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TGFBR1 Haplotypes and Risk of Non–Small-Cell Lung Cancer

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Abstract

Transforming growth factor β (TGF-β) receptors are centrally involved in TGF-β–mediated cell growth and differentiation and are frequently inactivated in non–small-cell lung cancer (NSCLC). Constitutively decreased type I TGF-β receptor (TGFBR1) expression is emerging as a novel tumor-predisposing phenotype. The association of *TGFBR1* haplotypes with risk for NSCLC has not yet been studied. We tested the hypothesis that single-nucleotide polymorphisms (SNP) and/or *TGFBR1* haplotypes are associated with risk of NSCLC. We genotyped six *TGFBR1* haplotype-tagging SNPs (htSNP) by PCR-RFLP assays and one htSNP by PCR-single-strand conformation polymorphism assay in two case-control studies. Case-control study 1 included 102 NSCLC patients and 104 healthy controls from Suzhou. Case-control study 2 included 131 patients with NSCLC and 133 healthy controls from Wuxi. Individuals included in both case-control studies were Han Chinese. Haplotypes were reconstructed according to the genotyping data and linkage disequilibrium status of these seven htSNPs. None of the htSNP was associated with NSCLC risk in either study. However, a four-marker CTGC haplotype was significantly more common among controls than among cases in both studies ($P = 0.014$ and $P = 0.010$, respectively), indicating that this haplotype is associated with decreased NSCLC risk {adjusted odds ratio [OR], 0.09 [95% confidence interval (95% CI), 0.01–0.61] and 0.11 [95% CI, 0.02–0.59], respectively}. Combined analysis of both studies shows a strong association of this four-marker haplotype with decreased NSCLC risk (adjusted OR, 0.11; 95% CI, 0.03–0.39). This is the first evidence of an association between a *TGFBR1* haplotype and risk for NSCLC. [Cancer Res 2009;69(17):7046–52]

Introduction

Lung cancer is one of the most common cancers worldwide. It is estimated that approximately 41.8 men and 19.3 women per 100,000 Chinese individuals die every year of lung cancer (1). Non–small-cell lung cancer (NSCLC) accounts for ~85% of all cases of lung cancer. Although smoking is considered a major risk factor for lung cancer, less than 20% of lifetime smokers develop lung cancer (2), suggesting that genetic susceptibility plays an important role in lung carcinogenesis.

Transforming growth factor β (TGF-β) is a potent inhibitor of normal epithelial cell growth *in vivo*. TGF-β signaling is mediated by two specific cellular serine/threonine kinase receptors, the type I (TGFBR1) and type II (TGFBR2) TGF-β receptors. TGF-β binds directly to TGFBR2 and is then recognized by TGFBR1, which is phosphorylated and activated by TGFBR2 (3).

Previous studies have shown that TGF-β signaling alterations significantly contribute to NSCLC progression (4). Furthermore, increased levels of circulating TGF-β are associated with poor prognosis (5), which indicates that TGF-β is ineffective in inhibiting the growth of lung tumors *in vivo* and may enhance disease progression. Alterations of TGF-β receptors are a potential mechanism underlying refractoriness to TGF-β growth inhibitory signals and the development and progression of human cancers, including NSCLC (6–10). Several studies have focused on the association between the polyalanine polymorphism of *TGFBR1* (*TGFBR1**6A) and several types of cancers (11–14). Another single-nucleotide polymorphism (SNP), Int7G24A (rs334354), may be associated with risk of kidney, bladder, and invasive breast cancers (15, 16). Given the fact that neither *TGFBR1**6A nor Int7G24A is associated with risk for NSCLC (17, 18), we decided to investigate the association of *TGFBR1* haplotypes with NSCLC risk.

To comprehensively study the genetic variants of *TGFBR1* associated with susceptibility to NSCLC, we genotyped six *TGFBR1* haplotype-tagging SNPs (htSNP) using PCR-RFLP and one htSNP using PCR-single-strand conformation polymorphism (SSCP) in two Chinese population-based case-control studies. The seven htSNPs, including three in the 5′ flanking region, three in intronic regions, and one in the 3′ flanking region of the *TGFBR1* gene, appropriately capture all the common haplotype blocks reconstructed in HapMap Phase II data.

