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Sulfotransferase1A1 and Risk of Postmenopausal Breast Cancer

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Abstract. *The detoxification enzyme sulfotransferase1A1 (SULT1A1) is implicated in the inactivation of estrogens and the activation of promutagens and procarcinogens. SULT1A1 activity varies among individuals, and this difference in phenotype is, in part, controlled by genetic polymorphism (Arg→His in codon 213). It is hypothesized that the His allele contributes to the risk of postmenopausal breast cancer. Frequencies of the Arg/His alleles were estimated in 229 postmenopausal breast cancer patients and 227 age-matched controls using a PCR-RFLP assay. Allele frequencies and genotype distributions were not statistically different between postmenopausal breast cancer patients and the population-based controls, i.e. neither of the alleles is associated with an increased risk of breast cancer in the present study.*

The genesis and progression of breast cancer are influenced by both environmental and genetic factors. A family history of breast cancer seems to be the greatest risk factor, and risk is a function of the number of affected relatives, the degree of relationship and the time of onset (1). Other significant and well known risk factors, which are also indicators of cumulative endogenous and/or exogenous estrogen exposure, are: low age at menarche, late menopause, nulliparity, advanced age at first full-time pregnancy, and use of estrogen replacement. Sulfotransferases (SULTs) play an important role in the modulation, inactivation and elimination of non-peptide hormones such as estrogens, androgens and catechols, as well as in the biosynthesis of steroids. Of the different human SULT isoforms (2), Sulfotransferase1A1 (SULT1A1) has been implicated in a number of cancers by activation of promutagens and procarcinogens (3-5). SULT activity displays large variations among individuals, and these phenotypic variations are in part controlled by genetic polymorphism. The most common polymorphism is a G to A transition, resulting in an arginine to histidine substitution at the conserved amino acid 213 (6). This A-allele (SULT1A1*2) is correlated with lower thermostability and a significantly decreased capacity of sulfation of SULT1A1 substrate in human liver and platelet cell extracts (6, 7).

Earlier studies on SULT1A1 demonstrated an age-related difference in genotype and inconsistent results in the relation between breast cancer risk and Arg/His polymorphism (8-10). The present study was performed to further assess the age-related genotype distribution and the significance of SULT1A1 polymorphism as a modulator of breast cancer risk in postmenopausal women.

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Patients and Methods

Patients. Postmenopausal patients, aged under 71 years, with a unilateral, operable stage II-III breast cancer, were included in the study. DNA from the 229 patients was isolated from fresh-frozen tumor tissues. The control group consisted of 227 women matched according to age (45 to 77 years, median age: 64 years) from a population-based DNA bank, collected in the counties of Östergötland, Kalmar and Jönköping, Sweden.

Methods. PCR was performed in a total reaction volume of 20 μl, containing 60 ng of DNA, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 unit of *Taq* polymerase (Invitrogen™), 1 μM each of forward (5'-GTTGGCTCTGCAGGGTTTCTAGGA-3') and reverse primer (5'-CCCAAACCCCCTGCTGGCCAGCACCC-3') in 1x PCR buffer. Forty cycles of PCR amplification were performed, with denaturation at 94˚C for 30 sec, annealing at 63˚C for 30 sec and extension at 72˚C for 40 sec. An initial 3-min denaturation step at 94˚C and a final extension period for 7 min at 72˚C were used. Fifteen μl PCR products were incubated for 5 h at 37˚C with 5 units of *Hae*II (New England BioLabs, MA, USA) in 20 μl reaction mixtures containing 1x NEBuffer 4 (supplied by the manufacturer), supplemented with 100 μg/mL BSA. After digestion, fragments

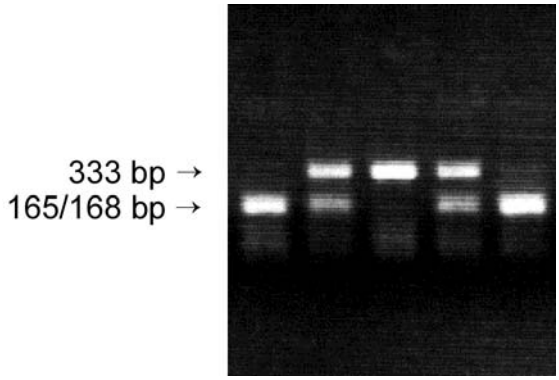

Figure 1. *Agarose gel showing PCR amplicon of the SULT1A1 gene which was incubated with HaeII enzyme. The HaeII enzyme digests *1 allele (wild-type), but not *2 allele. Lanes 1 and 5: *1/*1. Lanes 2 and 4: *1/*2. Lane 3: *2/*2.*

were separated by electrophoresis on a 3% (w/v) agarose gel containing ethidium bromide and then detected using UV light (Figure 1).

