

Original Article

Tankyrase 1 polymorphism associated with an increased risk in developing non-small cell lung cancer in a Chinese population: a proof-of-principle study

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Abstract: Objectives: Tankyrase 1 (TNKS1), a poly (ADP-ribose) polymerase, regulates telomere length and apoptosis in cells, overexpression of which occurred in non-small cell lung cancer (NSCLC). This study investigated TNKS1 single-nucleotide polymorphisms (SNPs) for association with a risk in NSCLC development in a Chinese population. Methods: NSCLC cases and healthy controls of 500 each were recruited for genotyping of 24 TNKS1 SNPs. The association between genotype and NSCLC risk was evaluated by computing the odds ratio (OR) and 95% confidence interval (CI) with multivariate unconditional logistic regression analyses. Haploview software was to analyze association between haplotypes and NSCLC risk. Results: TNKS1 rs6601328 A allele was associated with a lower risk in developing NSCLC and adenocarcinoma (ADC) (OR=0.71; 95% CI, 0.51-0.99 and OR=0.70; 95% CI, 0.50-0.99), whereas TNKS1 rs11991621 C allele (OR=1.44; 95% CI, 1.03-2.03), rs11991621 C/C (OR=1.44, 95% CI, 1.03-2.35; $P=0.03$), and rs10503380 G/G (OR= 1.56, 95% CI, 1.09-2.50, $P=0.02$) were associated with a higher risk in developing NSCLC or ADC in females and rs6601328 A/A major allele (OR=1.39; 95% CI, 1.00-1.92; $P=0.047$) and rs7015700 G/G (OR= 1.51, 95% CI, 1.04-2.21) was associated with an increased NSCLC or ADC risk in males but a reduced NSCLC risk (OR=0.63; 95% CI, 0.42-0.96) and ADC risk (OR=0.64; 95% CI, 0.42-0.97) in females. Haploview showed that there were three Haplotype Blocks associated with NSCLC risk. However, TNKS1 rs12541709 C/C was associated with protective effect against ADC (OR=0.75; 95% CI, 0.56-0.99; $P=0.04$) in this Chinese population. Conclusion: TNKS1 SNPs (rs11991621 rs10503380, and rs7015700) were associated with NSCLC risk, whereas rs6601328 and rs12541709 inversely associated with NSCLC or ADC risk in this Chinese population.

Keywords: Non-small cell lung cancer, genetic susceptibility, *TNKS1*, single nucleotide polymorphisms

Introduction

Lung cancer is a significant health problem and one of the leading causes of cancer death in the world [1]. Lung cancer is significantly increased in China [2], although lung cancer incidence has been declining in the US [3]. Histologically, non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, accounting for approximately 85% of all lung cancer, most of which are diagnosed at the advanced stages of disease and lead to poor five-year survival (16% of the overall survival rate [4]). Tobacco smoke is the sin-

gle most significant risk factor in developing lung cancer [5, 6], although certain population, like Chinese females with a lower prevalence of tobacco smoke (less than 4% adult smokers [7]) is more frequently developing lung cancer than that of certain European counterparts with an 20% adult tobacco smoke rate [8]. Lung cancer risk also occurs in different ethnic groups [9, 10]. These data indicate that gene-environment interaction played an important role in lung carcinogenesis. Thus, research focuses on individual genetic susceptibility could help us to better understand mechanism of lung cancer development.

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Table 1. Clinical characteristics of cases and controls

Population	Gender, n		Age (years)		TNM stage, n				ADC, n		SCC, n		Smoking status, n			
	Male	Female	Average	Range	I	II	III	IV	Male	Female	Male	Female	Smoking		Non-smoking	
NSCLC patients	350	150	56.66	33-80	42	73	138	247	189	142	161	8	280	21	70	129
Controls	259	240	50.75	29-73	-	-	-	-	-	-	-	-	189	14	70	226

Table 2. *TNKS1* allele frequency between female NSCLC patients and healthy controls

Gene allele	Female controls	Female NSCLC patients	P value	OR (95% CI)	Female ADC	P value	OR (95% CI)
rs6601328							
A	379 (79.0)	218 (72.7)			206 (72.5)		
G	101 (21.0)	82 (27.3)	0.04	0.71 (0.51-0.99)	78 (27.5)	0.04	0.70 (0.50-0.99)
rs11991621							
C	340 (70.8)	231 (77.0)			221 (77.8)		
T	140 (29.2)	69 (23.0)	0.06	1.37 (0.99-1.92)	63 (22.2)	0.03	1.44 (1.03-2.03)

