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Identification of *TRPCs* genetic variants that modify risk for lung cancer based on the pathway and two-stage study

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ABSTRACT

Objective: Store operated calcium channels (SOCCs) and Receptor-operated calcium channels (ROCCs) are important pathways participating in regulation of intracellular Ca^{2+} concentration in various cell types. The purpose of our study is to determine whether genetic variations in key components of SOCCs and ROCCs are associated with lung cancer risk.

Methods: We identified 236 tagSNPs in 9 key genes related to SOCCs and ROCCs (*TRPC1, TRPC3, TRPC4, TRPC6, TRPC7, ORAI1, ORAI2, STIM1*, and *STIM2*) and evaluated their association with lung cancer risk in a two-stage case-control study with a total of 2433 lung cancer cases and 2433 cancer-free controls using Illumina high throughput genotyping platform.

Results: We found consistently significant associations of *TRPC4* rs9547991 and rs978156, and *TRPC7* rs11748198 with increased risk of lung cancer among the three kinds of sources of populations (additive model in combined population: adjusted OR = 1.33, 95% CI = 1.11–1.59 for rs9547991; adjusted OR = 1.21, 95% CI = 1.08–1.35 for rs978156; and adjusted OR = 1.28, 95% CI = 1.10–1.47 for rs11748198). When combining the effects of *TRPC7* rs11748198, and *TRPC4* rs9547991 and rs978156, subjects carrying "≥1" variant alleles had a 1.29-fold increased risk of lung cancer (95% CI = 1.15–1.46), compared with those carrying "0" variant allele. Lung cancer risk significantly increased with the increasing number of variant alleles of the three SNPs in a dose-dependent manner (*P* for trend = 7.2×10^{-7}).

Conclusion: These findings suggested that *TRPC4* rs9547991 and rs978156, and *TRPC7* rs11748198 were candidate susceptibility markers for lung cancer in Chinese population. Our study provides the epidemiological evidence supporting a connection between *TRPC* members and lung cancer risks.

1. Introduction

Lung cancer is the most common malignant tumor in the world. Non-small cell lung carcinoma (NSCLC) constitutes approximately 80% of lung cancer, with a 5-year survival of only 15%. Further study on the pathogenetic mechanism of lung cancer is needed to establish novel diagnostic or treatment strategies for this lethal disease.

Ca^{2+} is a ubiquitous cellular signal mediating various cellular activities such as proliferation, differentiation, and gene transcription. Store-operated Ca^{2+} channels (SOCCs) and receptor-operated Ca^{2+} channels (ROCCs) are two important pathways regulating the basal intracellular Ca^{2+} concentration. Both channels are thought to be formed of transient receptor potential channel (*TRPCs*) family protein members (*TRPC1–7*) and/or Ca^{2+}-release-actived Ca^{2+} channel (CRAC/ORAI) family members (*ORAI1–3*), and are activated by stromal interacting molecules (*STIM1* and *STIM2*) (Lu et al., 2008). Recently, emerging evidence has uncovered that abnormal expression of *TRPCs* were related to the development of various kinds of tumors, such as renal cell carcinoma, hepatoma, prostatic carcinoma, neuroblastoma IMR-32 cells, and breast cancer (Nasman et al., 2006; Thebault et al., 2006; Veliceasa et al., 2007; El Boustany et al., 2008; Guilbert et al., 2008; Aydar et al., 2009; Saito et al., 2011). Recently, a study identified that *TRPC* expression correlates to lung cancer differentiation (Jiang et al., 2013). Especially, another study showed that higher levels of *TRPC3* expression in tumor cells are an independent predictor of a better prognosis in patients with adenocarcinoma of the lung (Ouadid-Ahidouch et al., 2012). Our previous study has also demonstrated that *TRPCs* played a role in the progresses of NSCLC (Zhang et al., 2010). Since *TRPCs* play an important role in the cell function including enzyme activity, emiocytosis, and cell proliferation and apoptosis (Liao et al., 2007; Peel et al., 2008), the underlying molecular mechanisms are still being elucidated.

