is a by chance finding or if a functionally relevant risk variant exists linked to this silent polymorphism. Due to the impact of aurora kinases in the loss of chromosomal integrity during human cancer development via mitotic subversion, this polymorphism may influence the therapy outcome in breast cancer.

Estrogen is a strong risk factor for the initiation and progression of breast cancer. The estrogen receptor ER-α is a member of the nuclear receptor family which activates gene expression through recruiting multiple co-activators. PPARGC1A, PPARGC1B and EP300 are transcriptional co-activators of nuclear receptors like the estrogen receptor (ER). Studies have reported that these genes are overexpressed in breast tumours. We investigated whether polymorphisms in these genes are associated with breast cancer risk. The non-synonymous *PPARGC1A* Thr612Met polymorphism revealed a significant association with familial breast cancer (OR = 1.35, 95% C.I. = 1.00-1.81, *P* = 0.024). The effect was even stronger in high-risk familial (OR = 1.49, 95% C.I. = 1.08-2.06, *P* = 0.013) and bilateral breast cancer (OR = 2.26, 95% C.I. = 1.27-4.00, *P* = 0.004), in an allele dose-dependent manner (P_{trend} = 0.028, P_{trend} = 0.014 and P_{trend} = 0.004, respectively). The *PPARGC1B* Ala203Pro variant revealed a significant association with familial breast cancer (OR = 1.5, 95% C.I. = 1.2-1.9, *P* = 0.002). *PPARGC1B* Pro388Pro showed also a significant association with high-risk familial breast cancer (OR = 1.37, 95 % CI 1.11-1.71, *P* = 0.003) following a dose-dependent mode (P_{trend} = 0.004). The joint effect of the *PPARGC1A* Thr612Met and the *PPARGC1B* Ala203Pro variants resulted in a dose-dependent risk (χ^2 = 14.8, P_{trend} = 0.0001). Due to the pivotal role of these genes in antiestrogen therapy resistance, these variants might also influence therapy outcome in breast cancer.

MicroRNAs (miRNAs) negatively regulate expression of target transcripts by hybridization to complementary sites of their mRNA targets. Chen and Rajewsky have described several putative functional SNPs in miRNA-target sites. Here, we selected 11 miRNA-binding site SNPs located in 3`UTRs of genes involved in cancer and breast cancer to analyze their impact on breast cancer risk using a large familial study population. Whereas no association was observed for 10 SNPs, a significant association was revealed for the variant affecting a miRNA-target site in the estrogen receptor one (ESR1). Age stratification showed that the association was stronger in premenopausal women (C versus T: OR = 0.60, C.I. = 0.41-0.89, *P* = 0.01). Furthermore, the effect was stronger in high risk familial cases (C versus T: OR =

0.42, C.I. = 0.25-0.71, *P* = 0.0009). The association always occurred in an allele dose-dependent manner. Clinical studies have shown that reduction of estrogen or elimination of ESR1 significantly reduces breast cancer risk. Thus, therapies that inhibit ESR1 are used for breast cancer treatment. According to in-silico analysis ESR1_ rs2747648 affects the binding capacity of miR-453, which is stronger when the C- allele is present. In contrast, the T-allele attenuates the binding of miR-453, which might lead to a reduced miRNA-mediated ERS1-repression, in consequence higher ESR1 protein levels and an increased breast cancer risk. Thus, the breast cancer protective effect observed for the C-allele in premenopausal women is biological reasonable. The analysis of large study populations in multicentre collaboration will be needed to verify the association and answer questions regarding the possible impact of this variant on therapeutic and clinical outcome.

According to present estimations, the unfavorable combination of alleles with low penetrance but high prevalence in the population might attribute for the major part (up to 80%) of the hereditary breast cancer risk. Deleted in Malignant Brain Tumours 1 (*DMBT1*) has been proposed as a tumour suppressor for breast cancer and other cancer types. Genome-wide mapping in mice further identified DMBT1 as a potential modulator of breast cancer risk. Here, we reported the association of the two frequent single nucleotide polymorphisms *DMBT1* -93C>T, located in the *DMBT1* promoter, and *DMBT1* A230C Thr42Pro, the latter, in complete LD ($r^2 = 1.0$) with *DMBT1* T267C Leu54Ser, with increased breast cancer risk in women above the age of 50 (OR = 1.31, C.I. = 1.08-1.59, *P* = 0.005; and OR = 1.30; C.I. = 1.07-1.58, *P* = 0.006) investigating a large familial breast cancer study cohort. The *DMBT1* -93T allele displays drastically reduced binding activity for a nuclear factor, which results in increased promoter activity. Likewise, the closely linked polymorphism in codon 54 (*DMBT1* T267C Leu54Ser) modulates the processing of the N-terminus and results in increased secretion. The data suggest that *DMBT1* polymorphisms in the 5`-region are associated with increased breast cancer risk. In contrast to previous results, however, the associated polymorphisms would be predicted to result in increased DMBT1 levels.

In conclusion, the polymorphisms may contribute to the inter-individual variability in susceptibility to breast cancer, and accumulation of variant alleles may have a high impact on breast cancer at the individual level. The identification of new predisposing gene variants and gene variant combinations are important for the understanding the etiology of a disease, for risk estimations and might give hints for new drug targets.