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Vitamin D and breast cancer risk – Association of dietary vitamin D, circulating 25-hydroxyvitamin D concentration, and polymorphisms in vitamin D pathway genes with pre- and postmenopausal breast cancer risk

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Antiproliferative effects of vitamin D by influencing cell differentiation, cell growth, and apoptosis in normal and malignant cells, including human breast cancer cells, are well established. Epidemiological and experimental studies suggest an inverse association of vitamin D with breast cancer risk. Most studies have assessed the association of breast cancer risk with dietary vitamin D intake. However, to account for endogenous vitamin D production, measurement of 25-hydroxyvitamin D [25(OH)D], the biomarker for vitamin D status in humans, is necessary. Furthermore, little is known about the differential effects of vitamin D regarding pre- and postmenopausal breast cancer risk. Genetic polymorphisms in vitamin D pathway genes may influence breast cancer risk by altering potential anticarcinogenic effects of vitamin D, yet epidemiologic studies have shown inconsistent results. Modification of the effect of vitamin D on breast cancer risk by genetic variants has been rarely examined.

In the present thesis, the association of dietary vitamin D and 25(OH)D with pre- and postmenopausal breast cancer risk was investigated. Potential effect modification of the associations of vitamin D and breast cancer risk by factors such as use of hormone therapy (HT) and receptor status of the tumor was assessed. Furthermore, genetic polymorphisms (FokI, TaqI, *VDR*-5132, and Cdx2) in the vitamin D receptor (VDR) and the vitamin D binding protein (Gc) (combined Gc genotypes for rs4588 and rs7041) were assessed with respect to their impact on postmenopausal breast cancer risk. An additional focus was on gene-environment interactions between vitamin D exposure and genotypes or haplotypes in the *VDR* and *Gc* gene with regard to breast cancer risk.

Data from two population-based case-control studies conducted in the Rhein-Neckar region were used to investigate the study questions on pre- and postmenopausal breast cancer risk, respectively. Comprehensive information on sociodemographic and breast cancer risk factors was available from questionnaire and personal interview for the pre- and postmenopausal study population, respectively. Dietary intake data were obtained by a validated food frequency questionnaire. Serum/plasma 25(OH)D was measured by an enzyme immunoassay. Genotyping was performed with restriction fragment length polymorphisms, sequencing analysis (Pyrosequencing^{TM}), and mass spectrometry. The final sample size consisted of 392 cases and 761 controls for the premenopausal and 1559 cases and 3008 controls for the postmenopausal study population. Odds ratios (OR) and 95% confidence intervals (CI) for breast cancer adjusted for potential confounders were calculated using conditional logistic regression. Statistical interaction was evaluated with the likelihood ratio test by including an interaction term of the interaction variables of interest.

Dietary vitamin D was inversely associated with both pre- and postmenopausal breast cancer risk comparing the highest (≥ 5 µg/day) with the lowest intake category (<2 µg/day)

[OR (95% CI) = 0.50 (0.26-0.96) and 0.75 (0.57-0.99) for pre- and postmenopausal breast cancer risk, respectively]. The level of dietary calcium intake did not affect the risk reduction associated with high vitamin D intake for premenopausal breast cancer. For postmenopausal breast cancer, however, an inverse association between dietary vitamin D and breast cancer risk was observed only when dietary calcium was low.

Serum/plasma 25(OH)D was inversely associated with breast cancer risk. Compared to the lowest category (<30 nmol/L), OR (95% CI) were 0.45 (0.29-0.70) for the highest category in premenopausal women (≥ 60 nmol/L) and 0.31 (0.24-0.42) for the highest category in postmenopausal women (≥ 75 nmol/L). Fractional polynomial analysis indicated a non-linear association with a stronger inverse association at the lower range of 25(OH)D concentrations.

The association for postmenopausal breast cancer risk was significantly stronger in women who had never used HT compared to women with past or current use and in women with increasing number of pregnancies. Subgroup analysis suggested an inverse association of dietary vitamin D and 25(OH)D in ER- or PR- tumors only. This observation was more pronounced in pre- than in postmenopausal women.

None of the analyzed polymorphisms in the *VDR* gene were individually associated with overall postmenopausal breast cancer risk. However, the TaqI polymorphism was associated with a significantly increased risk of ER+ tumors [OR (95% CI) = 1.18 (1.00-1.38) comparing t allele carriers with non-carriers] but not of ER- tumors [0.88 (0.69-1.13)]. Haplotype analysis revealed the haplotype FtCA (FokI F, TaqI t, *VDR*-5132 C, Cdx2 A) to be associated with a significantly higher breast cancer risk compared to the most frequent haplotype FTCG [OR (95% CI) = 1.43 (1.00-2.05)]. Gc2-2 genotype in the vitamin D binding protein was associated with a significantly decreased breast cancer risk with an OR (95% CI) of 0.72 (0.54-0.96) compared to homozygote Gc1s allele carriers, that was independent of vitamin D status. No significant interaction between VDR, Gc genotypes or VDR haplotypes and 25(OH)D or dietary vitamin D with regard to postmenopausal breast cancer risk was observed.

In conclusion, the present findings strongly suggest a protective effect of vitamin D as assessed by dietary intake and 25(OH)D status on both pre- and postmenopausal breast cancer. Since interactions with estrogen-related exposure variables and the receptor status of the tumor were observed, further epidemiological studies on vitamin D and breast cancer risk should take the receptor status of the tumor and the estrogen metabolism into account. The observed effects of VDR and Gc genotypes require confirmation in further epidemiological studies and functional studies to clarify the biological mechanisms involved. Overall, the present results strengthen the cumulative evidence of health beneficial effects of vitamin D with regard to breast cancer risk.