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

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ARTICLE INFO

Article history:
Received 8 December 2015
Revised 26 June 2016
Accepted 7 July 2016
Available online 8 July 2016

Keywords:
TRPCs
SOCCs
ROCCs
Genetic variants
Lung cancer

ABSTRACT

Objective: Store operated calcium channels (SOCCs) and Receptor-operated calcium channels (ROCCs) are important pathways participating in regulation of intracellular Ca2+ 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 rs95479991 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.

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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.

Ca2+ is a ubiquitous cellular signal mediating various cellular activities such as proliferation, differentiation, and gene transcription. Store-operated Ca2+ channels (SOCCs) and receptor-operated Ca2+ channels (ROCCs) are two important pathways regulating the basal intracellular Ca2+ concentration. Both channels are thought to be formed of transient receptor potential channel (TRPCs) family protein members (TRPC1–7) and/or Ca2+-release-activated Ca2+ 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; Velicu 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-Abidouch 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.
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 Xiangyang, 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 cases by sex and age (±5 years). The characteristics of the cases and 1011 controls were matched to the cases and 1011 controls by sex and age (±5 years) and their associations with lung cancer were described in Table S1 and their associations with lung cancer were described in Table S1. The polymorphism analysis was made by two persons independently. The genotypes of 236 selected tagSNPs in the first-stage analysis were confirmed by the Illumina Beadstudio software. To ensure quality control, genotyping information, and polymorphism analysis was made by two persons independently. 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%.

2.2. Blood sampling, SNP selection, and genotyping

After informed written consent was obtained, a ~5 ml venous blood sample with EGTA-Na2 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: TRPC1, TRPC3, TRPC4, TRPC6, TRPC7, ORAI1, ORAI2, STIM1, and STIM2. For each of them, we selected the tagSNPs by Haploviz 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

χ² test was used to evaluate differences in the distributions of demographic characteristics, selected variables, and genotypes of the variants

| Variables          | Discovery | Replication | Combined |
|--------------------|-----------|-------------|----------|
|                    | Cases     | Controls    | P        | Cases     | Controls    | P        | P        | Case      | Control    | P        |
|                    | (N = 1422)| (N = 1422)  |          | (N = 1011)| (N = 1011) |          |          | (N = 2433)| (N = 2433) |          |
| Age                |           |             |          |           |             |          |          |           |           |          |
| ≤60                | 688 (50.00)| 688 (50.00)| 0.9855  | 470 (48.60)| 497 (51.40) | 0.2294  | 0.3519  | 1158 (49.42)| 1185 (50.58)| 0.4467  |
| >60                | 733 (49.79)| 734 (50.03)| 0.9904  | 541 (51.28)| 514 (48.72) | 1.0000  | 0.9938  | 1274 (50.52)| 1248 (49.48)| 0.9927  |
| Sex                |           |             |          |           |             |          |          |           |           |          |
| Male               | 1006 (49.80)| 1007 (50.02)|         | 716 (50.00)| 716 (50.00) | 1.0000  | 1.0000   | 1722 (49.99)| 1723 (50.01)| <0.0001 |
| Female             | 415 (50.00)| 415 (50.00)| 0.9904  | 295 (50.00)| 295 (50.00) | 1.0000  | 1.0000   | 710 (50.00)| 710 (50.00) | <0.0001 |
| Smoking status     |           |             |          |           |             |          |          |           |           |          |
| Current            | 702 (54.49)| 519 (45.21)| <0.0001 | 558 (59.24)| 384 (40.76) | <0.0001 | 5.8 × 10⁻⁶| 1260 (58.25)| 903 (41.75) | <0.0001 |
| Former             | 168 (68.85)| 76 (31.15) | 0.4243  | 93 (47.21)| 104 (52.79) | 0.1667  | 1.0653   | 261 (59.18)| 180 (40.82) | <0.0001 |
| Never              | 551 (39.99)| 827 (60.01)| <0.0001 | 360 (40.77)| 523 (59.23) | <0.0001 | 1.667    | 911 (40.29)| 1350 (59.71) | <0.0001 |
| Pack year          |           |             |          |           |             |          |          |           |           |          |
| ≥25                | 650 (65.46)| 343 (34.54)| <0.0001 | 483 (61.22)| 306 (38.78) | <0.0001 | 1.667    | 1133 (63.58)| 649 (36.42) | <0.0001 |
| <25                | 220 (46.61)| 252 (53.39)| <0.0001 | 168 (48.00)| 182 (52.00) | <0.0001 | 1.0653   | 388 (47.20)| 434 (52.80) | <0.0001 |
| 0                  | 551 (39.99)| 827 (60.01)| <0.0001 | 360 (40.77)| 523 (59.23) | <0.0001 | 1.667    | 911 (40.29)| 1350 (59.71) | <0.0001 |
| Sex and smoking    |           |             |          |           |             |          |          |           |           |          |
| Male smokers       | 832 (58.47)| 591 (41.53)| <0.0001 | 609 (56.34)| 472 (43.66) | <0.0001 | 1.0653   | 1442 (57.55)| 1063 (42.45)| <0.0001 |
| Male non-smokers   | 174 (29.49)| 416 (70.51)| <0.0001 | 107 (30.48)| 244 (69.52) | <0.0001 | 1.0653   | 281 (29.86)| 660 (70.14) | <0.0001 |
| Female smokers     | 38 (90.48)| 4 (9.52)   | 0.2294  | 42 (72.41)| 18 (27.59)  | 0.0001  | 0.2294   | 80 (80.00)| 20 (20.00)  | 0.0001  |
| Female non-smokers | 377 (47.84)| 411 (52.16)| 0.3519  | 253 (47.56)| 279 (52.44) | 0.3519  | 0.3519   | 630 (47.73)| 690 (52.27) | 0.3519  |

