Sulfotransferase 1A1 is a Risk Factor for Breast Cancer in Young Women

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Objectives. SULT1A1, the major form of cytosolic sulfotransferase enzymes (SULTs), activates or metabolizes many chemicals and carcinogens. The effect of the SULT1A1 genotype on the development of cancers is still not clear. The purpose of this study was to analyze the relationship between SULT1A1 polymorphisms and cancer risk.

Methods. We determined *SULT1A1* polymorphisms by PCR-RFLP and then analyzed the frequencies of the *SULT1A1*1* and *SULT1A1*2* alleles from several cancerous cohorts.

Results. After analyzing 76 hepatoma patients, 180 breast cancer patients, 61 lung cancer patients, 52 oral cancer patients and 74 gastric cancer patients, the frequencies of *SULT1A1*1* were 96.1%, 94.2%, 95.1%, 96.1%, and 97.3%, respectively, whereas the frequencies of *SULT1A1*2* were 3.9%, 5.8%, 4.9%, 3.9%, and 2.7%, respectively. No *SULT1A1*3* alleles were found in these patients.

Conclusions. In comparison with the frequencies of SULT1A1*1 and SULT1A1*2 in healthy controls (96.0% and 4.0% for *SULT1A1*1* and *SULT1A1*2*, respectively), the allelic frequencies of *SULT1A1* polymorphisms in the cancer patients were not statistically significant. However, it appears to influence the age of onset among early-onset breast cancer patients ($p = 0.012$, OR = 3.35, 95% CI = 1.25 – 8.98). **(Mid Taiwan J Med 2003;8:59-65)**

Key words

breast cancer, polymorphism, SULT1A1

INTRODUCTION

Cytosolic sulfation is an important pathway for the biotransformation of drugs, xenobiotics, and endogenous compounds [1]. This phase II reaction is catalyzed by sulfotransferases (SULTs) which enzymatically transfer a sulfate moiety from a donor substrate, 3'-phosphoadenosine-5'-phosphosulfate (PAPS), to an acceptor substrate containing an amino or hydroxyl group [2]. The consequence of the modification of these substrates with a charged sulfate group either makes the substrates more readily excretable or less toxically active [3]. Consequently, sulfation directly participates in chemical defense and xenobiotic metabolism. However, a large number of promutagens have been shown to be the substrates of SULTs and are activated by SULTs in humans [4-6]. These enzymes also chemically induce diseases, such as cancers and

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cardiovascular diseases, as well as induce drug reactions which represent a great fraction of mortality and morbidity. Therefore, the functions of the SULT family play both good roles and bad roles in humans. The activities of SULTs presumably influence the causes of diseases. However, little information is known as to what extent the enzymes influence the development of cancers.

Three phenol SULTs (SULT1A1, SULT1A2, and SULT1A3) are expressed in human tissues [2,5]. Individual differences in the expression and activity of drug-metabolizing enzymes is a well established cause of adverse drug reactions and other toxic effects associated with exposure to both xenobiotic and endogenous chemicals. These differences may arise from a variety of genetic and/or environmental events [7,8]. Little information about the polymorphic expression of SULT enzymes is known compared to other drug-metabolizing enzymes, such as CYP450. In human liver, SULT1A1 is the major form of phenol SULT, and its molecular basis has been identified [9,10]. A single transition in the *SULT1A1* gene results in an Arg to His at codon 213 which alters the expression and activity of the enzyme, presumably through reduced protein stability. Platelet enzyme activity correlates strongly with protein expression, and individuals who are homozygous for the *SULT1A1*2* genotype have significantly reduced platelet sulfotransferase activity. In this study, we showed the correlation of the *SULT1A1* polymorphisms with the risk of developing cancers.

MATERIALS AND METHODS

DNA Preparation

Blood samples were collected from 200 healthy controls from the general population, 196 elderly normal controls, 180 breast intraductal carcinoma patients, 76 hepatoma patients, 52 oral squamous carcinoma patients, 74 gastric adenocarcinoma patients, and 61 lung squamous carcinoma patients. They all resided in central Taiwan. The age distributions of the patients with cancer are shown in Table 1. Total genomic DNA was isolated from peripheral leukocytes as previously described [11].