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In this study, we hypothesized that the polymorphisms in SOCCs and ROCCs component and regulatory genes might contribute to genetic susceptibility to lung cancer. To test this hypothesis, we conducted a two-stage case-control study with a total of 2433 lung cancer cases and 2433 cancer-free controls to evaluate the effects of gene polymorphisms in the 9 selected genes related to SOCCs and ROCCs (*TRPC1, TRPC3, TRPC4, TRPC6, TRPC7, ORAI1, ORAI2, STIM1*, and *STIM2*). To our knowledge, this is the first study to explore the associations between a comprehensive panel of polymorphisms in genes related to SOCCs and ROCCs and lung cancer risk, and to identify subgroups that would be more likely to have higher lung cancer risk.

2. Material and methods

2.1. Study population and design

The study design and subject recruitment have been described previously (Zhang et al., 2013). Briefly, in this study, we performed two independent case-control studies. The first-stage "Discovery" study included 1422 lung cancer cases and 1422 controls, which were genetically unrelated ethnic Han Chinese and were from The First Affiliated Hospital of Guangzhou Medical University (Guangzhou, Guangdong, China) as described in a previous study (Zhang et al., 2013). The second-stage "Replication" study was conducted on participants (1011 cases and 1011 controls) derived from Xianyang, Central Hospital (Xiangyang, Hubei, China) to verify the results from the first-stage analysis. All the total 2433 patients with histopathologically confirmed incident lung cancer were consecutively recruited from September 2009 to September 2013. The 2433 cancer-free controls, frequency matched to patients by sex and age (±5 years) were randomly selected from the Health Examination Center of the same hospital during the same time period when patients were recruited. Generally, all subjects met the following criteria: (1) both cases and controls were genetically unrelated Han Chinese; (2) all the lung cancer patients in the study were newly diagnosed and histopathologically confirmed; (3) all the control subjects were no self-reported cancer history and frequency matched to the cases by sex and age (±5 years). The characteristics of the cases and controls selected for this two-stage study with a total of 2433 cases and 2433 controls are summarized in Table 1. Structured questionnaires were performed by trained interviewers with the use of standardized protocol. Information on demographic data and environmental exposure history such as tobacco smoking were also obtained by the professional interviewers. Subjects were defined as current smokers, former smokers, and never smokers. Individuals who had smoked <100 cigarettes in the past lifetime were identified as never smokers; otherwise, they were defined as smokers (those smokers who stopped smoking for >1 year were identified as former smokers). Pack-years were calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. This study was approved by each participating center's Institutional Ethical Committee and was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.2. Blood sampling, SNP selection, and genotyping

After informed written consent was obtained, a ~5 ml venous blood sample with EGTA-Na_2 as anticoagulant was collected for each participant. The genomic DNA was extracted with QIAGEN Blood DNA Kit (Qiagen, Valencia, CA).

Nine genes related to SOCCs and ROCCs were selected: *TRPC 1, TRPC3, TRPC4, TRPC6, TRPC7, ORAI1, ORAI2, STIM1*, and *STIM2*. For each of them, we selected the tagSNPs by Haploview 4.2 software within 10 kb upstream of the transcriptional start site or 10 kb downstream of the transcriptional stop site. The genotypes of 236 selected tagSNPs and their associations with lung cancer were described in Table S1 ($P < 0.05$) and Fig. S1 SNP frequency and LD data were based on the International HapMap Project database, release 24, human genome build 36. The genotyping was performed using Illumina high throughput genotyping platform. Genotypes were analyzed and exported using the Illumina Beadstudio software. To ensure quality control, genotyping was done without knowledge of case/control status of the subjects, and the polymorphism analysis was made by two persons independently. >15% of the samples were randomly performed for confirmation, and the results were 100% concordant. The genotyping call rates for these polymorphisms were all above 95%.

3. Calculation

χ^2 test was used to evaluate differences in the distributions of demographic characteristics, selected variables, and genotypes of the variants

Table 1
Frequency distributions of selected variables in lung cancer patients and cancer-free controls.