Materials and Methods

Specimens. In case-control study 1, blood specimens were collected from 102 consecutive patients diagnosed with NSCLC at the First Affiliated Hospital of Soochow University between January 2005 and May 2008. None of NSCLC patients had received either radiotherapy or chemotherapy before blood sampling. As controls, we collected blood samples from 104 geographically matched individuals with the same age range and without a history of cancer at the First Affiliated Hospital of Soochow University between January and December 2005. In case-control study 2, blood specimens were collected from 131 patients with a diagnosis of NSCLC who had not received radiotherapy or chemotherapy and 133 geographically matched controls with the same age range at Wuxi Third People's Hospital between October 2004 and June 2007. Blood specimens

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Characteristics of the two case-control studies

	Case-control study 1			Case-control study 2		
	Cases (n = 102), n (%)	Controls (n = 104), n (%)	P*	Cases (n = 131), n (%)	Controls (n = 133), n (%)	P*
Age (mean ± SD)	61.5 ± 9.8	60.3 ± 10.1	0.38	61.0 ± 10.0	58.8 ± 10.7	0.09
Gender			0.06			0.09
Male	79 (77.5)	68 (65.4)		99 (75.6)	88 (66.2)	
Female	23 (22.5)	36 (34.6)		32 (24.4)	45 (33.8)	
Smoking history			<0.001			0.006
Yes	65 (63.7)	38 (36.5)		82 (62.6)	60 (45.1)	
No	37 (36.3)	66 (63.5)		49 (37.4)	73 (54.9)	
Histology						
AC	28 (27.5)			49 (37.4)		
SCC	45 (44.1)			61 (46.6)		
LCC	15 (14.7)			11 (8.4)		
ACC	10 (9.8)			8 (6.1)		
Carc	4 (3.9)			2 (1.5)		
TNM stage †						
Stage I	29 (28.4)			36 (27.5)		
Stage II	43 (42.2)			61 (46.6)		
Stage III	21 (20.6)			24 (18.3)		
Stage IV	4 (3.9)			6 (4.6)		

Abbreviations: AC, adenocarcinoma; SCC, squamous cell carcinoma; LCC, large cell carcinoma; ACC, alveolar cell carcinoma; Carc, carcinoid tumor.
*Two-sided χ^2 test was applied to gender and smoking status and independent t test was applied to age.
†There are five and four cases with missing TNM stage in study groups 1 and 2, respectively.

were obtained after informed consent from all subjects. This study was approved by the Academic Advisory Board of Soochow University. A standardized questionnaire was carried out to collect data on age, sex, and smoking history.

Tagging SNP selection. HapMap SNP Phase II data[7] were used to determine the frequency of SNPs among Han Chinese (CHB), and 74 SNPs were obtained from a 76-kb region of *TGFBR1* from 28 kb upstream of the transcriptional start site to 7 kb downstream of the 3′ untranslated region. Three haplotype blocks were reconstructed using these 74 SNPs with the Haploview program (19). htSNP selection was done using the Haploview program. The Haploview program implemented a htSNP selection method proposed by Carlson and colleagues (20), which selects a set of htSNPs such that each SNP considered has r^2 greater than a prespecified threshold with at least one of the htSNPs. In our selection, only SNPs with minor allele frequency >10% were considered and the threshold of pairwise linkage disequilibrium (LD) was set as $r^2 = 0.8$. A total of seven htSNPs within three blocks were selected among 47 SNPs considered across *TGFBR1*, including three in the 5′ flanking region, three in intronic regions, and one in the 3′ flanking region (Supplementary Table S1). The LD map of these seven htSNPs is shown in Supplementary Fig. S1.