Indeed, to date, a great number of studies have shown that genetic susceptibility is the most important factor in lung cancer development and contributes to individual lung cancer risk [11-13]. Our research has been focused on tankyrase 1 (TNKS1), which is a multifunctional poly (ADP-ribose) polymerase (PARP) that utilizes NAD⁺ as a substrate to transfer ADP-ribose polymers onto protein acceptors, including itself [14, 15]. Previous studies demonstrated that tankyrase 1 was able to regulate telomere length [16] and apoptosis [17] in cells, overexpression of which occurred in NSCLC tissue specimens [18]. Knockdown of TNKS1 expression using shRNA was shown to reduce lung cancer cell growth and repressed formation and growth of lung cancer cell xenografts in a murine lung cancer model [19]. tankyrase 1 as well as tankyrase 2 is also key mediators of the Wnt-signaling [20, 21] and the latter signaling pathway has been used as a potential therapeutic target for lung cancer [22-25]. Thus, detection of TNKS1 genetic polymorphisms could help us to evaluate and identify whether TNKS1 is a lung cancer susceptibility. Therefore, in this study, we recruited 500 each of NSCLC patients and healthy controls to genotype 24 *TNKS1* SNPs for association with a risk in developing NSCLC in a Chinese population.

Patients and methods

Study population

This study obtained 500 each of NSCLC patients and healthy controls, as described in

our previous study [10]. Specifically, 500 each of NSCLC patients and unrelated age-matched healthy controls were recruited from Zhejiang Cancer Hospital between March 2011 and April 2012. All patients were histologically diagnosed as NSCLC and any patients with history of primary cancer, other than lung cancer, were excluded from this study. The controls were independent of any lung-related diseases to avoid any probable interference from overlapping genes. All control subjects were matched to these patients by sex and age. Tobacco smoking was recorded and defined as one who had smoked more than ten packs of tobacco in life time and a current or former smoker was defined as a smoker who was still smoking in the current year or in a year before participation of this study according to a previous study [26]. This study was approved by the ethical committee of Zhejiang Cancer Hospital. All subjects provided an informed consent before participation of this study.

Selection and genotyping of *TNKS1* SNPs

TNKS1 SNPs were selected by using the SNP Browser version 3.5 (http://www.allsnps.com/snp_browser) based on previously published criteria [27], i.e., pairwise r^2 (≥ 0.8) among all common SNPs with minor allele frequency (MAF) ≥ 0.15 using the Tagger program implemented in Haploview version 4.1 (<http://www.broad.mit.edu/mpg/haploview>). After that, we utilized the Chinese HapMap database to select *TNKS1* SNPs for genotyping in this study,

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Table 3. *TNKS1* SNP genotypes between NSCLC patients and controls

Genotypes	NSCLC Controls		<i>p</i> value	OR (95% CI)	M NSCLC M Controls		<i>p</i> value	OR (95% CI)	F NSCLC F Controls		<i>p</i> value	OR (95% CI)
	N=500 n (%)	N=500 n (%)			N=350, n (%)	N=259, n (%)			N=150 n (%)	N=240 n (%)		
rs6601328												
AA	285 (57.0)	284 (56.8)			208 (59.4)	133 (51.4)			77 (51.3)	150 (62.5)		
AG	187 (37.4)	192 (38.4)			123 (35.1)	113 (43.6)			64 (42.7)	79 (32.9)		
GG	28 (5.6)	24 (4.8)	0.83		19 (5.4)	13 (5.0)	0.10		9 (6.0)	11 (4.6)	0.09	
AG+GG	215 (43.0)	216 (43.2)	0.95	1.01 (0.79-1.30)	142 (40.6)	126 (48.6)	0.047	1.39 (1.00-1.92)	73 (48.7)	90 (37.5)	0.03	0.63 (0.42-0.96)
rs11991621												
CC	267 (53.4)	265 (53.0)			178 (50.9)	148 (57.1)			89 (59.3)	16 (48.3)		
TT	35 (7.0)	28 (5.6)			27 (7.7)	12 (4.6)			8 (5.3)	16 (6.7)		
CT	198 (39.6)	207 (41.4)	0.61		145 (41.4)	99 (38.2)	0.16		53 (35.3)	108 (45.0)	0.11	
TT+CT	233 (46.6)	235 (47.0)	0.90	1.02 (0.79-1.30)	172 (49.1)	111 (42.9)	0.12	0.78 (0.56-1.07)	61 (40.7)	124 (51.7)	0.03	1.56 (1.03-2.35)
rs10503380												
GG	216 (43.2)	197 (39.4)			144 (41.1)	110 (42.5)			72 (48.0)	86 (35.8)		
AG	213 (42.6)	244 (48.8)			155 (44.3)	116 (44.8)			58 (38.7)	128 (53.3)		
AA	71 (14.2)	59 (11.8)	0.13		51 (14.6)	33 (12.7)	0.81		20 (13.3)	26 (10.8)	0.02	
AG+GG	284 (56.8)	303 (60.6)	0.22	1.17 (0.91-1.51)	206 (58.9)	149 (57.5)	0.74	0.95 (0.68-1.31)	78 (52.0)	154 (64.2)	0.02	1.65 (1.09-2.50)

M, male; F, female.

