Michael Wirtenberger
Dr.sc.hum.

Susceptibility genes for familial breast cancer

Geboren am 28.05.1970 in Absam, Österreich
Diplom der Fachrichtung Mikrobiologie am 10.10.2002 an der Universität Innsbruck, Österreich

Promotionsfach: DKFZ (Deutsches Krebsforschungszentrum)
Doktorvater: Prof. K. Hemminki, MD, PhD

According to the polygenic model of inherited breast cancer predisposition, combinations of genetic variants in different candidate genes are suggested to account for a large portion of the excess familial risk for breast cancer. The presented case-control studies were performed on a German and/or Polish study population and mainly focussed on the impact of putative functional and non-conservative amino acid exchanges on familial breast cancer in candidate genes with a strong prior probability to be involved in breast cancer carcinogenesis. Due to the large sample size and the use of familial, *BRCA1/ 2* mutation negative cases, we achieved a high power to detect even variants with a small individual effect and we excluded all effects derived from the two known high-penetrance breast cancer genes.

The mitogen effect of the ovarian steroid estrogen is a strong risk factor for breast cancer development, mainly mediated by the estrogen receptor α, a hormone inducible transcription factor that activates gene expression through recruiting multiple coactivators, such as NCOA3 PPARGC1A, PPARGC1B and EP300.

NCOA3 is overexpressed in ~ 60 % of primary human breast tumours and high levels of *NCOA3* expression are associated with tamoxifen resistance and worse survival rates. A joint analysis of the German and Polish study population showed a significant protective effect of *Gln*586*His* in *NCOA3* for familial breast cancer (1758G>C) (OR = 0.78, 95 % CI 0.63-0.98, P = 0.035) and *Thr*960*Thr* (2880A>G) (OR = 0.78, 95 % CI 0.61-0.99, P = 0.046). In addition, haplotype analysis revealed a protective effect of the 1758C-2880A and 1758G-2880G haplotypes (OR 0.79, 95 % CI 0.67-0.93, P = 0.004). Moreover, analyses of the German study cohort revealed a significant association of *PPARGC1A Thr*612*Met* (61463G>A) with familial breast cancer (OR = 1.35, 95 % CI 1.00-1.81, P = 0.049). The effect was even stronger in high-risk (OR = 1.51, 95 % CI 1.08-2.12, P = 0.017) and bilateral familial breast cancer (OR = 2.30, 95 % CI 1.24-4.28, P = 0.009), in an allele dose-dependent manner (P_{trend} = 0.014, P_{trend} = 0.010 and P_{trend} = 0.002, respectively). Additionally, linear regression analyses of polymorphisms in *PPARGC1B* determined an allele dose-dependent association of *Ala*203*Pro* (94749 G>C) with familial breast cancer (OR = 1.48, 95% CI 1.15-1.91, P= 0.002, P_{trend} = 0.002) and *Pro*388*Pro* with high-risk

familial breast cancer (OR = 1.43, 95 % CI 1.12-1.83, $P = 0.004$, $P_{trend} = 0.002$). The joint effect of the *PPARGC1A Thr*612*Met* and the *PPARGC1B Ala*203*Pro* variants resulted in a strong allele dose-dependent risk ($P_{trend} = 0.0001$), as well as the combined action of *PPARGC1B Ala*203*Pro* and *Pro*388*Pro* ($P_{trend} = 0.0004$). Due to the pivotal role of these genes in the ER pathway, the associated variants might also influence anti-estrogen therapy outcome and resistance.

The oncogene c-MYC is a multifaceted protein that regulates cell proliferation, differentiation and apoptosis. Its crucial role in diverse cancers has been demonstrated in several studies. A joint analysis of the Polish and the German study population revealed a 55 % increased risk for familial breast cancer associated with the *Asn*11*Ser* variant (OR = 1.47, 95 % CI 1.21-1.78, p = 0.028). *Asn*11*Ser* might interfere with c-MYC mediated apoptosis. Thus, this variant might affect the genetic susceptibility of other cancers as well.

The A-kinase anchor protein 13 (AKAP13, alias BRX and lbc) tethers cAMP-dependent protein kinase to its subcellular environment and catalyses Rho GTPases activity as a guanine nucleotide exchange factor. The crucial role of members of the Rho family of GTPases in carcinogenesis is well established and Rho proteins are targets of antineoplastic compounds. Analyses of the German study population revealed a significant association of the newfound *Lys*526*Gln* polymorphism with familial breast cancer (OR = 1.58, 95 % CI = 1.07-2.35, $P = 0.022$) and an even stronger association with high-risk familial breast cancer (OR = 1.85, 95 % CI = 1.19-2.88, $P = 0.005$). The important role of AKAP13 in the Rho GTPases signalling network suggests that this variant might influence anticancer therapies targeting RhoA and clinical outcome.

DNA helicases play an essential role in order to maintain genome stability. Mutations in human RecQ helicase genes, such as *WRN* and *BLM*, lead to rare autosomal recessive disorders, Werner and Bloom syndromes, respectively, associated with premature ageing and cancer predisposition. The analysis of the German study cohort revealed a significant association of the *WRN Cys*1367*Arg* polymorphism with familial breast cancer (OR = 1.28, 95 % CI 1.06-1.54, $P = 0.010$). Additionally, we have shown an increased familial breast cancer risk of *p53 Msp*I 1798G>A, which is completely linked to *p53PIN3*, a recently reported 16 bp insertion/duplication associated with reduced *p53* RNA levels (OR = 2.15, 95 % CI = 1.12-4.11, $P = 0.018$). The joint effect of both variants resulted in a strong allele dose-dependent risk ($P_{trend} = 0.0007$). Our results indicate the potential importance of inherited variations in the *TP53* and *WRN* gene interaction for familial breast cancer susceptibility.

The identification of breast cancer susceptibility genes and gene variants associated with disease risk is essential for the understanding of the pathogenesis of the disease, the development of medical diagnostics, prevention and therapeutic strategies.