Genotyping. Genomic DNA from blood specimens was isolated according to standard proteinase K digestion and phenol-chloroform extraction. The seven *TGFBR1* htSNPs were amplified by PCR. The sequences of PCR primers and annealing temperature are reported in Supplementary Table S2. The PCR reaction was carried out in a total volume of 25 μL, containing 50 to 100 ng of genomic DNA, 1 unit of Ex Taq DNA polymerase (Takara, Japan), 0.2 μmol/L of each primer, 1× Ex Taq Buffer (Mg2+ Plus), 0.25 mmol/L of each deoxynucleotide triphosphate. Genotyping for the htSNPs was done by RFLP with restriction endonucleases (Supplementary Table S2). The different alleles were identified on a 2.5% agarose gel and visualized with ethidium bromide. One htSNP (rs1888223) was genotyped using SSCP because of lack of restriction endonuclease. For SSCP, the PCR products were mixed at 1:1 ratio with loading buffer (95% formamide, 0.05% xylene cyanol, and 0.05% bromophenol blue), denatured at 95°C for 5 min, and cooled on ice for 2 min. Electrophoresis was done in 8% nondenaturing polyacrylamide gels and run at a constant 20 W for 5 h in 1× Tris-borate-EDTA running buffer, with the gel temperature maintained at 7°C. Ethidium bromide staining was used for detection of single-strand DNA in polyacrylamide gels.

LD and haplotype analysis. Pairwise measures of LD measured by Lewontin coefficient (D') and squared correlation coefficient (r^2) between the SNPs genotyped were calculated with the Haploview program (19). The frequencies of individual haplotypes were estimated from the genotype data using the SAS 9.1.3 PROC HAPLOTYPE and SHEsis programs (21), which implement an expectation-maximization algorithm and a Full-Precise-Iteration algorithm for reconstructing haplotypes, respectively. Haplotypes with a frequency of <0.05 were not considered in the analysis. Logistic regression analysis was done using SAS PROC LOGISTIC to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) of individual SNPs or haplotypes, with adjustment for age, sex, and smoking status.

Statistical analysis. Two-sided χ^2 test or independent-samples t test was used to compare the difference in gender, age, and smoking status between NSCLC cases and controls. Hardy-Weinberg equilibrium analysis for genotype distribution in controls was carried out by a χ^2 goodness-of-fit test. Differences in genotype and allele frequencies between cases and controls were determined using χ^2 test. Logistic regression was done to assess OR and 95% CI, which were adjusted for gender, age, and smoking status. All the statistical analyses were implemented with SAS 9.1.3. Statistical significance cutoff was $P < 0.05$.

Results

As shown in Table 1, there was no significant difference with respect to sex and age between patients with NSCLC and controls

[7] http://www.hapmap.org

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Table 2. Genotype and allelic frequencies of *TGFBR1* htSNPs among NSCLC cases and controls and associations with risk of NSCLC

	Case-control study 1			Case-control study 2		
	Cases/controls	OR (95% CI)*	$P^{\dagger}$	Cases/controls	OR (95% CI)*	$P^{\dagger}$
rs7040869						
Genotype						
GG	57/58	1.00		68/71	1.00	
GA	36/35	1.09 (0.58–2.03)		49/46	1.06 (0.62–1.80)	
AA	9/11	0.96 (0.36–2.60)	0.90	14/16	0.87 (0.39–1.97)	0.87
Allele						
G	150/151	1.00		185/188	1.00	
A	54/57	0.95 (0.62–1.47)	0.83	77/78	1.00 (0.69–1.46)	0.99
rs4743325						
Genotype						
TT	31/30	1.00		44/39	1.00	
TG	49/51	0.84 (0.43–1.64)		59/66	0.83 (0.47–1.47)	
GG	22/23	0.71 (0.31–1.61)	0.97	28/28	0.90 (0.45–1.79)	0.71
Allele						
T	111/111	1.00		147/144	1.00	
G	93/97	0.96 (0.65–1.41)	0.83	115/122	0.92 (0.66–1.30)	0.65
rs1888223						
Genotype						
AA	31/28	1.00		43/38	1.00	
AC	54/58	0.79 (0.41–1.53)		66/69	0.83 (0.47–1.46)	
CC	17/18	0.70 (0.29–1.69)	0.86	22/26	0.70 (0.34–1.45)	0.71
Allele						
A	116/114	1.00		152/145	1.00	
C	88/94	0.92 (0.62–1.36)	0.68	110/121	0.87 (0.62–1.22)	0.42
rs10819638						
Genotype						
CC	30/31	1.00		45/43	1.00	
CT	53/57	0.86 (0.45–1.66)		61/68	0.84 (0.48–1.46)	
TT	19/16	1.13 (0.48–2.71)	0.82	25/22	0.98 (0.47–2.03)	0.74
Allele						
C	113/119	1.00		151/154	1.00	
T	91/89	1.08 (0.73–1.59)	0.71	111/112	1.01 (0.72–1.43)	0.95
rs6478974						
Genotype						
TT	40/38	1.00		55/50	1.00	
TA	48/56	0.86 (0.46–1.59)		56/66	0.86 (0.51–1.48)	
AA	14/10	1.18 (0.45–3.08)	0.52	20/17	1.19 (0.55–2.58)	0.53
Allele						
T	128/132	1.00		166/166	1.00	
A	76/76	1.03 (0.69–1.54)	0.88	96/100	0.96 (0.67–1.37)	0.82
rs10733710						
Genotype						
GG	76/77	1.00		89/98	1.00	
GA	20/24	0.78 (0.39–1.58)		36/31	1.21 (0.68–2.14)	
AA	6/3	2.25 (0.52–9.99)	0.51	6/4	1.49 (0.39–5.61)	0.55
Allele						
G	172/178	1.00		214/227	1.00	
A	32/30	1.10 (0.64–1.90)	0.72	48/39	1.30 (0.82–2.07)	0.26
rs597457						
Genotype						
CC	31/32	1.00		45/43	1.00	
CA	51/52	0.92 (0.48–1.78)		60/65	0.89 (0.51–1.55)	
AA	20/20	1.01 (0.44–2.32)	1.00	26/25	0.90 (0.44–1.81)	0.88
Allele						
C	113/115	1.00		150/151	1.00	
A	91/93	1.02 (0.69–1.50)	0.94	112/115	0.98 (0.70–1.38)	0.91