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Table 4. *TNKS1* SNP genotypes between ADC patients and controls

Genotypes	ADC Controls		<i>p</i> value	OR (95% CI)	M ADC M Controls		<i>p</i> value	OR (95% CI)	F ADC F Controls		<i>p</i> value	OR (95% CI)
	N=331 n (%)	N=500 n (%)			N=189 n (%)	N=259 n (%)			N=142 n (%)	N=240 n (%)		
rs12541709												
CC	128 (38.7)	229 (45.8)			76 (40.2)	120 (46.3)			52 (36.6)	108 (45.0)		
GG	38 (11.5)	55 (11.0)			23 (12.2)	35 (13.5)			15 (10.6)	20 (8.3)		
CG	165 (49.8)	216 (43.2)	0.12		90 (47.6)	104 (40.2)	0.27		75 (52.8)	112 (46.7)	0.26	
GG+CG	203 (61.3)	271 (54.2)	0.04	0.75 (0.56-0.99)	113 (59.8)	139 (53.7)	0.20	0.78 (0.53-1.14)	90 (63.4)	132 (55.0)	0.11	0.76 (0.46-1.08)
rs7015700												
GG	167 (50.5)	236 (47.2)			99 (52.4)	109 (42.1)			68 (47.9)	126 (52.5)		
AA	26 (7.9)	42 (8.4)			12 (6.3)	20 (7.7)			14 (9.9)	22 (9.2)		
AG	138 (41.7)	222 (44.4)	0.66		78 (41.3)	130 (50.2)	0.09		60 (42.3)	92 (38.3)	0.68	
AA+AG	164 (49.5)	264 (52.8)	0.36	1.14 (0.86-1.50)	90 (47.6)	150 (57.9)	0.03	1.51 (1.04-2.21)	74 (52.1)	114 (47.5)	0.38	0.83 (0.55-1.26)
rs11991621												
CC	180 (54.4)	265 (53.0)			94 (49.7)	148 (57.1)			86 (60.6)	116 (48.3)		
TT	22 (6.6)	28 (5.6)			15 (7.9)	12 (4.6)			7 (4.9)	16 (6.7)		
CT	129 (39.0)	207 (41.4)	0.70		80 (42.3)	99 (38.2)	0.17		49 (34.5)	108 (45.0)	0.07	
TT+CT	151 (45.6)	235 (47.0)	0.70	1.06 (0.80-1.40)	95 (50.3)	111 (42.9)	0.12	0.74 (0.51-1.08)	56 (39.4)	124 (51.7)	0.02	1.64 (1.08-2.50)
rs6601328												
AA	187 (56.5)	284 (56.8)			114 (60.3)	133 (51.4)			73 (51.4)	150 (62.5)		
GG	17 (5.1)	24 (4.8)			8 (4.2)	13 (5.0)			9 (6.3)	11 (4.6)		
AG	127 (38.4)	192 (38.4)	0.98		67 (35.4)	113 (43.6)	0.17		60 (42.3)	79 (32.9)	0.10	
GG+AG	144 (43.5)	216 (43.2)	0.93	0.99 (0.75-1.31)	75 (39.7)	126 (48.6)	0.06	1.44 (0.99-2.11)	69 (48.6)	90 (37.5)	0.03	0.64 (0.42-0.97)
rs10503380												
GG	145 (43.8)	197 (39.4)			76 (40.2)	110 (42.5)			69 (48.6)	86 (35.8)		
AA	43 (13.0)	59 (11.8)			24 (12.7)	33 (12.7)			19 (13.4)	26 (10.8)		
AG	143 (43.2)	244 (48.8)	0.29		89 (47.1)	116 (44.8)	0.88		54 (38.0)	128 (53.3)	0.01	
AA+AG	186 (56.2)	303 (60.6)	0.21	1.20 (0.91-1.59)	113 (59.8)	149 (57.5)	0.63	0.91 (0.62-1.33)	73 (51.4)	154 (64.2)	0.01	1.69 (1.11-2.58)

Tankyrases1 SNPs and NSCLC risk

Table 5. Haplotype distribution and formation between patients and controls for association of haplotype blocks with NSCLC risk

