Journal of Food and Drug Analysis, Vol. 19, No. 4, 2011, Pages 403-409 藥物食品分析 第十九卷 第四期

Association of Low Activity of UGT1A7 with Lung Cancer in Taiwan: A Preliminary Case Control Study

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(Received: April 27, 2011; Accepted: July 27, 2011)

ABSTRACT

This study was aimed to evaluate the correlation between the polymorphisms of the *UDP-glucuronosyltransferases1A7 (UGT1A7)* gene and the risk of lung carcinogenesis in the Taiwanese population. A total of 230 lung cancer patients and 230 age- and gender-matched healthy individuals were enrolled in this case control study. *UGT1A7*1* was defined as a high activity allele, while *UGT1A7*2, UGT1A7*3* and *UGT1A7*4* were categorized as low activity alleles. The relationship between *EGFR* mutations and *UGT1A7* polymorphisms was investigated in this study. The frequency of *UGT1A7*2* and *UGT1A7*3* was significantly higher in the lung cancer group than in the control group ($p = 0.03$, odds ratio (OR) = 1.44, 95% confidence interval (CI) = 1.04 - 1.99 for *UGT1A7*2*; and $p = 0.01$, OR = 1.56, 95% CI = 1.11 - 2.18 for *UGT1A7*3*). The frequency of lower activity alleles was significantly higher in the lung cancer group than in the control group ($p = 0.03$, OR = 1.58, 95% CI = 1.04 - 2.40 for the high-activity allele/low-activity allele (H/L) group; and $p < 0.01$, OR = 2.23, 95% CI = 1.29 - 3.84 for the low-activity allele/low-activity allele (L/L) group). This difference was only significant in the male subgroup, with odds ratio of 1.87 (95% CI = 1.05 - 3.36, $p = 0.03$) for the H/L group and 2.638 (95% CI = 1.28 - 5.42, $p < 0.01$) for the L/L allele group. Yet, pathologic type and epidermal growth factor receptor (EGFR) mutations did not affect the distribution of *UGT1A7* in the patient group. The results suggested that the polymorphisms of the metabolic gene, *UGT1A7,* may contribute to reduced enzyme activity and subsequently affect the detoxification of carcinogens. It is therefore concluded that *UGT1A7* polymorphism is associated with lung carcinogenesis for the Taiwanese population.

Key words: *UGT1A7* polymorphisms, lung cancer, risk factor, EGFR mutation, gene, enzyme

INTRODUCTION

Lung cancer is responsible for 1.3 million deaths worldwide annually[(1)]. It is ranked the number-one cause of all cancer deaths in both men and women in Taiwan[(2)]. Tobacco smoking is an important risk factor for lung cancer. In particular, the activity of xenobiotic enzymes has been linked to the modulation of risk[(3,4)]. Tobacco smoke contains more than 60 known carcinogens such as nicotine, nitrosamines and polycyclic aromatic hydrocarbons. These carcinogens are metabolized by both hepatic and extrahepatic enzymes, such as cytochrome P450 enzymes or uridine 5'-diphospho-glucuronosyltransferases (UGTs)[(4-6)]. The *UGT1A* gene complex is located on human chromosome 2 at 2q37, which encoded nine functional proteins (UGT1, UGT1A3-UGT1A10)[(7,8)]. UGT1A7, a member of the UGT1 family, plays important roles in the conjugation and detoxification of several tobacco carcinogens, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)[(9)] and benzo-[α]-pyrene (BaP)[(10,11)]. The catalytic activity of UGT1A7 is determined by its polymorphisms at codon 129, 131 and 208. *UGT1A7*1* (wild type) has the highest activity, followed by *UGT1A7*2* ($K^{129}K^{131}W^{208}$), *UGT1A7*4* ($N^{129}R^{131}R^{208}$) and *UGT1A7*3* ($K^{129}K^{131}R^{208}$)[(11)]. In recent reports in Japan, the presence of *UGT1A7* polymorphisms has been suggested to be a risk factor in orolaryngeal cancer[(12)], proximal GI tract cancer[(13)], hepatocellular carcinoma[(14)], colorectal cancer[(15,16)], pancreatic cancer[(17)] and lung cancer[(6)].