*Adjusted for age, gender, and smoking status.
$^{\dagger}P$ value for χ^2 analysis.

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Table 3. D' and r^2 between pairs of seven *TGFBR1* htSNPs in NSCLC cases and controls

htSNP pairs		Case-control study 1		Case-control study 2	
		D' Cases/controls	r^2 Cases/controls	D' Cases/controls	r^2 Cases/controls
rs7040869	rs4743325	0.607/0.848	0.111/0.237	0.849/0.816	0.234/0.234
rs7040869	rs1888223	0.203/0.091	0.011/0.003	0.189/0.193	0.011/0.013
rs7040869	rs10819638	0.317/0.147	0.029/0.006	0.159/0.247	0.008/0.018
rs7040869	rs6478974	0.333/0.205	0.067/0.027	0.255/0.194	0.047/0.026
rs7040869	rs10733710	0.250/0.456	0.004/0.013	0.244/0.138	0.006/0.001
rs7040869	rs597457	0.342/0.045	0.034/0.001	0.239/0.205	0.018/0.013
rs4743325	rs1888223	0.042/0.087	0.002/0.007	0.151/0.045	0.021/0.002
rs4743325	rs10819638	0.124/0.174	0.015/0.026	0.211/0.157	0.042/0.021
rs4743325	rs6478974	0.070/0.210	0.002/0.022	0.381/0.113	0.066/0.007
rs4743325	rs10733710	0.008/0.269	0.000/0.014	0.064/0.101	0.001/0.002
rs4743325	rs597457	0.170/0.004	0.028/0.000	0.223/0.042	0.047/0.002
rs1888223	rs10819638	0.754/0.799	0.536/0.579	0.818/0.841	0.658/0.616
rs1888223	rs6478974	0.784/0.884	0.227/0.371	0.968/0.889	0.392/0.397
rs1888223	rs10733710	1.000/0.783	0.141/0.085	1.000/0.742	0.162/0.079
rs1888223	rs597457	0.755/0.618	0.537/0.368	0.833/0.718	0.673/0.470
rs10819638	rs6478974	1.000/0.916	0.478/0.361	1.000/0.938	0.425/0.386
rs10819638	rs10733710	1.000/0.759	0.150/0.073	0.920/0.791	0.140/0.078
rs10819638	rs597457	0.980/0.851	0.961/0.682	0.968/0.885	0.923/0.748
rs6478974	rs10733710	0.889/0.383	0.087/0.014	0.902/0.608	0.105/0.038
rs6478974	rs597457	0.963/0.709	0.443/0.229	0.970/0.817	0.406/0.307
rs10733710	rs597457	1.000/0.883	0.150/0.104	0.922/0.899	0.142/0.106

NOTE: Values of D' and r^2 were calculated with the Haploview program.

in both studies. However, there was a higher proportion of smokers among patients with NSCLC than among controls ($P < 0.001$ and $P = 0.006$, respectively).