* P values for a two-sided χ² 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 χ²-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 r² (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 ≥ 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 (Pcombined = 1.6 × 10⁻³, OR = 1.33 at 5q31.1; Pcombined = 6.6 × 10⁻³, OR = 1.21 13q13.3, respectively), and TRPC7 rs11748198 (Pcombined = 1.3 × 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 × 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 $P_{\text{max}} = 0.0328$ (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
connection between comprehensive panel of polymorphisms of TRPC 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. We conducted a two-stage case-control study with a total of 2433 lung cancer cases and 2433 controls, we found that rs9547991 and rs978156, calculated from genotyping data of 2433 controls of the combined study, was D = 0.91 and r² = 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 TRPC 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. We conducted a 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 TRPC4 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 Ca²⁺ 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 Ca²⁺ entry signal transduction channel regulating vascular tone (Abramowitz and Birnbaumer, 2009). Similarly, vascular endothelial cells derived from TRPC4 knock-out (TRPC4 −/−) mice showed impaired Store-operated Ca²⁺ entry channels (SOCE) (Freichel et al., 2001). Therefore, studies showed that

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

| SNP     | Study            | Case¹   | Control¹ | MAF² case | Control | Adjusted³ | ORuest(95%CI) | ORadd(95%CI) | Padd  |
|---------|------------------|---------|----------|-----------|---------|-----------|---------------|--------------|-------|
| rs1748198 TRPC7 5q11.1G/T⁴ | 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 |
|         | Replication      | 820/148/7  | 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 | 2070/149/14 | 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 13q13.3 A/G⁵ | 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 |
|         | 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     | 2114/311/7 | 2202/191/12 | 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 13q13.3C/T⁶ | 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 |
|         | Replication      | 693/236/56 | 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 | 1746/607/68 | 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 |

ORuest: heterozygote versus wild-type homozygote; ORadd: variant homozygote versus wild-type homozygote; ORadd, Padd: calculated by additive model.