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Non-smoking women have a high incidence rate of lung adenocarcinoma in Taiwan and other Asian countries, indicating that there may be risk factors other than smoking, which leads to lung cancer. Alteration of genes such as over-expression of oncogenes[18], silencing of tumor suppressor genes[19,20] and defects in DNA repair mechanism[21] may also contribute to lung carcinogenesis. Most Asian female patients with lung cancer are non-smokers and they often represent a distinct type. Adenocarcinoma and epidermal growth factor receptor (EGFR) overexpression are the most predominant[22]. EGFR has been identified as an important prognostic factor in the advanced stage of lung cancer. The clinical outcomes after the therapy of EGFR tyrosine kinase inhibitor can be predicted by *EGFR* mutations[23].

The purpose of this study was to examine the relationship between *UGT1A7* polymorphism and *EGFR* mutations in the Taiwanese population and to determine whether *UGT1A7* polymorphisms are potentially associated with a higher risk of lung cancer. The distribution of *UGT1A7* alleles, genotypes and their predicted phenotypes in lung cancer patients were compared with the controls. *EGFR* mutations in the lung cancer patients were also analyzed.

MATERIALS AND METHODS

I. *Patients and Clinical Specimens*

A total of 230 lung cancer patients from Wan Fang Hospital, Taipei Medical University were studied between 2004 and 2009 according to a protocol approved by the Institutional Review Board (IRB). All patients who participated in this study signed the letter of informed consent and had the willingness and ability to give informed consent. A confirmed diagnosis of primary lung cancer and ages between 18 and 90 were the specific inclusion criteria in this study. The peripheral blood and clinical information of the patients were collected after obtaining informed consent. Of the 230 patients, 197 cancer-containing tissues were collected from bronchoscopic biopsies (70.6%), proven malignant pleural effusion (26.9%) or lobectomy (2.5%). A total of 230 healthy volunteers who were matched with the cancer patients by age and sex, and had no history of cancer or disease associated with *UGT1A7* polymorphism, such as Gilbert's syndrome, pancreatic diseases, hyperbilirubinemia and hepatitis C, were randomly recruited into the study over the same period of time.

II. *Determination of Genotypes*

In order to determine the *UGT1A7* genotyping, genomic DNA from peripheral blood mononuclear cells was extracted by proteinase K digestion, followed by the conventional phenol-chloroform method as previously described[24]. Genomic DNA from paraffin-embedded tissues was extracted by commercial kits (DEXPAT^{TM}, Takara, Shiga, Japan) for the detection of *EGFR* mutation.

The identification of each subject's *UGT1A7* alleles was performed by PCR-restriction fragment length polymorphism (RFLP) analysis for the nucleotide 622 polymorphic sites and nested PCR was performed for nucleotide 387 polymorphic positions. The use of these combined analyses allowed the differentiation of all genotypes formed by *UGT1A7*1, UGT1A7*2, UGT1A7*3 and UGT1A7*4* alleles, except *UGT1A7*1/UGT1A7*3* and *UGT1A7*2/UGT1A7*4*. Since the frequency of the *UGT1A7*4* allele in the Chinese population was less than 3%[11,17,25], the subjects who exhibited either the *UGT1A7*1/UGT1A7*3* or *UGT1A7*2/UGT1A7*4* genotypes were considered to have the *UGT1A7*1/UGT1A7*3* genotypes, according to previous reports[12,26].

The PCR reaction for generating *UGT1A7* 622T→C and *UGT1A7* 387T→G DNA fragments was performed in a thermal cycler (PC806, ASTEC, Japan) in the presence of 0.2 mM of dNTPs (Protech, Taiwan), 1 x Taq buffer (Protech, Taiwan), 0.04 U/μL of Taq polymerase (Protech, Taiwan), 1 μL of forward and reverse primers (Table 1) and 0.5 μL of DNA prepared in a total volume of 25 μL[26]. The reaction was performed under the following conditions: 1 cycle at 94°C for 3 min, followed by 34 cycles at 94°C for 30 s; 55°C for 30 s; 72°C for 1 min, and 1 cycle at 72°C for 10 min, and then cooling down at 4°C. Primary and nested PCR amplifications were utilized to determine UGT1A7 387T→G DNA fragments and to further confirm by restriction enzymes