The allele and genotype distributions for seven *TGFBR1* htSNPs among NSCLC cases and controls are summarized in Table 2. The genotype frequencies of these polymorphisms were in Hardy-Weinberg equilibrium in controls in both studies. No significant difference in allele and genotype frequencies at any of these seven polymorphic sites was observed between NSCLC patients and controls in either study.

D' value and r^2 for these seven polymorphisms were calculated according to the genotyping data reported in Table 2. The different degrees of LD between cases and controls are summarized in Table 3, and their LD maps measured by D' in cases and controls are shown in Fig. 1. In case-control study 1, four polymorphisms consisting of rs10819638, rs6478974, rs10733710, and rs597457 were in LD with each other in cases ($D' > 0.8$). In contrast, the D' values of rs107733710 with rs10819638 and rs6478974 and the D' value of rs6478974 with rs597457 were <0.80 in controls. Especially, LD between rs6478974 and rs10733710 was very weak in controls ($D' = 0.383$, $r^2 = 0.014$). Moreover, two htSNPs in the 5′ flanking region, rs7040869 and 4743325, had weaker LD in cases ($D' = 0.607$, $r^2 = 0.111$) than they had in controls ($D' = 0.848$, $r^2 = 0.237$). The LD findings in study 2 are similar to those in study 1 (Table 3; Fig. 1).

Accordingly, 4-SNP haplotypes (rs10819638, rs6478974, rs10733710, and rs597457) and 2-SNP haplotypes (rs7040869 and 4743325) were reconstructed according to the genotyping data in NSCLC patients and controls. Using haplotypes with frequencies of >0.05 for further analysis, four 4-SNP haplotypes accounted for 90.0% and 92.2% of the corresponding haplotypes in controls of study 1 and study 2, respectively; three 2-SNP haplotypes accounted for 98.1% and 97.5% of the corresponding haplotypes in controls of study 1 and study 2, respectively (Table 4). After adjustment for gender, age, and smoking status, a 4-SNP CTGC haplotype was significantly more common in controls than in cases in both case-control studies ($P = 0.014$; adjusted OR, 0.09; 95% CI, 0.01–0.61; and $P = 0.010$; adjusted OR, 0.11; 95% CI, 0.02–0.59, respectively) whereas the frequencies for all of 2-SNP haplotypes were not significantly different between NSCLC patients and controls. Moreover, as summarized in Table 5, combined analysis of both studies shows an association of this 4-SNP haplotype with decreased NSCLC risk (adjusted OR, 0.11; 95% CI, 0.03–0.39). Interestingly, four individuals were homozygous for the 4-SNP haplotype among controls (4 of 237) and none among cases (0 of 233; $P = 0.124$). We did not observe any association between the 4-SNP haplotype and gender ($P = 0.745$); age, assessed either as a categorical ($P = 0.584$) or a continuous ($P = 0.317$) variable; histology ($P = 0.599$); and tumor-node-metastasis (TNM) stage ($P = 0.804$). Importantly, we found that the pairwise LD values between these four SNPs were quite strong, especially for cases in both studies (Supplementary Table S3). These findings provide strong support for the novel notion that the CTGC haplotype is associated with lung cancer risk.

Discussion

The morbidity and mortality of lung cancer have dramatically increased in China in the past decade. It has been suggested that NSCLC results from accumulation of multiple genetic and/or epigenetic aberrations, and genetic variants that modulate susceptibility to complex diseases may be identified through association studies. Recent studies have confirmed that variants in DNA and cell cycle pathway are weakly associated with risk of lung cancer (22). A recent genome-wide association study has identified two

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markers at 5p15.33 that are associated with risk of lung cancer (23). Recent studies suggest that, compared with single SNP approaches for genetic association studies, analyses based on haplotypes can significantly improve the power of mapping disease genes (24).