Variables	Discovery			Replication				Combined		
	Cases (N = 1422) n (%)	Controls (N = 1422) n (%)	P^a	Cases (N = 1011) n (%)	Controls (N = 1011) n (%)	P^a	P^b	Case (N = 2433) n (%)	Control (N = 2433) n (%)	P^a
Age			0.9855			0.2294	0.3519			0.4467
≤60	688 (50.00)	688 (50.00)		470 (48.60)	497 (51.40)			1158 (49.42)	1185 (50.58)	
>60	733 (49.97)	734 (50.03)		541 (51.28)	514 (48.72)			1274 (50.52)	1248 (49.48)	
Sex			0.9904			1.0000	0.9938			0.9927
Male	1006 (49.98)	1007 (50.02)		716 (50.00)	716 (50.00)			1722 (49.99)	1723 (50.01)	
Female	415 (50.00)	415 (50.00)		295 (50.00)	295 (50.00)			710 (50.00)	710 (50.00)	
Smoking status			<0.0001			<0.0001	5.8×10^{-6}			<0.0001
Current	702 (54.49)	519 (42.51)		558 (59.24)	384 (40.76)			1260 (58.25)	903 (41.75)	
Former	168 (68.85)	76 (31.15)		93 (47.21)	104 (52.79)			261 (59.18)	180 (40.82)	
Never	551 (39.99)	827 (60.01)		360 (40.77)	523 (59.23)			911 (40.29)	1350 (59.71)	
Pack year			<0.0001			<0.0001	0.1667			<0.0001
≥25	650 (65.46)	343 (34.54)		483 (61.22)	306 (38.78)			1133 (63.58)	649 (36.42)	
<25	220 (46.61)	252 (53.39)		168 (48.00)	182 (52.00)			388 (47.20)	434 (52.80)	
0	551 (39.99)	827 (60.01)		360 (40.77)	523 (59.23)			911 (40.29)	1350 (59.71)	
Sex and smoking			<0.0001			<0.0001	0.4243			<0.0001
Male smokers	832 (58.47)	591 (41.53)		609 (56.34)	472 (43.66)			1442 (57.55)	1063 (42.45)	
Male non-smokers	174 (29.49)	416 (70.51)		107 (30.48)	244 (69.52)			281 (29.86)	660 (70.14)	
Female smokers	38 (90.48)	4 (9.52)		42 (72.41)	16 (27.59)			80 (80.00)	20 (20.00)	
Female non-smokers	377 (47.84)	411 (52.16)		253 (47.56)	279 (52.44)			630 (47.73)	690 (52.27)	

[a] *P* values for a two-sided χ^2 test.
[b] *P* values for the test of homogeneity by Breslow day test.

between the cases and controls. Goodness of fit to the Hardy-Weinberg equilibrium (HWE) expectation in control was also evaluated by the χ^2-test for each SNP. Akaike's information criteria (AIC) (Akaike, H., IEEE Trans. Automat. Contr. AC-19, 716-723 (1974)) were applied to select the most parsimonious genetic model for each SNP. Odds ratios (ORs) and its corresponding 95% confidence intervals (CIs) were measured by an unconditional logistic regression model with adjustment for age, sex, and pack-year of smoking. Stratification analyses were also done by variables of interest, such as age, sex, smoking status, pack year, and sex-smoking. The pairwise LD among the SNPs was calculated using Lewontin's standardized coefficient D' and LD coefficient r^2 (Lewontin, 1988) and haplotype blocks were defined by the method of Gabriel et al. (Gabriel et al., 2002) in the publicly available Haploview software with default settings (http://www.broad.mit.edu/personal/jcbarret/haplo/). Each common haplotype was compared between all cases and the controls. In addition, PHASE 2.1 Bayesian algorithm was used to validate the haplotype frequencies estimated by Haplo.stats (Stephens and Donnelly, 2003) (http://mayoresearch.mayo.edu/mayo/research/schaid_lab/index.cfm). Homogeneity test between discovery and replication populations was assessed by Breslow day test. Statistic power (Gauderman, 2002) was done by QUANTO 1.2 (http://hydra.usc.edu/gxe). All statistical analyses were performed with the SAS 9.2 software. $P = 0.05$ was the criterion of statistical significance and all statistical tests were two-sided.

4. Results

4.1. Characteristics of the study populations

As shown in Table 1, concordant results were observed in both Discovery and Replication populations with significant differences identified in the smoking status, pack years, and sex-smoking ($P < 0.001$ for all) and no significant deviation in distributions of age and sex between case and control groups ($P > 0.05$ for all). The frequency distributions of smoking status were not homogeneous (Breslow-Day Test $P = 5.8 \times 10^{-6}$), reflecting different lifestyle between the Discovery and Replication populations.

We further combined the two populations into stratification analysis and cumulative effect analysis in order to increase the study power. In addition, these variables were further adjusted by age, sex and pack-year of smoking in the multivariate logistic regression model to control possible confounding on the main effects of the studied polymorphisms.