Table 1. Primer sequences used in *UGT1A7* genotyping

Position (cDNA)	Primers	Sequence	Restriction Enzyme	Result (bp)
622 T→C	U7F3	5'-TGTCCCCAGACTTCTCTTAG-3'	RsaI	WT: 447
	U7R3	5'-GCTACCCAACAATTAAGTGA-3'		MT: 54+393
387 T→G	1st PCR			
	U7F1	5'-TGAATGAATAAGTACACGCC-3'		
	U7R2	5'-TAGGGGCAAAATAAATGTTC-3'		
	Nested PCR			
	387F	5'-AAATTGCAGGAGTTTGC*TTA-3'	AflII	WT: 159
	387R	5'-TGGCAAAATATTCCCCTGGC-3'		MT: 142+17

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digestion. The UGT1A7 622T→C DNA fragments were digested by RsaI (Promega, USA). DNA fragments were separated by 3% agarose gel electrophoresis at 100 V for 40 min. While UGT1A7 387T→G DNA fragments were digested by AflIII (New England BioLabs, UK), sizes were fractioned by 8% polyacrylamide gel electrophoresis at 100 V for 95 min. Both were then visualized by ethidium bromide staining. According to previous literatures on UGT1A7 enzyme activity, *UGT1A7*1* was defined as a higher activity allele and *UGT1A7*2, UGT1A7*3* and *UGT1A7*4* were categorized as lower activity alleles[10,11]. The predicted phenotypes were categorized into high-activity allele/high-activity allele (H/H), high-activity allele/low-activity allele (H/L), and low-activity allele/low-activity allele (L/L)[27].

The detection of *EGFR* mutations in exon 18-21 was performed by PCR. Table 1 represents the primers used in the study and the representative results have been previously described[28]. Direct sequencing of the mutant bands was also used to validate the accuracy of detection.

III. *Statistical Analysis*

SPSS 13.0 (SPSS Inc, Chicago, IL, USA) for Windows was used to analyze continuous and categorical data. The continuous data were analyzed by Student's *t*-test. Multivariable logistic regression was applied to calculate the odds ratios for comparing the differences in the predicted phenotypes, genotypes and variant allele frequencies between patients and normal controls. Two-tailed $p < 0.05$ was considered as statistically significant. The Hardy-Weinberg equilibrium of alleles at individual loci was also evaluated.

RESULTS

I. *Clinical and Histological Data*

Table 2 depicts the demographic characteristics of cancer patients and normal controls. Both groups of lung cancer patients and healthy individuals consisted of 128 males (55.7%) and 102 females (44.3%). The majority of patients (n = 223, 96.9%) were diagnosed with non-small cell lung cancer (NSCLC) at an advanced stage. Among the patients, 77 (33.5%) were diagnosed with stage III B and 113 (49.1%) with stage IV.

II. *Association of UGT1A7 Polymorphism with Lung Cancer*

The alleles at the individual loci fulfilled the Hardy-Weinberg distribution in both patient and control groups ($p > 0.05$). The frequencies of *UGT1A7*2* and *UGT1A7*3* were significantly higher in the lung cancer group. ($p = 0.03$, odds ratio (OR) = 1.44, 95% confidence interval (CI) = 1.04 - 1.99 for *UGT1A7*2*; and $p = 0.01$, OR = 1.56, 95% CI = 1.11 - 2.18 for *UGT1A7*3*, respectively, Table 3). Subgroup analysis that compared male lung cancer patients with male controls revealed a similar result, with an odds ratio of 1.59 (95% CI = 1.06 - 2.43, $p = 0.03$) for *UGT1A7*2*, and 1.63 (95% CI = 1.05 - 2.54, $p = 0.03$) for *UGT1A7*3*. However, the frequencies of *UGT1A7*2* and *UGT1A7*3* were not significantly different between the female lung cancer patients and their corresponding controls.