This is the first study investigating the association of *TGFBR1* haplotypes with risk for NSCLC. We performed tagging SNP and haplotype analyses to comprehensively capture the various genetic variants of *TGFBR1* in the Chinese population. No significant differences in allele and genotype frequencies were observed between NSCLC patients and controls, which suggests that none of the individual *TGFBR1* SNPs examined in this study is associated with NSCLC risk. However, a 4-SNP *TGFBR1* CTGC haplotype was significantly higher in controls (10.4% for study 1 and 8.8% for study 2) than in NSCLC patients (2.9% for study 1 and 3.1% for study 2), indicating that this haplotype may confer protection against NSCLC (combined adjusted OR, 0.11; 95% CI, 0.03–0.39). To test the hypothesis that constitutively decreased TGFBR1 signaling enhances cancer risk, we have developed a novel mouse model of *Tgfbr1* haploinsufficiency (25). We observed that $Tgfbr1^{+/-}$ mice do not exhibit an obvious phenotype but, when crossed with $Apc^{Min/+}$ mice, have a dramatically increased susceptibility to develop colorectal cancer. These findings led us to validate this hypothesis in humans and led to the discovery that constitutively decreased TGFBR1 signaling in humans is also associated with dramatically increased colorectal cancer susceptibility (26). This allele-specific quantitative trait is dominantly inherited, and two *TGFBR1* haplotypes are associated with a substantially increased risk of colorectal cancer in Caucasians (26). We hypothesize that constitutively decreased TGFBR1 signaling may be associated with increased cancer susceptibility that is not limited to colorectal cancer. Because of the observed protective effect of the *TGFBR1* CTGC haplotype with respect to NSCLC risk, we predict that the CTGC haplotype is associated with increased TGF-β signaling.

TGF-β is a potent naturally occurring inhibitor of cell growth. Decreased TGF-β signaling may increase susceptibility to cancer development (27, 28). There is compelling evidence supporting the concept that *TGFBR1* is a tumor suppressor gene, and *TGFBR1* mutations are associated with various human cancers, including head and neck cancers and cervical and ovarian carcinomas (11, 29–31). However, such an association has not been found in