4.2. The genetic variants in TRPC4 and TRPC7 are associated with lung cancer risk

We selected 236 tagSNPs from nine genes related to SOCCs and ROCCs: *TRPC1, TRPC3, TRPC4, TRPC6, TRPC7, ORAI1, ORAI2, STIM1,* and *STIM2*. Table S1 showed the one with "$P < 0.05$" in any of the Discovery, Replication, and combined populations. Among these tagSNPs, we found consistently significant associations of *TRPC4* rs9547991 and rs978156 and *TRPC7* rs11748198 with lung cancer risk among the above three kinds of groups ($P < 0.05$ for all). All observed genotype distributions among these groups agreed with the HWE ($P \geq 0.05$ for all). When combined discovery and replication populations, the significances were more significant than any of them in additive model. In Table S2, the results of the first-stage study revealed that *TRPC4* rs9547991 and rs978156 variant genotypes significantly increased the lung cancer risk in additive model (adjusted OR = 1.29, 95%CI = 1.03–1.62; adjusted OR = 1.21, 95%CI = 1.05–1.40, respectively). *TRPC7* rs11748198 significantly increased the lung cancer risk in additive model (rs11748198: adjusted OR = 1.26, 95%CI = 1.04–1.53). In the second-stage study, the associations of *TRPC4* rs9547991 and rs978156, and *TRPC7* rs11748198 with lung cancer risk were validated with ORs of 1.38 and 1.21, and 1.29, respectively. Of course, the associations remained significant after all subjects were combined (rs9547991: adjusted OR = 1.33, 95%CI = 1.11–1.59; rs978156: adjusted OR = 1.21, 95%CI = 1.08–1.35; rs11748198: adjusted OR = 1.28, 95%CI = 1.10–1.47). Then, further detailed analysis was taken about the relationship between these three SNPs and the three groups by pooling all of the discovery and validation stage in additive models. We achieved significant associations for *TRPC4* rs9547991 and rs978156 ($P_{combined} = 1.6 \times 10^{-3}$, OR = 1.33 at 5q31.1; $P_{combined} = 6.6 \times 10^{-4}$, OR = 1.21 13q13.3, respectively), and *TRPC7* rs11748198 ($P_{combined} = 1.3 \times 10^{-3}$, OR = 1.28 13q13.3) (Table 2). The genotype distributions of the three significant SNPs were also in Table 2.

Furthermore, we evaluated combined effects of the three SNPs variants (*TRPC4* rs9547991 A>G and rs978156 C>T, *TRPC7* rs11748198 G>T) on lung cancer risks. As shown in Table 3, lung cancer risk was significantly increased with the increasing number of variant alleles of the three SNPs in a dose-dependent manner (P for trend $= 7.2 \times 10^{-7}$). Compared with those carrying "0" variant allele, subjects carrying "≥1" variant alleles had a 1.29-fold increased risk of lung cancer (95%CI = 1.15–1.46).

4.3. Stratification analysis on the three SNPs in combined study

For further study, the relationships between combined variant alleles and environmental characteristics in combined population were also taken. We found that individuals with "1–6" variant alleles had a more significantly increased lung risks than "0" variant alleles in younger people (age ≤ 60, $P = 1.9 \times 10^{-5}$); in both sex ($P = 1.5 \times 10^{-3}$ for male; $P = 5.0 \times 10^{-3}$ for female); in current ($P = 1.1 \times 10^{-3}$) and never smokers ($P = 12.0 \times 10^{-3}$); in both ≥25 pack year and 0 pack year ($P = 2.9 \times 10^{-3}$ and $P = 12.0 \times 10^{-3}$, respectively); in both male smokers and female non-smoker (both $P > 0.7 \times 10^{-3}$) as well as in discovery population. We also found that there were significant multiplicative interactions between pack year, sex-smoking, source of population and allele genes with $P_{max} \leq 0.0328$ (Table 4).