There was a significant difference in the distribution of genotypes between the patients and controls. Cancer patients had a higher percentage of *UGT1A7**1/*3, *UGT1A7**2/*2 and *UGT1A7**3/*3 genotypes than the controls, suggesting that genotypes of lower activity might be a potential risk factor for the development of lung cancer ($p = 0.02$, OR = 1.83, 95% CI = 1.10 - 3.04 for *UGT1A7**1/*3; $p < 0.01$, OR = 3.89, 95% CI = 1.44 - 10.51 for *UGT1A7**2/*2; and $p = 0.04$, OR = 2.92, 95% CI = 1.04 - 8.20 for *UGT1A7**3/*3). Results of further analysis on the predicted phenotypes showed that the frequency of cancer patients having at least one low-activity allele was significantly higher than the controls ($p = 0.03$, OR = 1.58, 95% CI = 1.04 - 2.40 for the H/L group; and $p < 0.01$, OR = 2.23, 95% CI = 1.29 - 3.84 for the L/L group, Table 4).

Comparing the subgroup analysis of male patients with that of male controls, similar results were revealed for the H/L and L/L allele groups, with an OR of 1.87 (95% CI = 1.05 - 3.36, p = 0.03) and 2.64 (95% CI = 1.28 - 5.42, $p < 0.01$), respectively. However, this trend was not observed in female patients. These results suggested a positive

Table 2. Demographic characteristics of enrolled patients with lung cancer and the control participants

	Lung cancer (n = 230)	Control (n = 230)
Sex (%)		
Male	128 (55.7)	128 (55.7)
Female	102 (44.3)	102 (44.3)
Age (year)	67.74 ± 12.23	67.70 ± 13.15
Pathology Classification (%)		
Non-small cell lung cancer	223 (96.9)	
Adenocarcinoma	181 (78.8)	
Squamous cell carcinoma	32 (13.9)	
Others	10 (4.3)	
Small cell lung cancer	7 (3.0)	
Lung cancer stage (%)		
IA	14 (6.1)	
IB	9 (3.9)	
IIA	2 (0.9)	
IIB	5 (2.2)	
IIIA	3 (1.3)	
IIIB	77 (33.5)	
IV	113 (49.1)	
Limited (small cell type)	1 (0.4)	
Extensive (small cell type)	6 (2.6)	

Table 3. Comparison of the allelic frequency of *UGT1A7* between lung cancer patients and the control group

Allele Frequency	Lung cancer (n = 460)	Control (n = 460)	OR	95% CI	*p* - value
*1	0.516 (237)	0.613 (282)	ref	-----	-----
*2	0.250 (115)	0.207 (95)	1.44	1.04 - 1.99	0.03*
*3	0.230 (106)	0.176 (81)	1.56	1.11 - 2.18	0.01*
*4	0.004 (2)	0.004 (2)	1.19	0.17 - 8.51	0.86
Female (n = 204 in each group)					
*1	0.554 (113)	0.627 (128)	ref	-----	-----
*2	0.225 (46)	0.202 (41)	1.27	0.78 - 2.08	0.34
*3	0.211 (43)	0.162 (33)	1.48	0.88 - 2.48	0.14
*4	0.010 (2)	0.009 (2)	1.13	0.16 - 8.17	0.90
Male (n = 256 in each group)					
*1	0.484 (124)	0.602 (154)	ref	-----	-----
*2	0.270 (69)	0.211 (54)	1.59	1.04 - 2.43	0.03*
*3	0.246 (63)	0.187 (48)	1.63	1.05 - 2.54	0.03*
*4	0.000 (0)	0.000 (0)	-----	-----	-----

* $p < 0.05$ compared with control.

Table 4. Comparison of predicted phenotypes between lung cancer patients and the control group

UGT1A7 Predicted phenotype	Lung cancer (n = 230) (%)	Control (n = 230) (%)	OR	95% CI	*p* - value
H/H	59 (25.7)	86 (37.4)	ref	-----	-----
H/L	119 (51.7)	110 (47.8)	1.58	1.04 - 2.40	0.03*
L/L	52 (22.6)	34 (14.8)	2.23	1.29 - 3.84	< 0.01*
Female (n = 102 in each group)					
H/H	31 (30.4)	38 (38.3)	ref	-----	-----
H/L	51 (50.0)	50 (49.0)	1.28	0.70 - 2.37	0.43
L/L	20 (19.6)	13 (12.7)	1.94	0.83 - 4.50	0.13
Male (n = 128 in each group)					
H/H	28 (21.9)	47 (36.7)	ref	-----	-----
H/L	67 (52.3)	60 (46.9)	1.87	1.05 - 3.36	0.03*
L/L	33 (25.8)	21 (16.4)	2.64	1.28 - 5.42	< 0.01*

* $p < 0.05$ compared with control.

correlation between these alleles and the presence of lung cancer. These alleles encoded protein with low catalytic activity of UGT1A may contribute to increased risk of lung cancer in male but not female patients.