PCR-RFLP Assays

The differences between *SULT1A1*1, SULT1A1*2* and *SULT1A1*3* are shown at nucleotides 638 and 667. G638 and A667 represent *SULT1A1*1*, A638 and A667 represent *SULT1A1*2*, and G638 and G667 represent *SULT1A1*3*. We used 5'-GGTTGAGGAGTT GGCTCTGC-3' and 5'-ATGAACTCCTGG GGGACGGT-3' as the upstream and downstream primers for the genotyping. A fragment of 281 bp was synthesized after the primers were used in the PCR reaction. PCR amplification was performed in a 50 μL reaction volume containing 200 ng of genomic DNA as templates, 1X Taq buffer, 0.2 mM dNTP, 0.2 μM of each primer, and 0.4 U Taq polymerase (Protech, Taipei, Taiwan). PCR reaction was started by incubating the samples at 94°C for 5 min. The amplification was carried out in 35 cycles of 3 stages: denaturing at 94°C for 1 min, annealing at 60°C for 1 min, and elongation at 72°C for 2 min. To determine *SULT1A1*2* and *SULT1A1*3*, the PCR products were subjected to *Hha* I or *Nla* III enzyme digestion before electrophoresis on a 3.5% agarose gel.

Sequencing Analysis of PCR-Amplified Fragments

The PCR-amplified products were purified using a PCR purification kit (QIAquick; Qiagen Inc., Valencia, CA, USA). To verify the accuracy of the PCR-RFLP assay, PCR products were subjected to direct sequencing by an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, San Francisco, USA). Primers used for sequencing were the same as those used for PCR as described above.

Statistical Analysis

The differences in distribution of *SULT1A1* genotypes between tumor patients and healthy controls were determined by Chi-Square test. Probability values < 0.05 were regarded as statistically significant. After adjusting for age and gender, odds ratios (ORs) with 95% confidence intervals (CI) were calculated by unconditional logistic regression to estimate the association between certain genotypes and

Table 1. The average age of cancer patients

Cancer (N)		Genotype	
		*SULT1A1*1/*1*	*SULT1A1*1/*2*
		Average age (range) (n)	
HCC	(76)	58 (29−81) (70)	55 (39−64) (6)
Breast Ca.	(180)	51 (15−84) (159)	48 (23−76) (21)
Lung Ca.	(61)	61 (24−78) (55)	67 (60−72) (6)
Oral Ca.	(52)	56 (36−75) (48)	55 (36−82) (4)
Gastric Ca.	(74)	64 (36−83) (70)	61 (47−70) (4)

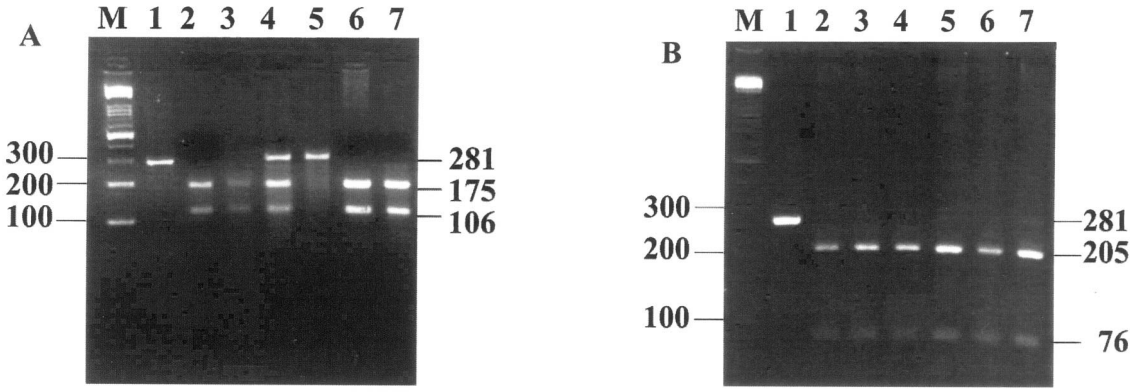

Figure. PCR-RFLP analysis of SULT1A1 polymorphisms. A: The PCR products containing codon 213 Arg (CGC) were digested into two fragments (lanes 2, 3, 6 and 7 for homozygotes, lane 4 for heterozygote), and only one fragment of 281 bp was observed for the one with codon 213 His (CAC) (lane 5) after Hha I digestion; B: The PCR products of codon 223 Met (ATG) released two fragments of 205 bp and 76 bp (lanes 2-7 for homozygotes), and the uncut fragment 281 bp was observed for the one with codon 223 Val (GTG) after Nla III digestion. In summary, lanes 2, 3, 6 and 7 are homozygous SULT1A1*1, lane 4 is heterozygous SULT1A1*2, and lane 5 is a selected case of homozygous SULT1A1*2. M: marker; Lane 1: uncut.

diseases. All of the statistical analyses were performed with Statistical Analysis System software (SAS Institute, Cary, NC, USA).