We further assessed the associations of SNPs rs9547991, rs978156 and rs11748198 variant genotypes with lung cancer risk stratified by selected variables. As shown in Fig. S2 and Table S3, compared with the common wild-type homozygous genotype, the adverse effects of rs9547991 and rs978156 were more evident in male (rs9547991: adjusted OR = 1.53 and 95% CI = 1.23–1.92; rs978156: adjusted OR = 1.21 and 95% CI = 1.04–1.41); in former smokers (rs9547991: adjusted OR = 2.77 and 95% CI = 1.33–5.57; rs978156: adjusted OR = 1.63 and 95% CI = 1.07–2.49); in male smokers (rs9547991: adjusted OR = 1.68 and 95% CI = 1.29–2.17; rs978156: adjusted OR = 1.24 and 95% CI = 1.04–1.498) than the rest of all. Similar association strengths were observed among younger subjects (age ≤ 60) compared with that in older subjects (age > 60) between all subgroups for the three SNPs (rs9547991: adjusted OR = 1.61 and 95% CI = 1.23–2.09; rs978156: adjusted OR = 1.38 and 95% CI = 1.55–1.66; rs11748198: adjusted OR = 1.38 and 95% CI = 1.11–1.73). Interestingly, stronger effects of rs11748198 were shown among female (adjusted OR = 1.67, 95% CI = 1.25–2.24) and never smokers (adjusted OR = 1.40, 95% CI = 1.11–1.76) than the rest of all.

4.4. Effect of haplotypes and combined genotypes of TRPC4 on lung cancer risk

We also performed haplotype analysis in the combined population to assess the effect of the haplotype containing rs9547991 and rs978156 variant alleles on lung cancer risks (Table S4). When compared with the most frequent AC haplotype, the haplotype carrying any of the variant allele all showed significant risk effects (adjusted OR > 1), which were consistent with that in the analysis of single SNP. Then the combined genotypes risk was also evaluated. We found that there was a significantly increased risk of lung cancer as the risk

Table 2
Summary of discovery and replication studies for the 3 SNPs.

SNP	Study	Case[a]	Control[a]	MAF[b]	Control	Adjusted[c]	OR_{hom}(95%CI)	OR_{add}(95%CI)	P_{add}
				case		OR_{het}(95%CI)			
rs11748198									
TRPC7	Discovery	1156\254\11	1205\210\11	0.10	0.08	1.25 (1.01–1.53)	1.94 (0.74–5.14)	1.26 (1.04–1.53)	0.0163
5q31.1G/T[d]	Replication	820\184\7	865\139\7	0.10	0.08	1.36 (1.06–1.74)	0.93 (0.30–2.85)	1.29 (1.02–1.62)	0.0319
	Combined all	1976\438\18	2070\349\14	0.10	0.08	1.29 (1.10–1.51)	1.42 (0.67–2.95)	1.28 (1.10–1.47)	1.3×10^{-3}
rs9547991									
TRPC4	Discovery	1225\193\4	1273\142\7	0.07	0.05	1.39 (1.10–1.77)	0.57 (0.16–2.02)	1.29 (1.03–1.62)	0.0253
13q13.3 A/G[d]	Replication	889\120\3	929\77\5	0.06	0.04	1.58 (1.15–2.15)	0.35 (0.07–1.81)	1.38 (1.03–1.85)	0.0295
	Combined all	2114\313\7	2202\219\12	0.07	0.05	1.47 (1.21–1.77)	0.47 (0.17–1.27)	1.33 (1.11–1.59)	1.6×10^{-3}
rs978156									
TRPC4	Discovery	948\427\43	1021\354\40	0.18	0.15	1.30 (1.10–1.55)	1.12 (0.70–1.74)	1.21 (1.05–1.40)	0.0100
13q13.3C/T[d]	Replication	693\256\56	725\253\29	0.18	0.15	1.09 (0.89–1.35)	1.87 (1.17–3.01)	1.21 (1.02–1.42)	0.0239
	Combined all	1641\683\99	1746\607\69	0.18	0.15	1.22 (1.06–1.39)	1.43 (1.03–1.97)	1.21 (1.08–1.35)	6.6×10^{-4}

OR_{het}: heterozygote versus wild-type homozygote; OR_{hom}: variant homozygote versus wild-type homozygote; OR_{add}, P_{add}: calculated by additive model.
[a] Wild-type homozygote\heterozygote\variant homozygote.
[b] Minor allele frequency.
[c] Adjusted by age, sex and pack-year of smoking.
[d] Major/minor alleles;

genotype number increased compared with those with "0" variants, implying that these variants might have a joint effect on the risk of lung cancer (P for trend $= 9.6 \times 10^{-5}$). LD information of these two SNPs (*TRPC4* rs9547991 and rs978156), calculated from genotyping data of 2433 controls of the combined study, was $D' = 0.91$ and $r^2 = 0.04$.