A stratified analysis of smoking rate in the male lung cancer patients was performed to evaluate the effect of smoking on these patients. Of the 128 male patients, 108 (85.3%) were smokers. The smoking rates of the male lung cancer patients were 82.1%, 86.6% and 93.3% in the H/H, H/L and L/L groups, respectively, showing no statistically significant difference ($p = 0.43$). This suggests that smoking was not the confounding factor for the observed differences in *UGT1A* genotypes in males.

Although the frequencies of genotypes and predicted alleles were different between the lung cancer patients and the controls, further analysis failed to demonstrate a significant association between *UGT1A7* polymorphisms and pathologic types or stages. The patient numbers identified with H/H, H/L and L/L *UGT1A7* genotypes were 26 (23.0%), 58 (51.3%) and 29 (25.7%) for those with stage IV NSCLC, and 34 (30.9%), 57 (51.8%) and 19 (17.3%) for earlier stages of NSCLC, respectively ($p = 0.21$). Of the 181 patients with adenocarcinoma, 52 (28.7%), 92 (50.8%) and 37 (20.4%) patients were identified with H/H, H/L and L/L *UGT1A7* genotypes, while in other types of lung cancer, the patient numbers were 8 (16.3%), 28 (57.1%) and 13(26.5%), respectively ($p = 0.19$).

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III. *Association between UGT1A7 Polymorphism and EGFR Mutations*

Of the 197 tissue samples collected from the cancer patients, 72 (36.5%) cancer patients showed *EGFR* mutations. The mutations of exon 19, exon 20 and exon 21 accounted for 36.1% (n = 26), 4.1% (n = 3) and 59.7% (n = 43), respectively. The results were consistent with prior studies, which indicated that the female gender and non-smoking status were risk factors for $EGFR^{mut}$ lung cancer. The mutation occurred in 46 (51%) out of 91 females and 26 (25%) out of 106 males, showing a positive association with lung cancer (OR = 3.15, 95% CI = 1.72 - 5.75, $p < 0.01$). In addition, the mutation rate was 57.3% (n = 55) in the non-smokers, which was significantly higher than the smokers (16.8%, n = 7, $p < 0.01$; OR = 6.63; 95% CI = 3.43 - 12.82). However, there was no significant difference in the distribution of *UGT1A7* polymorphisms for the patients with or without *EGFR* mutations.

DISCUSSION

The relationship between *UGT1A7* genotypes and the risk of lung cancer in the Taiwanese population was evaluated in this study. Our results were consistent with a previous Japanese study that showed a strong correlation between the *UGT1A7* genotype and lung cancer[6]. In addition to reports revealed by the previous study, our results indicated that patients with predicted low-activity phenotypes, i.e. one or two of the *UGT1A7*2, UGT1A7*3* and *UGT1A7*4* alleles, were associated with an increased risk of lung cancer in the Taiwanese population. This association was found in the male subgroup for the first time by subgroup analysis in this study. The stratified analysis showed that the smoking rates among the three groups with *UGT1A7* H/H, H/L and L/L genotypes were similar, indicating that smoking was not a confounding factor of genotype variables. Since the smoking rate of male lung cancer patients (85.3%) was much higher than the rate of male adults (35.4%) in Taiwan, the accumulation of tobacco carcinogens was expected to be increased in those with low activity of UGT1A7 expression, leading to elevated lung carcinogenesis in these patients[29].