RESULTS

SULT1A1*1 is the Major Allele in Taiwanese

In order to determine the *SULT1A1* polymorphisms, we used the PCR-RFLP method to detect the transition of G to A at nucleotide 638 in the 213th codon in the *SULT1A1* coding region. This G to A transition causes a change from Arg in *SULT1A1*1* or *SULT1A1*3* to His at codon 213 in *SULT1A1*2*. Consequently, the Hha I recognition site will be abolished if a chromosome contains the *SULT1A1*2* allele (Fig. A). To distinguish *SULT1A1*1* from *SULT1A1*3*, the transition of A (*SULT1A1*1*) to G (*SULT1A1*3*) at nucleotide 667 in the 223th codon was determined by Nla III enzyme digestion (Fig. B). A total of 76, 180, 61, 52 and 74 hepatoma, breast, lung, oral and gastric cancer patients were genotyped, respectively, as well as 200 healthy subjects (Table 2). Among the 643 individuals, there were no homozygous SULT1A1*2 cases detected in these samples.

SULT1A1 Genotype and Age

After analysis of the data from the patients and the healthy controls, the average age of individuals with the *SULT1A1*1/*1* or *SULT1A1*1/*2* appeared to be different (Table 1). The age distributions in the cancerous groups were further analyzed, and the results showed that the young patients with breast cancer (< 39 years old) had higher allelic frequencies of *SULT1A1*2*, which was statistically significant (p = 0.012, OR = 3.35, 95% CI = 1.25−8.98) (Table 3). However no difference was observed between breast cancer patients and normal controls in the older-age group (p = 0.320) (Table 4). Since only a few cases of *SULT1A1*2* were

Table 2. The genotypic frequencies of *SULT1A1* polymorphisms in different cancers in Taiwanese

Cancer (N)	Genotype†		*p*
	*SULT1A1*1/*1*	*SULT1A1*1/*2*	
HCC (76)	70/76 (92.1%)	6/76 (7.9%)	0.764
Breast Ca. (180)	159/180 (88.3%)	21/180 (11.7%)	0.241
Lung Ca. (61)	55/61 (90.2%)	6/61 (9.8%)	0.659
Oral Ca. (52)	48/52 (92.1%)	4/52 (7.9%)	0.935
Gastric Ca. (74)	70/74 (94.6%)	4/74 (5.4%)	0.472
Normal (200)	184/200 (92%)	16/200 (8.0%)	

†no case of *SULT1A1*3* was found in this study.

Table 3. Age-related variation in *SULT1A1* allelic frequencies in Taiwanese breast cancer patients

Age range	Mean	No. of cases	*SULT1A1*1/*1*	*SULT1A1*1/*2*
15–39	33	31	77.4% (24)	22.6% (7)†
40–49	45	58	89.3% (52)	10.7% (6)
50–59	55	40	92.5% (37)	7.5% (3)
60–69	63	35	88.6% (31)	11.4% (4)
70–84	75	16	93.8% (15)	6.3% (1)
15–84	51	180	88.3% (159)	11.7% (21)
Control		200	92% (184)	8% (16)ǂ

†$p = 0.012$, OR = 3.35, 95% CI = 1.25–8.98; ǂno difference between different age groups.

Table 4. Comparison of *SULT1A1* allelic frequencies of the old age group between breast cancer patients and normal controls

	Age range	Chromosomes	*SULT1A1*1/*1*	*SULT1A1*1/*2*
Old age-normal	70–80	308	92.2% (284)	7.8% (24)†
	> 85	84	97.6% (82)	2.4% (2)
Breast cancer	70–84	32	96.9% (31)	3.1% (1)

†$p = 0.320$.

found in hepatoma, lung, oral and gastric cancer patients, we neither stratified the age group nor analyzed the effect for these cohorts.

DISCUSSION

SULT1A1 is a drug-metabolizing enzyme which catalyzes the sulfation conjugation in phase II metabolism [12]. The frequency of this genotype varies widely among different ethnic groups. The major polymorphism of *SUL1A1* in Taiwanese is *SULT1A1*1*, which completely dominates the other *SULT1A1* polymorphisms, *SULT1A1*2* and *SULT1A1*3*. Chinese women selected from Shanghai, PRC, showed a completely dominant *SULT1A1*1* allele (91.6%) with very few *SULT1A1*3* [10], which was not observed in the Taiwanese population in this study. From the data that have been reported, Taiwanese have the highest frequency of the *SULT1A1*1* allele (approximately 96.0%). Although *SULT1A1*1* is also the major form of *SULT1A1* in Caucasians (65.6%) and African Americans (47.7%), *SULT1A1*2* and *SULT1A1*3* are also significantly present in these populations [10].