Statistical analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by logistic regression to evaluate the risk of breast cancer for individuals homozygous or heterozygous for the predicted risk allele, SULT1A*2 and *p*-values were calculated by χ^2-analysis, the latter also being used to investigate age-related genotype distribution.

Results

The distribution of SULT1A1 alleles *1 and *2 in the control population were 0.60 and 0.40, respectively, and in postmenopausal breast cancer patients 0.61 for *1 and 0.39 for *2. The frequencies of SULT1A1 alleles and genotypes in the group of patient and controls are shown in Table I. No significant difference in genotypes or allele frequencies between cases and controls were found. For women of the *2/*2 genotype, the risk of breast cancer in relation to individuals carrying at least one wild-type allele, was calculated as 0.69 (95 % CI, 0.40-1.21). In the comparison between *1/*1 and *2/*2 genotypes, the odds ratio for the latter genotype was 0.76 (95 % CI, 0.41-1.42). Genotype distribution according to age was tested using median age as the cut-off value in both the case and control groups (Table II). No significant difference was found in any of the groups.

Discussion

Genotype distributions and allele frequencies, obtained in the present study, were not statistically different between postmenopausal breast cancer patients and the population-based controls in the present study, *i.e.* neither of the alleles

Table I. *Distribution of SULT1A1 genotypes and allele frequencies in postmenopausal breast cancer patients and in the population-based control group.*

	SULT1A1 genotype frequency			Allele frequency	
	*1/*1 (%)	*1/*2 (%)	*2/*2 (%)	*1	*2
Patients (n=229)	80 (34.9)	121 (52.8)	28 (12.2)	0.61	0.39
Controls (n=227)	83 (36.6)	106 (46.7)	38 (16.7)	0.60	0.40

Table II. *Age-related genotype frequency in controls and the breast cancer population. Median age was used as cut-off value. No statistical difference was found between age groups.*

	Control population		Breast cancer population	
Genotype	Age ≥64 (%)	Age <64 (%)	Age ≥60 (%)	Age <60 (%)
*1/*1	44 (38)	40 (36)	33 (31)	47 (38)
*1/*2	53 (46)	53 (47)	57 (54)	64 (52)
*2/*2	19 (16)	19 (17)	15 (14)	13 (10)

was associated with an increased risk of breast cancer. Earlier reports on the SULT1A1 genotype and breast cancer risk have shown somewhat inconsistent results. In two large case control studies (9, 10) of Caucasian and Taiwanese women, no association between SULT1A1 genotype and breast cancer risk was found. A similar result was also obtained by Seth *et al.* for early-onset breast (11). However, Seth *et al.* found a positive association between genotype and the age of onset of breast cancer, where the *1 allele contributed to an earlier onset. In a study by Zheng *et al.* of postmenopausal women (12), the risk of developing breast cancer was shown to be elevated by 80% for those who carried the SULT1A1*2 genotype. The patient population studied by Zheng *et al.* was similar in size to the present study, and was the first to be published showing an association between the SULT1A1 polymorphism and breast cancer risk in postmenopausal women.

The allele frequencies in our control population were 0.60 and 0.40 for SULT1A1*1 and SULT1A1*2, respectively. In earlier studies of Caucasians, the SULT1A1*1 allele was reported to exist at a frequency ranging from 0.659 to 0.692 (6, 8, 11-14).

Contrary to the present study, Coughtrie *et al.* reported an age-related difference in SULT1A1 genotypes (8). They found that the number of Caucasian *1 homozygotes was significantly increased with increasing age. The proportion of

*1 homozygotes was 39% in a group with the lowest mean age (12-39 years) and 59% in individuals aged between 70 and 99 years. In another study by Carlini and coworkers, no significant age-related effect on allele frequency in Caucasians was found (14). The only exception was in Chinese subjects, where the opposite trend, *i.e.* the proportion of *1 homozygotes decreased with age, was found.

In conclusion, this study supports the hypothesis of Langsenlehner *et al.* (9) and Seth *et al.* (11) that the allelic variants of SULT1A1 do not seem to play a major role in breast cancer susceptibility in the Caucasian population. Thus, evidence of a relationship between the *2 allele and an elevated risk of breast cancer, as hypothesized by Zheng *et al.* (12), has not been confirmed.

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