Our finding was in line with the known consequence of low UGT1A7 activity, leading to the accumulation of carcinogens as a result of reduced capacity of conjugation and detoxification against them. In addition to lung cancer, low-activity alleles of *UGT1A7* have also been implicated in colorectal cancer[15,16], with dietary carcinogen intake[30], smoking and alcohol consumption[25] as modifying factors. Likewise, similar trends have been found in the susceptibility of proximal gastrointestinal tract cancers and orolaryngeal cancers for the smokers in particular[12,23]. The frequency of the wild type *UGT1A7*1* allele in the present study was more than 50% of the population, which was similar to previous studies on the *UGT1A7* genotypes in the Chinese, Taiwanese and Japanese populations[6,25,26], but higher than that in Caucasians[12]. This suggested that ethnic differences in the *UGT1A7* genotypes may exist between Caucasians and Asians.

The association between lung cancer and *UGT1A7*, a gene that metabolizes carcinogens from smoking, was not significant in female patients and this finding is disclosed for the first time in this report. The finding echoes a previous molecular epidemiologic discovery on a pathogenesis of lung cancer unrelated to the detoxication of carcinogens, especially derived from female patients who smoke[31]. The smoking rate of the female patients was 11.8% and not significantly different from that of Taiwanese female adults[29], indicating that the *UGT1A7* gene had a low impact on interrupting the cancer development in the female patients. Previous research also found that the female gender was a sole risk factor independent of smoking for $EGFR^{mut}$ NSCLC[32]. With significantly higher incidence of EGFR mutations found in Asian female patients as shown in our study and previous reports, other risk factors and carcinogenesis correlated to EGFR mutations should be considered to prevent lung cancer in this subpopulation[33]. On the other hand, the suspicion that low activity alleles of *UGT1A7* may be more common among the patients with *EGFR* wild type than those with *EGFR* mutations was ruled out by the observation that the mutations of *EGFR* and the polymorphisms of *UGT1A7* were unrelated.

No single factor or class of genes shall determine the development of lung cancer as it is a complex disease involving several genetic, biological, psychological, social and environmental factors. For example, inherited germline mutation in tumor suppressor gene *P53* significantly increases the risk of developing lung cancer[19]. A higher risk in non-small cell lung cancer was associated with functional polymorphism in the promoter region of the *MDM2* gene, one that controls *MDM2* transcription and P53 activity[20]. Alteration in DNA repair proteins, such as ERCC2, results in impaired DNA repair capability and also increases the risk of lung cancer[21]. Apart from *UGT1A7*, other carcinogen metabolic genes, such as cytochrome P450, particularly the *CYP1A1* subfamily polymorphisms, are also involved in increasing the risk of lung cancer[4,5]. Individuals with combined *NAT1* rapid and *NAT2* slow genotype seemed to have a significantly higher risk for lung adenocarcinoma[34], or the *NAT2* slow genotype when combined with the *GSTM1* null genotype may increase the susceptibility to adduct formation, gene mutation and lung cancer risk[35]. With the consideration of the significant genes involving the development of lung cancer, better prediction and prevention of the disease may be achieved. Correlations of toxin-metabolizing genes and risk factors should be investigated to predict the risk of lung cancer in future studies.

The results of the current study suggest that *UGT1A7* polymorphism is associated with lung cancer in a Taiwanese subpopulation. Lower activity alleles and predicted phenotypes are significantly associated with an elevated risk of lung cancer, with the exclusion of female patients. Based on this observation, the determination of *UGT1A7* polymorphisms

may provide additional information for high risk groups and support in developing preventive strategies against lung cancer, especially in male smokers. Further studies on a larger population should be performed to confirm these findings.

ACKNOWLEDGMENTS

The authors thank the patients and their families for their participation and contribution in this research. This work was supported by a joint research grant from the Taipei Medical University and Wan Fang Hospital (96TMU-WFH-21) as well as a grant from the Department of Health of Taiwan (DOH99-TD-B-111-003). The sponsoring organization was not involved in the study design, data analysis and interpretation. The authors bear all responsibility and have no conflict of interest with regard to this work.