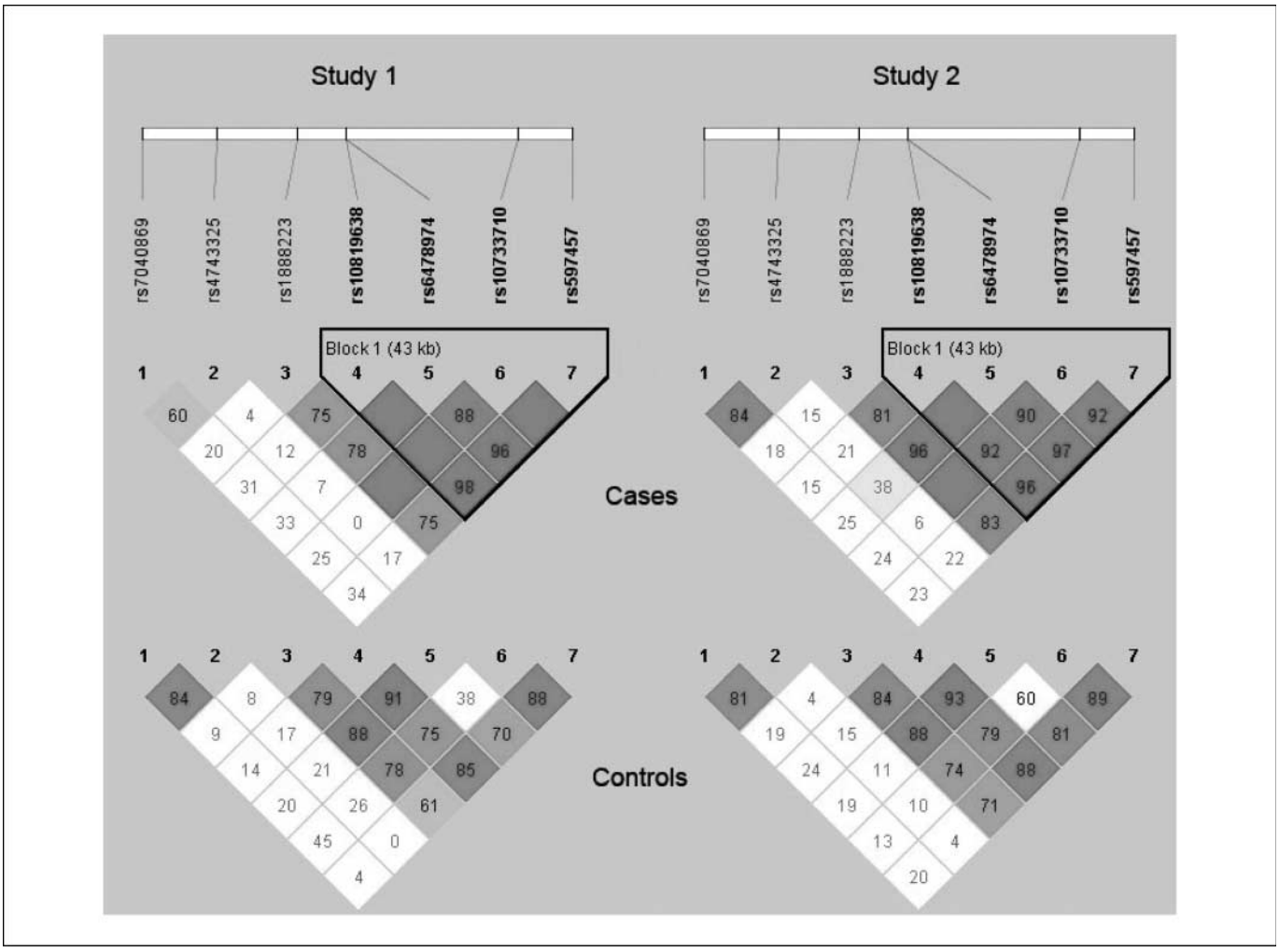

Figure 1. LD maps of seven htSNPs in NSCLC cases and controls in two studies. The value in each diamond is measured as *D′* corresponding to the dark grey-to-white color gradient. Dark grey diamonds without a number indicate that the value of *D′* was 1. In study 1 (*left*), four polymorphisms consisting of rs10819638, rs6478974, rs10733710, and rs597457 were in LD with each other in cases (*D′* > 0.8). In contrast, the value of *D′* of rs107733710 with rs10819638 and rs6478974 and the value of *D′* of rs6478974 with rs597457 were <0.80 in controls. The LD status in study 2 (*right*) is similar to that in study 1.

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Table 4. Frequencies of estimated 4-SNP and 2-SNP haplotypes of *TGFBR1* in NSCLC cases and controls

	4-SNP haplotype*				2-SNP haplotype*		
	TTGA	CAGC	CTAC	CTGC	GG	GT	AT
Case-control study 1							
Cases (%)/controls (%)	44.1/38.6	36.3/29.2	15.2/11.8	2.9/10.4	40.8/44.7	32.7/27.9	21.7/25.5
Crude OR (95% CI) †	1.08 (0.72–1.61)	1.21 (0.80–1.85)	1.20 (0.68–2.13)	0.24 (0.09–0.60)	0.90 (0.60–1.33)	1.31 (0.86–2.00)	0.84 (0.53–1.33)
P	0.707	0.365	0.526	0.001	0.588	0.291	0.464
Adjusted OR (95% CI) ‡	1.48 (0.64–3.42)	1.88 (0.76–4.63)	1.80 (0.60–5.37)	0.09 (0.01–0.61)	0.59 (0.26–1.31)	1.81 (0.74–4.39)	0.83 (0.32–2.18)
P	0.363	0.171	0.293	0.014	0.195	0.191	0.706
Case-control study 2							
Cases (%)/controls (%)	41.6/38.8	35.9/31.9	17.2/12.7	3.1/8.8	41.9/43.4	28.7/27.3	27.4/26.8
Crude OR (95% CI) †	1.02 (0.72–1.45)	1.10 (0.76–1.58)	1.34 (0.82–2.17)	0.31 (0.14–0.70)	0.93 (0.66–1.32)	1.06 (0.73–1.56)	1.02 (0.70–1.50)
P	0.911	0.626	0.239	0.003	0.695	0.752	0.908
Adjusted OR (95% CI) ‡	1.16 (0.58–2.34)	1.66 (0.78–3.53)	1.94 (0.74–5.12)	0.11 (0.02–0.59)	0.93 (0.47–1.84)	1.19 (0.54–2.63)	1.00 (0.47–2.15)
P	0.679	0.185	0.181	0.01	0.823	0.668	0.993