Block	loci	Cases/controls	Haplotype	Frequency (%)		OR (95% CI) [#]	P value [#]		
				Case	Control				
Block 1	rs11991621 and rs4840426	Male NSCLC patients	CT	31.9	37.5	0.78 (0.62-0.99)	0.04		
			Male controls	CG	39.7	38.8	1.04 (0.82-1.31)	0.75	
				TT	28.4	23.5	1.29 (0.99-1.67)	0.06	
		Male ADC patients	Male controls	CT	31.0	37.5	0.75 (0.57-0.99)	0.04	
				CG	39.9	38.7	1.05 (0.80-1.38)	0.73	
				TT	29.0	23.5	1.33 (0.99-1.80)	0.06	
Block 2	rs11991621, rs4840426 and rs10503380	Female NSCLC patients	CTG	28.0	20.9	1.47 (1.06-2.06)	0.02		
			Female controls	TTA	22.2	28.0	0.73 (0.52-1.03)	0.07	
				CGG	38.5	40.5	0.92 (0.69-1.24)	0.59	
		CTA		9.6	9.2	1.06 (0.65-1.73)	0.83		
		Female ADC patients	Female controls	TTA	21.4	28.0	0.70 (0.49-0.99)	0.04	
				CTG	28.2	20.9	1.49 (1.06-2.09)	0.02	
				CGG	38.6	40.5	0.92 (0.68-1.25)	0.61	
		Block 3	rs12542420, rs11774818, rs4075359, rs7015700 and rs7006406	Male ADC patients	GAAGG	22.7	16.6	1.48 (1.06-2.07)	0.02
					Male controls	AAGGA	38.3	37.3	1.04 (0.79-1.37)
AGAAA	18.9					21.4	0.86 (0.62-1.20)	0.37	
GAAGA	10.1			9.5		1.07 (0.68-1.67)	0.77		
SCC patients	Controls			AAAAA	6.7	9.2	0.71 (0.43-1.17)	0.18	
				GAAAA	0.8	1.4	0.58 (0.15-2.27)	0.44	
				AGAGA	0.9	1.2	0.70 (0.19-2.68)	0.61	
				GAAGA	13.9	9.6	1.52 (1.05-2.21)	0.03	
				AAGGA	36.5	37.7	0.95 (0.74-1.23)	0.71	
				AGAAA	21.2	20.5	1.04 (0.77-1.41)	0.78	
Female SCC patients	Female controls			GAAGG	17.1	18.3	0.92 (0.66-1.27)	0.61	
				AAAAA	5.8	8.5	0.67 (0.40-1.11)	0.12	
				AAAGA	1.1	1.5	0.73 (0.23-2.31)	0.59	
				AGAGA	1.9	0.9	2.13 (0.77-5.86)	0.15	
				GAAGA	25.0	9.8	3.08 (0.95-9.93)	0.048	
		AAGGA	37.5	37.8	0.99 (0.35-2.76)	0.98			
Female SCC patients	Female controls	AGAAA	18.7	19.9	0.93 (25.9-3.32)	0.91			
		GAAGG	12.5	20.1	0.57 (0.13-2.54)	0.46			
		AAAAA	0.0	7.6	-	0.25			
			AAAGA	0.0	1.9	-	0.57		

[#]Compared with other haplotypes.

i.e., population = CHB (Han-Chinese, Beijing), resulting in 24 *TNKS1* Tag SNPs.

To genotype these 24 SNPs, we extracted genomic DNA from whole blood of these participants using the AxyPrep Blood Genomic DNA Miniprep kit (Axygen Biosciences, Union City, CA) and then genotyped these 24 SNPs using the SEQUENOM MassARRAY matrix-assisted

laser desorption ionization-time of flight mass spectrometry platform (Sequenom, San Diego, CA). PCR primers and single base extension primers were designed using the Assay Designer's software version 3.0 (Sequenom) and synthesized by Sangon Biotech (Shanghai, China). A total of 10% samples were randomly selected and repeated PCR amplification; PCR data showed 100% of repeatability.