REFERENCES

1. World Health Organization. Cancer Fact Sheet N°297; February 2009. http://www.who.int/mediacentre/factsheets/fs297/en/. [Accessed May 14, 2010].
2. Death Statistics in Taiwan, 2008.Department of Health, Executive Yuan, Taiwan. R.O.C. http://www.doh.gov.tw/CHT2006/DisplayStatisticFile.aspx?d=71701&s=1. [Accessed May 14, 2010].
3. Biesalski, H. K., Bueno de Mesquita, B., Chesson, A., *et al*. 1998. European Consensus Statement on Lung Cancer: risk factors and prevention. Lung Cancer Panel. CA Cancer J. Clin. 48: 167-176; discussion 164-166.
4. Gemignani, F., Landi, S., Szeszenia-Dabrowska, N., *et al*. 2007. Development of lung cancer before the age 50: the role of xenobiotic metabolizing genes. Carcinogenesis 28: 1287-1293.
5. Oyama, T., Sugio, K., Uramoto, H., *et al*. 2007. Cytochrome P450 expression (CYP) in non-small cell lung cancer. Front. Biosci. 12: 2299-2308.
6. Araki, J., Kobayashi, Y., Iwasa, M., *et al*. 2005. Polymorphism of UDP-glucuronosyltransferase 1A7 gene: a possible new risk factor for lung cancer. Eur. J. Cancer 41: 2360-2365.
7. Clarke, D. J., Cassidy, A. J., See, C. G., Povey, S., and Burchell, B. 1997. Cloning of the human UGT1 gene complex in yeast artificial chromosomes: novel aspects of gene structure and subchromosomal mapping to 2q37. Biochem. Soc. Trans. 25: S562.
8. Gong, Q. H., Cho, J. W., Huang, T., *et al*. 2001. Thirteen UDP- glucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics 11: 357-368.
9. Nguyen, N., Strassburg, C. P., Yeuh, M. F., *et al*. 2000. Human UDP-glucuronosyltransferases 1A7 and 1A9 are responsible for N- and N-OH linked glucuronidation reactions. Proc. Am. Assoc. Cancer Res. 41: 445.
10. Grove, A. D., Kessler, F. K., Metz, R. P. and Ritter, J. K. 1997. Identification of a rat oltipraz-inducible UDP-glucuronosyltransferase (UGT1A7) with activity towards benzo(a)pyrene-7,8-dihydrodiol. J. Biol. Chem. 272: 1621-1627.
11. Guillemette, C., Ritter, J. K., Auyeung, D. J., Kessler, F. K. and Housman, D.E. 2000. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. Pharmacogenetics 10: 629-644.
12. Zheng, Z., Park, J. Y., Guillemette, C., Schantz, S. P. and Lazarus, P. 2001. Tobacco carcinogen-detoxifying enzyme UGT1A7 and its association with orolaryngeal cancer risk. J. Natl. Cancer Inst. 93: 1411-1418.
13. Vogel, A., Ockenga, J., Ehmer, U., *et al*. 2002. Polymorphisms of the carcinogen detoxifying UDP-glucuronosyltransferase UGT1A7 in proximal digestive tract cancer. Z. Gastroenterol. 40: 497-502.
14. Vogel, A., Kneip, S., Barut, A., *et al*. 2001. Genetic link of hepatocellular carcinoma with polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene. Gastroenterology 121: 1136-1144.
15. Strassburg, C. P., Vogel, A., Kneip, S., Tukey, R. H. and Manns, M. P. 2002. Polymorphisms of the human UDP-glucuronosyltransferase (UGT) 1A7 gene in colorectal cancer. Gut 50: 851-856.
16. Tang, K. S., Chiu, H. F., Chen, H. H., *et al*. 2005. Link between colorectal cancer and polymorphisms in the uridine-diphosphoglucuronosyltransferase 1A7 and 1A1 genes. World J. Gastroenterol. 11: 3250-3254.
17. Ockenga, J., Vogel, A., Teich, N., Keim, V., Manns, M. P. and Strassburg, C. P. 2003. UDP glucuronosyltransferase (UGT1A7) gene polymorphisms increase the risk of chronic pancreatitis and pancreatic cancer. Gastroenterology 124: 1802-1808.
18. Frattini, M., Ferrario, C., Bressan, P., *et al*. 2004. Alternative mutations of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene 23: 7436-7440.
19. Hwang, S. J., Cheng, L. S., Lozano, G., Amos, C. I., Gu, X. and Strong, L. C. 2003. Lung cancer risk in germline p53 mutation carriers: association between an inherited cancer predisposition, cigarette smoking, and cancer risk. Hum. Genet. 113: 238-243.
20. Lind, H., Zienolddiny, S., Ekstrøm, P. O., Skaug, V. and Haugen, A. 2006. Association of a functional polymorphism in the promoter of the MDM2 gene with risk of nonsmall cell lung cancer. Int. J. Cancer 119: 718-721.
21. Zhou, W., Liu, G., Miller, D. P., *et al*. 2003. Polymorphisms in the DNA repair genes XRCC1 and ERCC2, smoking, and lung cancer risk. Cancer Epidemiol. Biomarkers Prev. 12: 359-365.
22. Tang, X., Shigematsu, H., Bekele, B. N. 2005. EGFR tyrosine kinase domain mutations are detected in histologically normal respiratory epithelium in lung cancer patients. Cancer Res. 65: 7568-7572.
23. Pao, W., Miller, V., Zakowski, M. 2004. EGF receptor gene mutations are common in lung cancers from "never

smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc. Natl. Acad. Sci. U.S.A. 101: 13306-13311.

24. Santella, R. M. 2006. Approaches to DNA/RNA Extraction and whole genome amplification. Cancer Epidemiol. Biomarkers. Prev. 15: 1585-1587.
25. Chen, K., Jin, M., Zhu, Y., *et al*. 2006. Genetic polymorphisms of the uridine diphosphate glucuronosyltransferase 1A7 and colorectal cancer risk in relation to cigarette smoking and alcohol drinking in a Chinese population. J. Gastroenterol. Hepatol. 21: 1036-1041.
26. Huang, M. J., Yang, S. S., Lin, M. S. and Huang, C. S. 2005. Polymorphisms of uridine-diphosphoglucuronosyltransferase 1A7 gene in Taiwan Chinese. World J. Gastroenterol. 11: 797-802.
27. Wang, Y., Kato, N., Hoshida, Y., *et al*. 2004. UDP-glucuronosyltransferase 1A7 genetic polymorphisms are associated with hepatocellular carcinoma in japanese patients with hepatitis C virus infection. Clin. Cancer Res. 10: 2441-2446.
28. Lee, C. N., Yu, M. C., Bai, K. J., *et al*. 2009. NAT2 fast acetylator genotypes are associated with an increased risk for lung cancer with wildtype epidermal growth factor receptors in Taiwan. Lung Cancer 64: 9-12.
29. Bureau of Health Promotion Annual Report. 2009. Department of Health. http://www.health99.doh.gov.tw/Media/public/zip/21618.zip. [Accessed August 24, 2010].
30. Butler, L. M., Duguay, Y., Millikan, R. C., *et al*. 2005. Joint effects between UDP-glucuronosyltransferase 1A7 genotype and dietary carcinogen exposure on risk of colon cancer. Cancer Epidemiol. Biomarkers Prev. 14: 1626-1632.
31. Sun, S., Schiller, J. H. and Gazdar, A. F. 2007. Lung cancer in never smokers--a different disease. Not. Rev. Cancer 7: 778-790.
32. Matsuo, K., Ito, H., Yatabe, Y., *et al*. 2007. Risk factors differ for non-small-cell lung cancers with and without EGFR mutation: assessment of smoking and sex by a case-control study in Japanese. Cancer Sci. 98: 96-101.
33. Mitsudomi, T. and Yatabe, Y. 2007. Mutations of the epidermal growth factor receptor gene and related genes as determinants of epidermal growth factor receptor tyrosine kinase inhibitors sensitivity in lung cancer. Cancer Sci. 98: 1817-1824.
34. Wikman, H., Thiel, S., Jager, B., *et al*. 2001. Relevance of N-acetyltransferase 1 and 2(NAT1, NAT2) genetic polymorphisms in non-small cell lung cancer susceptibility. Pharmacogenetics 11: 157-168.
35. Hou, S. M., Falt, S., Yang, K., *et al*. 2001. Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency in lung cancer patients and population controls. Cancer Epidemiol. Biomarkers Prev. 10: 133-140.