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 | P² | Crude OR (95%CI) | Adjusted OR (95%CI)² |
|--------------|--------|----------|----|-----------------|---------------------|
| Total no. of subjects | 2433   | 2433     |    |                 |                     |
| Total no. of alleles  | 4866   | 4866     |    |                 |                     |
| No. of risk alleles  |        |          | 3.3 × 10⁻⁶ | 1.00 (ref.) | 1.00 (ref.)  |
| 0             | 1322 (47.21)   | 1478 (52.79)  |     |                 |                     |
| 1             | 670 (52.10)    | 616 (47.90)   |     | 1.22 (1.07-1.39) | 1.20 (1.05-1.38)    |
| 2             | 313 (54.43)    | 262 (45.57)   |     | 1.33 (1.11-1.59) | 1.33 (1.11-1.61)    |
| 3-6           | 118 (65.73)    | 66 (34.27)    |     | 2.00 (1.47-2.73) | 1.92 (1.39-2.65)    |
| Trend test P value | 8.7 × 10⁻⁸  | 7.2 × 10⁻⁷    |    |                 |                     |
| Combined no. of risk alleles | 5.2 × 10⁻⁶ | 7.2 × 10⁻⁷    |    |                 |                     |
| 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)    |

Bold numbers indicate significance at P < 0.05.

¹ P values for a two-sided Global test.

² 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 OR (95%CI) | P<sub>corr</sub> |
|--------------------|------------------|---------------------|---------------------|-----------------|
|                   | 0 risk allele n (%) | 1–6 risk alleles n (%) | 0 risk allele n (%) | 1–6 risk alleles n (%) |
| **Age**            |                  |                     |                    |                 |
| ≤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 (40.30)     | 1.14 (0.96–1.35) | 0.1296          |
| **Sex**            |                  |                     |                    |                 |
| 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** |                  |                     |                    |                 |
| Current            | 663 (52.58)       | 598 (47.42)         | 541 (59.91)        | 362 (40.9)      | 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**      |                  |                     |                    |                 |
| ≤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**|                  |                     |                    |                 |
| Male smokers       | 765 (53.05)       | 677 (46.95)         | 636 (59.83)        | 427 (40.17)     | 1.22 (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**     |                  |                     |                    |                 |
| Discovery          | 840 (53.67)       | 725 (46.33)         | 933 (61.58)        | 582 (38.42)     | 1.35 (1.16–1.57) | 7.5 × 10⁻³      |
| Replication        | 487 (56.11)       | 381 (43.89)         | 552 (60.13)        | 366 (39.87)     | 1.19 (0.98–1.45) | 0.0858          |

*P<sub>corr</sub>* calculated by Gene-Environment interaction; Bold numbers indicate significance at P < 0.05.

*Adjusted in a logistic regression model that included age, sex and pack-year of smoking.*

TRPC4-dependent Ca²⁺ 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 Ca²⁺-selective, while TRPC3 and TRPC7 do not appear to be. TRPC7 has demonstrable sensitivity to SKF96365 (a novel inhibitor of receptor-mediated Ca²⁺ 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 (IP₃) receptors to suppress their activity. Alternatively, TRPC7 may also be localized in the endoplasmic reticulum (ER) membrane and contribute to passive Ca²⁺ release from stores. It has been suggested that TRPC7 plays key roles in the Ca²⁺ 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 examine 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.

Funding

This work was supported by Guangdong Natural Science Foundation Team Grant (1035101200300000), the National Natural Science Foundation of China (81170582, 81071917, 81173112, 81520108001, and 81220108001), the Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme to W. Lu (2014) and the Key Project of Department of Education of Guangdong Province (cxzdk1025). Guangzhou Department of Education Team Grant for Innovation (13C08), Guangzhou Department of Education Research Grant (cxzkd1025), Guangzhou Municipal University Research Projects (1201430298). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

None.

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