Tankyrases1 SNPs and NSCLC risk

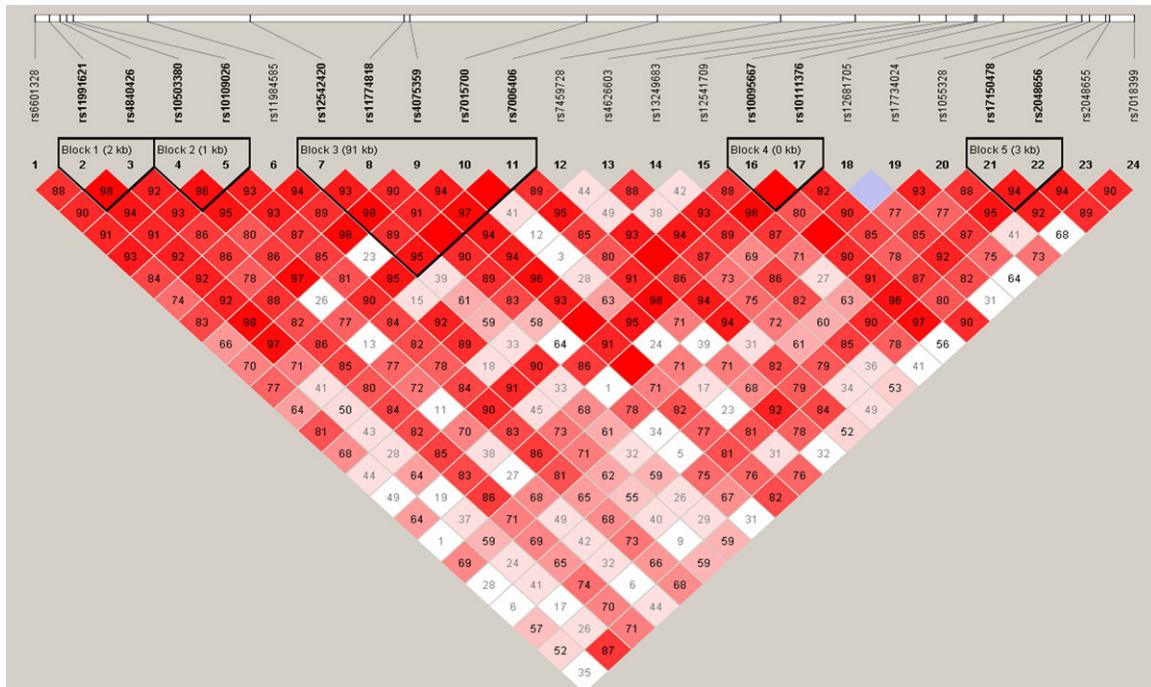


Figure 1. Structure of haplotype block 1, including rs11991621 and rs4840426 among male NSCLC, ADC, and healthy controls. Haplotype structure of Block 3, including rs12542420, rs11774818, rs4075359, rs7015700, and rs7006406 between male ADC and healthy controls. The darker color indicates a higher level of the linkage disequilibrium (LD), while the lighter color indicates less LD.

Statistical analysis

All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL). Hardy-Weinberg equilibrium (HWE) was performed to analyze TNKS1 allele and genotype frequencies between cases and control using χ^2 test and a $P < 0.001$ value was selected for a HWE discrepancy. We also performed the χ^2 test to assess frequencies of the selected allele and genotype for association with lung cancer risk. The association between SNPs and NSCLC risk and link between tobacco smoke and lung cancer risk were evaluated by computing the odds ratio (OR) and 95% confidence interval (CI) from multivariate unconditional logistic regression analysis. Haploview software version 4.1 was to analyze association between haplotypes and NSCLC. A two-sided p value less than 0.05 was considered statistically significant.

Results

Clinical characteristics of our study population

The clinical characteristics of patients and controls are shown in **Table 1**. Specifically, the mean

age of patients was 56.6 years (ranged between 33 and 80 years) and 350 males and 150 females with 115 stage I/II and 385 stage III/IV diseases. There were 381 adenocarcinoma and 169 squamous cell carcinoma and 301 tobacco smokers and 199 non-smokers. In contrast, the mean age of the healthy controls was 50.7 years (ranged between 29 and 73 years) and 259 males and 240 females with 203 tobacco smokers and 296 non-smokers.

TNKS1 polymorphisms between NSCLCs and healthy controls

In this study, we selected and genotyped 24 TNKS1 SNPs (rs10109026, rs1055328, rs12541709, rs4840426, rs7459728, rs4075359, rs10111376, rs11991621, rs7006406, rs13249683, rs17150478, rs10095667, rs2048656, rs11774818, rs4626603, rs12681705, rs7015700, rs7018399, rs11984585, rs12542420, rs2048655, rs6601328, rs17734024, and rs10503380) in both cases and controls. We found that all of these 24 SNPs were within the Hardy-Weinberg equilibrium between cases and controls. However, frequency of rs6601328 A allele was 79.0% and G