5. Discussion

Lung cancer remains to be a challenging disease due to a 5-year survival of only 15% and the accompanied high medical costs. Using genetic markers for determining risk may help to identify high risk population for early screening, diagnosis, and therapy, which may also improve clinical outcome. This is the first study to explore the associations between a comprehensive panel of polymorphisms of *TRPCs* genes and lung cancer risks and to explore subgroups that would more likely have higher cancer risks. We evaluated 236 tagSNPs in the 9 selected genes from the SOCC and the ROCC pathways for their associations with lung cancer risks. In this two-stage case-control study with a total of 2433 lung cancer cases and 2433 controls, we found that rs9547991 and rs978156 in *TRPC4* and rs11748198 in *TRPC7* were potentially susceptibility markers of lung cancer in Chinese population. Especially, our study provides the epidemiological evidence supporting a connection between comprehensive *TRPCs* SNPs and lung cancer risks.

The mammalian *TRPCs* channels are encoded by at least 28 genes (Bodding, 2007). Most of these proteins have a putative topology of six transmembrane domains with a pore loop between the fifth and sixth segments. Both the C- and N-termini are presumably located intracellular. Evidences of genetic linkage to diseases and studies from numerous independent laboratories have strongly suggested that *TRPCs* channels have substantial importance in mammalian biology and might be valuable therapeutic drug targets. Members of *TRPCs* have been found to be implicated in abnormal proliferation, differentiation, and cancer formation (Bodding, 2007). There are examples of *TRPCs* gene mutations linked to human disease. For example, *TRPC6* mutations cause familial focal segmental glomerulosclerosis (Winn et al., 2005), and another study has been linked a SNP in *TRPC6* to idiopathic pulmonary hypertension (Yu et al., 2009). Gain-of-function mutation in *TRPC4* protects against myocardial infarction (MI) in diabetes (Jung et al., 2011). In the present study, two of the three significant SNPs were in *TRPC4*, which is known to have important functions contributing to lung cancer risk. *TRPC4* is widely expressed in the vasculature (Yip et al., 2004), where it participates in the generation of intracellular Ca^{2+} signals that regulate functions such as endothelial permeability (Tiruppathi et al., 2002) and smooth muscle proliferation (Zhang et al., 2004). Up to now, the best-characterized physiological role for *TRPC4* is in the regulation of endothelial cell function. *TRPC4* has been demonstrated that is a required component of SOCC channels in vascular endothelial cells and that *TRPC4* is part of the Ca^{2+} entry signal transduction channel regulating vascular tone (Abramowitz and Birnbaumer, 2009). Similarly, vascular endothelial cells derived from *TRPC4* knockout (*TRPC4* −/−) mice showed impaired Store-operated Ca^{2+} entry channels (SOCE) (Freichel et al., 2001). Therefore, studies showed that

Table 3
Cumulative effect of risk alleles of SOC-related pathway on lung cancer risk in combined set (3 SNPs in SOC-related pathway).

Risk alleles	Cases	Controls	P[a]	Crude	Adjusted	P[b]
	n (%)	n (%)		OR (95%CI)	OR (95%CI)[b]	
Total no. of subjects	2433	2433				
Total no. of alleles	4866	4866				
No. of risk alleles			3.3×10^{-6}			
0	1322 (47.21)	1478 (52.79)		1.00 (ref.)	1.00 (ref.)	
1	670 (52.10)	616 (47.90)		1.22 (1.07–1.39)	**1.20 (1.05–1.38)**	**7.9×10^{-3}**
2	313 (54.43)	262 (45.57)		1.33 (1.11–1.59)	**1.33 (1.11–1.61)**	**2.2×10^{-3}**
3–6	118 (65.73)	66 (34.27)		2.00 (1.47–2.73)	**1.92 (1.39–2.65)**	**6.7×10^{-5}**
Trend test P value				8.7×10^{-8}	**7.2×10^{-7}**	
Combined no. of risk alleles			5.2×10^{-6}			
0	1322 (47.21)	1478 (52.79)		1.00 (ref.)	1.00 (ref.)	
1–6	1101 (53.84)	944 (46.16)		1.30 (1.16–1.46)	**1.29 (1.15–1.46)**	**2.3×10^{-5}**

Bold numbers indicate significance at $P < 0.05$.
[a] P values for a two-sided Global test.
[b] Adjusted in a logistic regression model that included age, sex, and pack-year of smoking.

Table 4
Stratification analysis of the number of risk alleles in SOC-related pathway by selected variables in lung cancer patients and controls.