Products of the *SULT1A1*1* show much higher activity and greater thermostability than those of the other alleles, indicating that it may increase the excretion of xenobiotics as well as activation of procarcinogens [4,9]. We compared the frequencies of five cancerous groups with those of healthy individuals. In comparison with the normal subjects, we observed that the frequencies of *SULT1A1*1* and *SULT1A1*2* were not significantly different in these patients, indicating that the risk of hepatoma, lung cancer,

breast cancer, oral cancer, and gastric cancer does not correlate with the *SULT1A1* genotype. However, in terms of lung cancer, our results differed from Wang et al [13]. They found that the genetic polymorphism of SULT1A1 may be associated with increased lung cancer risk in Caucasians. Our results were consistent with those reported by Seth et al [14], but different from the data presented by Zheng et al [15]. Seth et al demonstrated that the *SULT1A1* genotype does not effect the risk of breast cancer; however, they revealed that the *SULT1A1*1* allele behaves as a dominant allele in early onset breast cancer patients; Zheng et al suggested that homozygosity for the SULT1A1 His 213 allele may be a risk factor for breast cancer. These discrepencies in observation may be due to ethnic and lifestyle differences. Environmental factors and food consumption habits may also be important reasons for the differences. In addition, among the normal population, Coughtrie et al also observed a statistically significant increase in the frequency of the *SULT1A1*1* allele with increasing age, compared with the *SULT1A1*2* allele [16]. After analyzing the average age of the cancer patients with the *SULT1A1*1/*1* and those with the *SULT1A1*1/*2* genotypes, we found the same trend among breast cancer patients: the average age of the *SULT1A1*1/*1* patients was about 4 years older than that of *SULT1A1*1/*2* patients; however, it was not statistically significant. Bamber et al suggested that the *SULT1A1*1* genotype reduced the risk of colorectal cancer in subjects under 80 years old [17]. The data provided by Nowell et al, Wang et al, and our study were not in concordance with their results, suggesting that the environment-gene interaction may play an important role in cancer risk [18,19].

Numerous drug-metabolizing genes have been shown to influence the development of different cancers. Consequently, the risk of cancer may not be dependent on a single gene. In addition, diet also influences the risk of cancer. For example, soya products, tea, and many fruits are known to protect against a variety of human cancers [20]. It has been shown that phenolic dietary compounds such as flavonoids and isoflavonoids competitively inhibit the activation of procarcinogens by SULTs [20]. On the contrary, well-done steak containes PAH, a carcinogen, so individuals with the active allelle *SULT1A1*1* who eat well-done meat have a greater risk of developing cancer than those without the allele [15]. In humans, high expression of the SULT1A1 enzyme was regarded as efficient metabolism and excretion of xenobiotics, and high risk of the bioactivation of toxins including procarcinogens. It is still not clear whether SULTs do more good than harm, and determining the answer is difficult because genotoxins may vary depending on environment and diet. In this study, we also showed that the *SULT1A1* genotype is not associated with the risk of hepatoma, breast, lung, oral, and gastric cancer in Taiwan. However, we did not demonstrate the correlation between the *SULT1A1*1* allele with the age of onset for these cancers due to the small sample size. Therefore further study is warranted.

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Sulfotransferase 1A1是年輕女性乳癌的危險因子

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目的 SULT1A1是細胞中主要的轉醯酵素，它活化或代謝很多的化學物及致癌物，SULT1A1多形性與癌症的相關性尚未清楚。

方法 我們利用PCR-RFLP的方法分析SULT1A1的多形性變異，並分析此變異與幾種癌症的相關性。

結果 在分析76個肝癌，180個乳癌，61個肺癌，52個口腔癌及74個胃癌病人後，*SULT1A1*1*在上述病人的發生率分別爲96.1%，94.2%，95.1%，96.1%及97.3%；而*SULT1A1*2*的發生率則分別爲3.9%，5.8%，4.9%，3.9%及2.7%；並沒有發現*SULT1A1*3*的存在。

結論 比較正常人SULT1A1的發生率(*SULT1A1*1*爲96.0%，而*SULT1A1*2*爲4.0%)，發現*SULT1A1*的多形性與上述各種癌的形成無關，但與年輕女性乳癌有關(p = 0.012，OR = 3.35，CI = 1.25 – 8.98)。（中台灣醫誌 **2003;8:59-65**）

關鍵詞

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