NOTE: Haplotypes with frequencies of >5% were included.
*Four htSNPs alleles from left to right (i.e., rs10819638, rs6478974, rs10733710, and rs597457) and two htSNPs (rs7040869 and rs4743325) were used for 4-SNP and 2-SNP reconstruction of haplotypes. Haplotype bases are depicted from the coding strand of *TGFBR1*.
†Calculated with SHEsis program.
‡Adjusted for gender, age, and smoking status using SAS software.

lung cancer (18). Although polymorphisms of the *TGFBR1* gene, including *TGFBR1**6A and Int7G24A, are associated with cancer risk in some studies (18, 32, 33), only limited data exist on the role of *TGFBR1* htSNP in NSCLC. *TGFBR1**6A, located in exon 1 of *TGFBR1*, has a deletion of three alanines within a stretch of nine alanines (12). There is accumulating evidence showing that *TGFBR1**6A may be associated with risk of breast, ovarian, and cervix cancers as well as with risk for abdominal aortic aneurysm (11–14, 34). Moreover, *TGFBR1**6A is somatically acquired in 29.5% of liver metastases from colorectal cancer (35) and enhances MCF-7 breast cell migration (36). Nevertheless, we previously reported no association between *TGFBR1**6A and lung cancer (17). Although the common SNP Int7G24A may modify risk of kidney, bladder, and invasive breast cancers (15, 16), it was not associated with risk for NSCLC (18). In this study, we selected a total of seven tagging SNPs. No single htSNP was significantly associated with risk of NSCLC, suggesting that interplay between these htSNP is related to predisposition to NSCLC, similarly to what we observed in colorectal cancer (26). In summary, our results suggest that a 4-SNP haplotype of *TGFBR1* is associated with significantly decreased risk for NSCLC. These finding warrant additional functional studies as well as validation studies in large series of NSCLC cases and matched controls in various ethnic groups.

Table 5. Frequencies of estimated 4-SNP and 2-SNP haplotypes of *TGFBR1* in combined cases and controls

Haplotype*	Combined case-control study		Crude OR (95% CI) †	*P*	Adjusted OR (95% CI) ‡	*P*
	Cases (%)	Controls (%)				
4-SNP						
TTGA	42.7	38.7	1.05 (0.80–1.36)	0.736	1.24 (0.72–2.12)	0.440
CAGC	36.0	30.7	1.14 (0.87–1.51)	0.338	1.64 (0.93–2.90)	0.090
CTAC	16.3	12.3	1.28 (0.88–1.85)	0.193	2.07 (1.00–4.28)	0.050
CTGC	3.0	9.5	0.27 (0.15–0.51)	0.00001	0.11 (0.03–0.39)	0.0007
2-SNP						
GG	41.5	44.0	0.92 (0.71–1.19)	0.521	0.78 (0.47–1.31)	0.348
GT	30.4	27.6	1.16 (0.88–1.54)	0.298	1.43 (0.80–2.58)	0.230
AT	25.0	26.2	0.95 (0.71–1.27)	0.722	0.91 (0.50–1.66)	0.767

NOTE: Haplotypes with frequencies of >5% were included.
*Four htSNPs alleles from left to right (i.e., rs10819638, rs6478974, rs10733710, and rs597457) and two htSNPs (rs7040869 and rs4743325) were used for 4-SNP and 2-SNP reconstruction of haplotypes. Haplotype bases are depicted from the coding strand of *TGFBR1*.
†Calculated with SHEsis program.
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Disclosure of Potential Conflicts of Interest

B. Pasche reports that he has filed patents related to *TGFBR1* in colorectal cancer. The other authors disclosed no potential conflicts of interest.

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