Variables	Cases (N = 2433)		Controls (N = 2433)		Adjusted		P_{int}
	0 risk allele n (%)	1–6 risk alleles n (%)	0 risk allele n (%)	1–6 risk alleles n (%)	OR (95%CI)[a]	P^a	
Age							0.7544
≤60	624 (53.89)	534 (46.11)	740 (62.45)	445 (37.55)	**1.44 (1.22–1.71)**	**1.9×10^{-5}**	
>60	703 (55.14)	572 (44.86)	745 (59.70)	503 (40.90)	1.14 (0.96–1.35)	0.1296	
Sex							0.4919
Male	926 (53.74)	797 (46.26)	1027 (59.61)	696 (40.39)	**1.26 (1.09–1.45)**	**0.0015**	
Female	401 (56.48)	309 (43.52)	458 (64.51)	252 (35.49)	**1.36 (1.10–1.69)**	**0.0050**	
Smoking status							0.5826
Current	663 (52.58)	598 (47.42)	541 (59.91)	362 (40.09)	**1.34 (1.12–1.59)**	**0.0011**	
Former	142 (54.41)	119 (45.59)	107 (59.44)	73 (40.56)	1.23 (0.84–1.80)	0.2979	
Never	522 (57.30)	389 (42.70)	837 (62.00)	513 (38.00)	**1.25 (1.05–1.49)**	**0.0120**	
Pack year							**3.2×10^{-4}**
≥25	592 (52.20)	542 (47.80)	390 (60.09)	259 (39.91)	**1.35 (1.11–1.65)**	**0.0029**	
<25	213 (54.90)	175 (45.10)	258 (59.45)	176 (40.55)	1.22 (0.92–1.62)	0.1587	
0	522 (57.30)	389 (42.70)	837 (62.00)	513 (38.00)	**1.25 (1.05–1.49)**	**0.0120**	
Sex and smoking							**0.0328**
Male smokers	765 (53.05)	677 (46.95)	636 (59.83)	427 (40.17)	**1.32 (1.13–1.55)**	**0.0007**	
Male non-smokers	161 (57.30)	120 (42.70)	391 (59.24)	269 (40.76)	1.03 (0.78–1.38)	0.8109	
Female smokers	40 (50.00)	40 (50.00)	12 (60.00)	8 (40.00)	1.54 (0.56–4.21)	0.4013	
Female non-smoker	361 (57.30)	269 (42.70)	446 (64.64)	244 (35.36)	**1.37 (1.10–1.71)**	**0.0054**	
Population							**0.0048**
Discovery	840 (53.67)	725 (46.33)	933 (61.58)	582 (38.42)	**1.35 (1.16–1.57)**	**7.5×10^{-5}**	
Replication	487 (56.11)	381 (43.89)	552 (60.13)	366 (39.87)	1.19 (0.98–1.45)	0.0858	

P_{int}: calculated by Gene-Environment interaction;
Bold numbers indicate significance at $P < 0.05$.
[a] Adjusted in a logistic regression model that included age, sex and pack-year of smoking.

TRPC4-dependent Ca^{2+} entry is a key determinant of increased permeability in the mouse pulmonary vasculature (Tiruppathi et al., 2002). In a study of the properties of lung endothelial cells derived from the same *TRPC4*$^{-/-}$ mice, Tiruppathi et al. (Tiruppathi et al., 2002) expanded the observations of Freichel et al. (Freichel et al., 2001), and identified that absence of *TRPC4* was associated with a loss of endothelial cell responses to thrombin, suggesting a critical involvement of *TRPC4* in microvascular permeability. *TRPC4* antisense oligonucleotides were shown to partially inhibit SOCE in mouse mesangial cells, implying that *TRPC4* might also form part of endogenous SOCCs in that kind cells (Wang et al., 2004). Furthermore, *TRPC4* appears to be involved in mediating some aspects of hypoxia-induced gene expression and cell proliferation. Culture of human pulmonary artery endothelial cells under hypoxic conditions results in increased *TRPC4* expression of mRNA and protein, enhanced SOCE (Fantozzi et al., 2003). In addition, haplotype analysis was also evaluated to further explore effects of haplotypes and combined genotypes of *TRPC4* on lung cancer risks, because haplotype-based analysis might be more informative than single SNP analysis and resequencing DNA samples carrying the high-risk haplotypes might be able to improve risk assessment. Especially, the two most significant *TRPC4* SNPs we identified are all located in the intron region, which may contribute to alterations in gene expression or splicing. Alternatively, it is also possible that these SNPs are linked to other causal variants in *TRPC4*.