Tankyrases1 SNPs and NSCLC risk

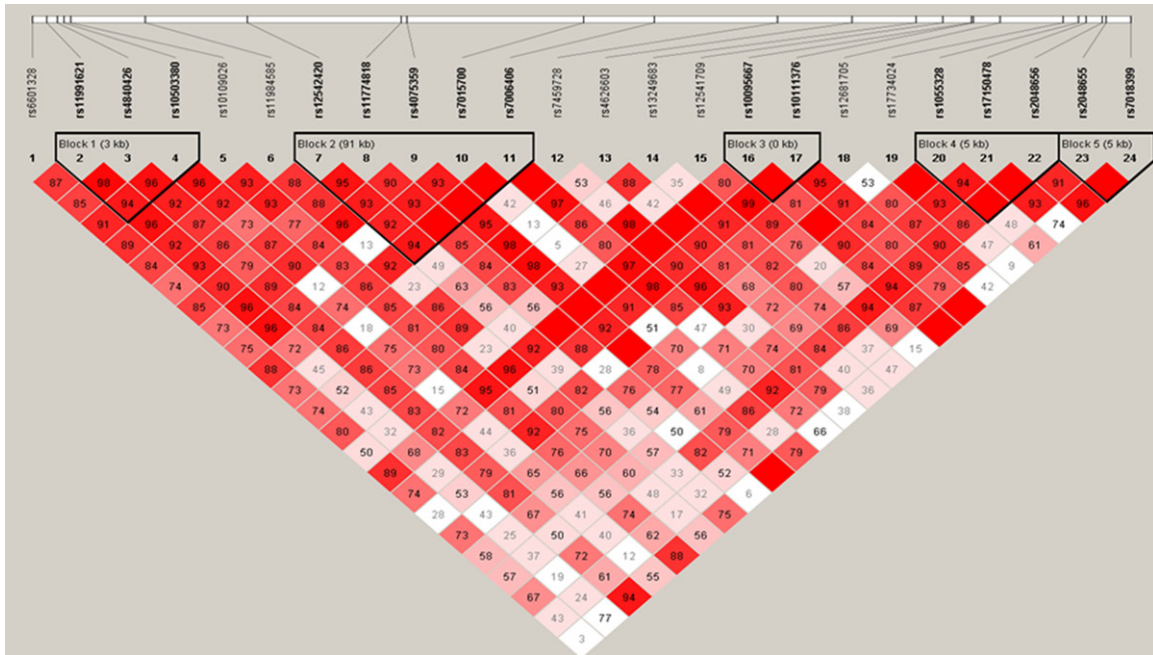


Figure 2. Haplotype structure of rs11991621, rs4840426, and rs10503380 among female NSCLC, ADC, and healthy controls. The darker color indicates a higher level of linkage disequilibrium (LD), while the lighter color indicates less LD.

allele was 21.0% in female controls, whereas they were 72.7% and 27.3%, and 72.5% and 27.5% in Female NSCLCs and ADCs, respectively ($P=0.04$). Logistic regression analysis revealed that rs6601328 A allele associated with a reduced risk in developing NSCLC and ADC (OR=0.71; 95% CI, 0.51-0.99 and OR=0.70; 95% CI, 0.50-0.99, respectively) in females. Similar, frequency of rs11991621 T and C allele was 29.2% and 70.8%, and 22.2% and 77.8% in female controls and female ADC, respectively ($P=0.03$). Logistic regression analysis revealed that rs11991621 C allele associated with a greater risk in developing ADC (OR=1.44; 95% CI, 1.03-2.03) in females (Table 2).

Genotype analysis showed that rs6601328 A/A genotype was associated with an increased NSCLC risk in males (59.4% vs. 51.4% between male NSCLC and controls, OR=1.39; 95% CI, 1.00-1.92; $P=0.047$). In contrast, A/A genotype was associated with a reduced NSCLC risk in females (51.3% vs. 62.5% between female NSCLC and controls, OR=0.63; 95% CI, 0.42-0.96; $P=0.03$; Table 3). Furthermore, rs11991621 C/C and rs10503380 G/G genotypes had a 1.56- and 1.65-fold increased risk in developing female NSCLC (95% CI, 1.03-2.35;

$P=0.03$ and 95% CI, 1.09-2.50, $P=0.02$, respectively; Table 3). However, rs12541709 C/C genotype did have a protective role to reduce ADC risk in both male and female (OR, 0.75; 95% CI, 0.56-0.99; $P=0.04$; Table 4). In addition, rs7015700 G/G genotype had 1.51-fold risk in developing male ADC (95% CI, 1.04-2.21; Table 4). Similarly, rs11991621 C/C and rs10503380 G/G genotypes had 1.64-fold (95% CI, 1.08-2.50) and 1.69-fold (95% CI, 1.11-2.58) risk in developing female ADC, respectively (Table 4). In contrast, rs6601328 A/A genotype had 0.64-fold reduction of risk in developing female ADC (95%, 0.42-0.97; Table 4).

Synergy of TNKS1 polymorphisms in association with NSCLC risk

We next utilized Haploview software to identify the Haplotype Blocks and then performed logistic regression analyses to associate haplotype blocks with NSCLC risk. We found that rs11991621 and rs4840426 were identified a Block 1 (the length was 2kb) in males and that their CT haplotype was associated with a decreased risk in developing NSCLC (OR, 0.78; 95%, 0.62-0.99; $P=0.04$), more obviously in