In our previous study, we have not found the expression of *TRPC7* in lung in human (Zhang et al., 2010), although studies have demonstrated that it is expressed in the heart, lung, and eye in mice (Okada et al., 1999). The main reason was that the relatively small sample size we chose might be difficult to detect the probably low expression of *TRPC7*. In the present study, one of the three most significant SNPs was in *TRPC7*. Unlike *TRPC4, TRPC7* have been detected less frequently and also have not been studied extensively. *TRPC7*, the final member of *TRPC* family, demonstrates properties very similar to *TRPC3* and *TRPC6* with regard to its voltage-current relationship (*TRPC7* is most closely related to *TRPC3* with 81% identity, and demonstrates 75% identity with *TRPC6* in mice), and activated by diacylglycerol (DAG) (Beck et al., 2006). The differences between the three channel types may lie in their ion selectivity, in which *TRPC6* is reported to be somewhat Ca^{2+}-selective, while *TRPC3* and *TRPC7* do not appear to be. *TRPC7* has demonstrable sensitivity to SKF96365 (a novel inhibitor of receptor-mediated Ca^{2+} entry), and is relatively insensitive to lanthanides. Up to now, the component(s) required for coupling of the *TRPC7* to store depletion is unknown. It is unlikely that *TRPC7* alone is responsible for specific biological function among *TRPCs*, since *TRPC7* is co-expressed with other *TRPCs* in most of the tissues. It is possible that *TRPC7* interacts with inositol triphosphate (IP_3) receptors to suppress their activity. Alternatively, *TRPC7* may be also localized in the endoplasmic reticulum (ER) membrane and contribute to passive Ca^{2+} release from stores. It has been suggested that *TRPC7* plays key roles in the Ca^{2+} signaling pathway because of its unique activation properties such as constitutive activity (Okada et al., 1999). A study revealed that *TRPC7* mediated angiotensin II-induced myocardial apoptosis (Satoh et al., 2007). However, another report showed that *TRPC3* and *TRPC6*, but not *TRPC7*, were essential for angiotensin II-induced cardiac hypertrophy. But *TRPC7* can display some functions via association with other proteins, such as *TRPC6*, which positively regulates calcineurin-NFAT (nuclear factor of activated T cells) signaling, and was related with cardiac hypertrophy (Nishida et al., 2010). *TRPC7* may also form heteromeric channels with *TRPC6*, and be involved in cardiac failure. In this study, it is possible that the variant allele of *TRPC7* rs11748198 may affect gene transcription thus altering protein level. Alternatively, it may be linked to other causal variants in *TRPC7*. Overall, our study suggested the association of *TRPC4* and *TRPC7* polymorphisms with lung cancer risks.

We applied a gene sets-based approach to comprehensively evaluate the effect of the three significant SNPs on the risk of lung cancer. When combined the effects of the three significant SNPs, subjects carrying "≥1" variant alleles had a higher increased risk of lung cancer compared with those carrying "0" variant allele. Lung cancer risks significantly increased with the increasing number of variant alleles of the three SNPs in a dose-dependent manner. Those with 2 risk genotypes had the highest risk of lung cancer, suggesting combined variations were detrimental and had a larger effect than any single variant. This emphasizes the importance of including multiple SNPs within a shared pathway for examining joint effects in the risk assessment.

Despite the strengths and biologic plausibility of the associations observed in the present study, inherited biases in our study may have led to spurious findings. Firstly, further fine mapping and functional assays

are necessary to reveal potential molecular mechanisms. Secondly, only Chinese Han populations were included in this study. However, subjects in the two-stage study covered the population of Southeastern and Northern Chinese, which made the population representativeness more stable and reasonable. In addition, it would be interesting to exam these SNPs in minority populations. Finally, although our data are largely internally validated, future replication studies in independent populations are needed to validate some of the results.

6. Conclusion

Our study provided evidence indicating that rs9547991 and rs978156 in *TRPC4* and rs11748198 in *TRPC7* were potentially susceptibility markers of lung cancer in Chinese population. These findings need to be substantiated by larger scale studies in different ethnic populations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2016.07.005.

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Conflict of interest

None.

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