Tankyrases1 SNPs and NSCLC risk

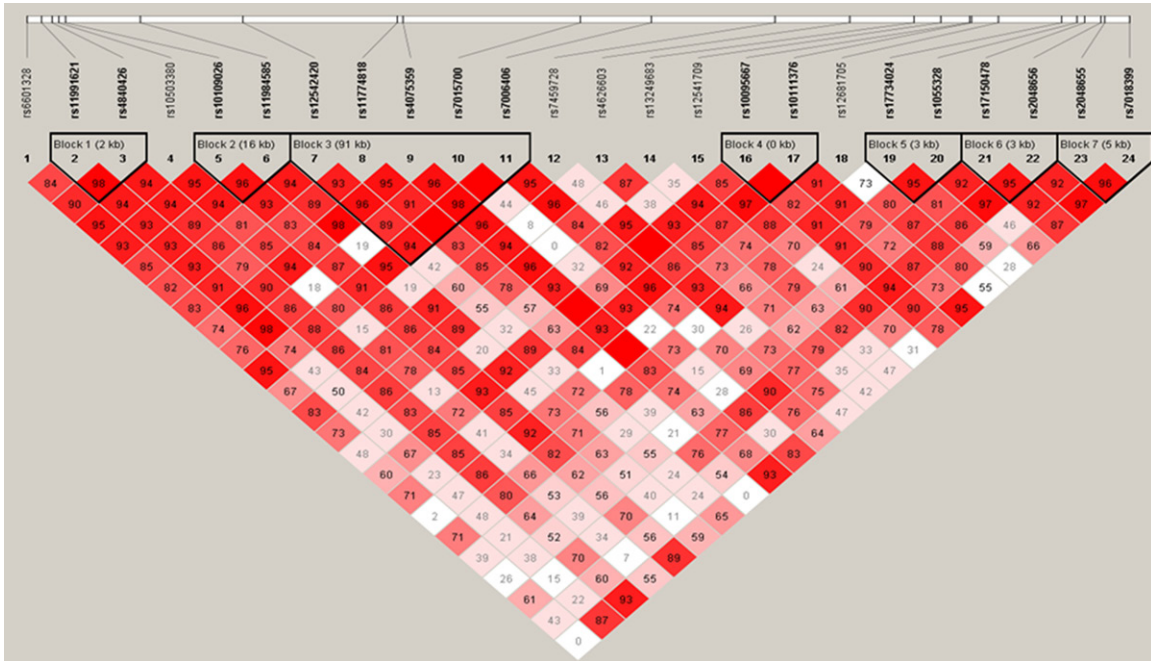


Figure 3. Haplotype structure of all markers among SCC patients and controls. The darker color indicates a higher level of linkage disequilibrium (LD), while lighter color indicates less LD.

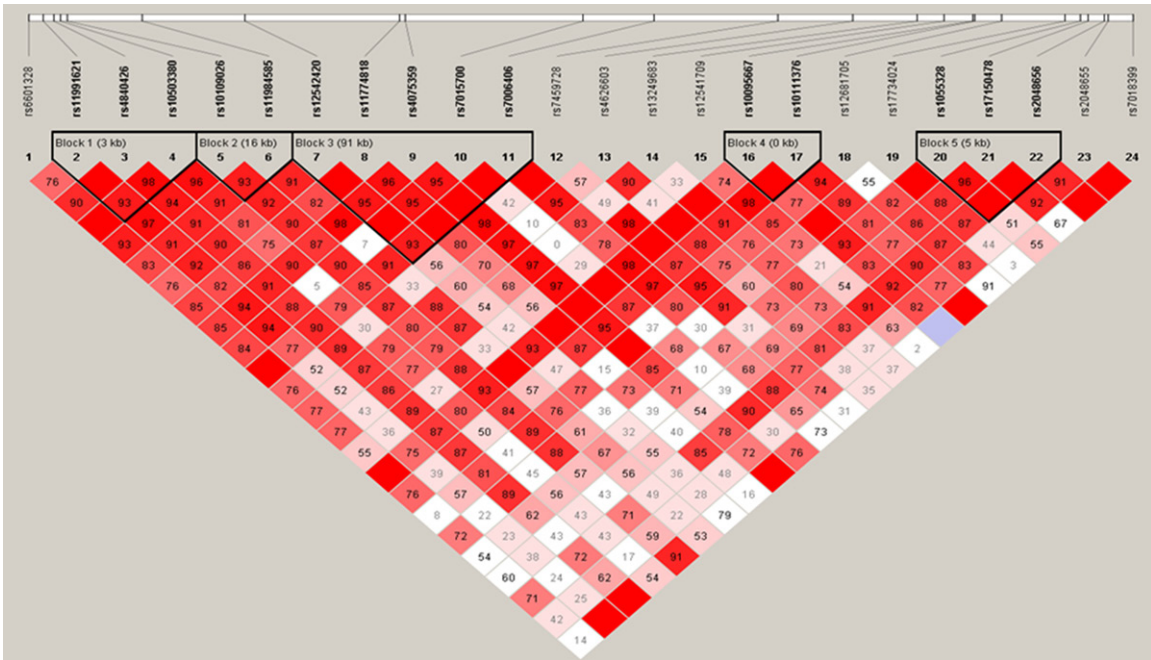


Figure 4. Haplotype structure of all markers between female SCC and healthy controls. The darker color indicates a higher level of linkage disequilibrium (LD), while lighter color indicates less LD.

reduction of ADC risk (Block 1 in Table 5 and Figure 1). Similarly, CTG haplotype was associated with increased risk in developing female NSCLC (OR, 1.47; 95%, 1.06-2.06; $P=0.02$),

especially in developing female ADC (OR, 1.49; 95%, 1.06-2.09; $P=0.02$). Furthermore, TTA haplotype was associated with decreased risk in developing ADC (OR, 0.70; 95%, 0.49-0.99;

$P=0.04$) in Block 2 (the length was 3kb, including rs11991621, rs4840426, and rs10503380; **Table 5** and **Figure 2**). GAAGG haplotype was associated with increased risk of developing male ADC (OR, 1.48; 95%, 1.06-2.07; $P=0.02$) and SCC (OR, 1.52; 95%, 1.05-2.21; $P=0.03$), but more obviously in developing female SCC (OR, 3.08; 95%, 0.95-9.93; $P=0.048$) in Block 3 (the length was 91 kb, including rs12542420, rs11774818, rs4075359, rs7015700, and rs7006406; **Table 5** and **Figures 1, 3, and 4**).

Discussion

The gene-environment interaction plays a role in pathogenesis of human diseases and genetic susceptibility is the most important factor in lung cancer development and contributes to individual lung cancer risk [28]. In the current study, we selected and analyzed 24 TNKS1 SNPs between NSCLC and healthy control for association with NSCLC risk in a Chinese population. We found that TNKS1 rs6601328 reduced the risk in developing NSCLC and adenocarcinomas (ADC) in females but increased NSCLC risk in males. Moreover, TNKS1 rs11991621 and rs10503380 were associated with an increased risk in developing NSCLC and ADC in females, while TNKS1 rs7015700 had an increased risk in developing ADC in males and rs12541709 C/C has a protective effect to reduce ADC risk in both male and female. These data indicate that different TNKS1 SNPs could affect TNKS1 transcription, protein expression, or enzymatic activity, and therefore, differently modulate NSCLC risk. Furthermore, our current data also showed that TNKS1 SNPs associated differently with male and female risk in developing NSCLC, suggesting that different environmental risk factors interact TNKS1 for genetic susceptibility in male and female lung cancer development.

Indeed, previous studies showed a different gene-environment interaction of histological type of NSCLC and gender [29, 30]. Molecular studies can better characterize genetic effects on disease risk and increase our understanding of the apparent heterogeneity of effects across sex and histological type [30]. In our current study, we found that TNKS1 rs6601328 SNPs had a protective role in females but was a risk factor for male in developing NSCLC. To

the best of our knowledge, up to date, there was no such a report in the literature; however, the underlying molecular mechanism remains unclear. There may be two reasons, i.e., firstly, the sample size is moderate and this association of NSCLC risk in males was just passed statistical threshold; thus, further confirmation studies are needed to verify our current data, and secondly, this is the first genetic polymorphism study of such phenomenon and future study will disclose TNKS1 SNPs for TNKS1 transcription and Tankyrase 1 expression and enzymatic activity for the gene-environment interaction, such as tobacco smoke.

At the chromosome level, TNKS1 is localized at the centromere of chromosome 8, function of which is to prevent over-elongation of centrioles and ensure a proper centrosome function by promoting degradation of centrosomal P4.1-associated protein (CPAP) in early G1 phase of cell cycle [31]. Thus, silencing of Tankyrase 1 expression was able to promote human cells to apoptosis and inhibit cell proliferation [32]. In lung cancer, Tankyrase 1 was highly expressed [18], indicating that Tankyrase 1 enzyme could associate with lung cancer development or progression. Indeed, previous studies demonstrated that function of Tankyrase 1 protein is to maintain cell telomere length and cell division capacity through Tankyrase 1-mediated PARsylation because Tankyrase 1 protein contains multiple binding sites for telomeric repeat binding factor 1 (TRF1) and a novel acceptor, tankyrase-binding protein (TAB182), which will promote TRF1 release from the telomeres, allowing telomerase access to telomeres and enhancing telomere elongation [33]. Indeed, a previous study reported by Riffell JL et al showed that Tankyrase-1 inhibitors were able to increase a risk of critically shortened telomere and promote the crisis and mortality of lung cancer cells [34]. Tankyrase 1 or tankyrase 2 is a key mediator in regulation of the Wnt signaling [20, 21]. Tankyrase-1-mediated PARsylation of Axin 1 and 2 (regulators of the Wnt signaling pathway) resulted in their degradation through the ubiquitin-proteasome pathway [20]. Moreover, the Wnt pathway proteins were activated or overexpressed in the resected NSCLC tissue samples, while aberrant methylation of various Wnt pathway components or downregulation of Wnt pathway inhibitors were

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associated with poor prognosis of lung cancer [35]. Thus, these data indicate that TNKS1 SNPs may affect TNKS1 transcription and tankyrase 1 expression and enzymatic activity and in turn lose the ability to block the Wnt-signaling during lung carcinogenesis, depending further investigation for confirmation.

However, our current study does have some limitations; for example, there were many more cases of male smokers than nonsmokers, which led to a difficulty to stratify and analyze the data according to smoking status. Overall, it is just a proof-of-principle study and our data showed interesting findings: TNKS1 SNPs associated with risk in developing NSCLC and TNKS1 SNPs could affect TNKS1 transcription, protein expression, or enzymatic activity, and therefore, differently modulate NSCLC risk. Furthermore, our current data also showed that but further studies will need to confirm our current data and applicability to other populations. For sure, future investigation of tankyrase 1 and its role in lung cancer development and progression could help us to better understand TNKS1 SNPs in regulation of TNKS1 transcription and tankyrase 1 expression and enzymatic activity and therefore, disclose TNKS1 interaction with environmental risk factors (tobacco smoke or gender difference) in modulation of NSCLC risk.

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Disclosure of conflict of interest

None.

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