Research Article

Association of BTLA Polymorphisms with Susceptibility to Non-Small-Cell Lung Cancer in the Chinese Population

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Studies have reported that B- and T-lymphocyte attenuator (BTLA) polymorphisms may be associated with the risk to different cancers. However, the correlation between those variations and non-small-cell lung cancer (NSCLC) is still unclear. A total of 1,003 NSCLC patients and 901 noncancer controls were recruited in the study, to confirm the association of variations in BTLA gene with the risk of NSCLC. The SNPscanTM genotyping assay was used to obtain the genotypes of the four BTLA polymorphisms (BTLA rs1982809 G>A, rs16859629 T>C, rs2171513 G>A, and rs3112270 A>G). It was found that BTLA rs1982809 polymorphism reduced the risk of NSCLC (GA vs. GG: adjusted odds ratio (OR) = 0.81, 95% confidence interval (CI) = 0.66–0.99, and P = 0.043). However, the BTLA rs16859629, rs2171513, and rs3112270 polymorphisms showed no significant association between NSCLC patients and controls in overall comparison. In subgroup analyses, we found that BTLA rs1982809 polymorphism reduced the risk of NSCLC (non-squamous cell carcinoma: GA vs. GG: adjusted OR = 0.79, 95%CI = 0.64–0.97, and P = 0.026; AA/GA vs. GG: adjusted OR = 0.81, 95%CI = 0.66–0.99, and P = 0.037; ≥59 years: GA vs. GG: P = 0.036; never alcohol consumption: GA vs. GG: P = 0.013; GA/AA vs. GG: P = 0.016; body mass index (BMI) ≥ 24 kg/m²: GA vs. GG: P = 0.030; GA/AA vs. GG: P = 0.041). The BTLA rs16859629 polymorphism increased the risk of the development of squamous cell carcinoma (CC vs. TT: adjusted OR = 9.85, 95%CI = 1.37–71.03, and P = 0.023; CC vs. TT/TC: adjusted OR = 9.55, 95%CI = 1.32–68.66, and P = 0.025). Taken together, the findings of the present suggest that BTLA rs1982809 and rs16859629 polymorphisms may influence the susceptibility to NSCLC in the Chinese population.

1. Introduction

Non-small-cell lung cancer (NSCLC) accounts for 80 to 85% of all the lung cancer and is the main pathological type of lung cancer (LC). LC has imposed huge diseases burden on human population and accounts for significant number of mortalities across the globe [1–3]. As per estimates, LC is currently ranked as first in terms of incidence and mortality [4]. The currently used treatment strategies for LC include surgery combined with adjuvant therapy. Owing to the recent advancements made in the diagnosis and treatment, the clinical outcomes have significantly improved [5]. However, the identification of the potential risk factors for the occurrence of LC is considered essential as it will permit early diagnosis and immediate management.

The B- and T-lymphocyte attenuator (BTLA), an immunosuppressive receptor, was identified after the cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD-1) and is thus the third member of the CD28 immunoglobulin superfamily (IgSF) [6]. The BTLA upon binding with herpesvirus entry mediator (HVEM) inhibits the T cell response. In contrary, blocking of BTLA may in turn activate T cells [7–9]. Evidences suggest that BTLA plays a crucial immune regulatory role in human malignancies. Liu et al. [10] have reported that BTLA/HVEM pathway plays an important immune suppressive role in the regulation of T cells in peripheral blood of hepatocellular carcinoma patients. Quan et al. [11] and Li et al. [12] found that the level of BTLA expression may act as a biomarker for the prognosis of diffuse large B cell lymphoma and NSCLC. In
yet another study, Chen et al. [13] concluded that chemotherapy in combination with anti-BTLA antibody improves the prognosis of ovarian cancer in a mouse model. Similarly, Zhang et al. [14] reported that BTLA could directly intervene the effects of miR-32 on cancer cells. Collectively, these studies point towards a correlation between BTLA and the development and progression of human cancers.

Single nucleotide polymorphism (SNP) mutations constitute an important form of genetic variations. Some recent studies have revealed a significant correlation between BTLA SNPs and development of cancer [15–19]. Fu et al. reported that BTLA rs1844089 G>A and rs2705535 A>G variation could increase the risk of breast cancer in human [15]. Partyka et al. and Karabon et al. found that the variation on BTLA rs1982809 G>A gene polymorphism might be a potential risk factor for the development of chronic lymphocytic leukemia (CLI) and renal cell carcinoma [16, 17]. In a previous study, we reported that BTLA rs1982809 SNPs increased the susceptibility of esophageal squamous cell carcinoma (ESCC) [19]. Given this background, we hypothesize that some associations may exist between BTLA polymorphisms and NSCLC pathogenesis.

Combined with these previous studies, the BTLA tagging SNPs (rs1982809, rs16859629, rs2171513, and rs3112270) were selected for analysis. Consistently, the present study is

### Table 1: Primary information for BTLA tagging polymorphisms.

<table>
<thead>
<tr>
<th>Genotyped polymorphisms</th>
<th>rs2171513 G&gt;A</th>
<th>rs3112270 A&gt;G</th>
<th>rs1982809 G&gt;A</th>
<th>rs16859629 T&gt;C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Position_38</td>
<td>112466080</td>
<td>112461797</td>
<td>112463893</td>
<td>112471533</td>
</tr>
<tr>
<td>Region</td>
<td>3’-UTR</td>
<td>Promoter</td>
<td>3’-UTR</td>
<td>Intron_variant</td>
</tr>
<tr>
<td>MAF in database (1000 genomes—Chinese Han populations)</td>
<td>0.198</td>
<td>0.278</td>
<td>0.195</td>
<td>0.082</td>
</tr>
<tr>
<td>MAF in our controls (n = 901)</td>
<td>0.194</td>
<td>0.295</td>
<td>0.270</td>
<td>0.079</td>
</tr>
<tr>
<td>P value for HWE test in our controls</td>
<td>0.554</td>
<td>0.259</td>
<td>0.584</td>
<td>0.747</td>
</tr>
<tr>
<td>% genotyping value</td>
<td>99.00%</td>
<td>98.84%</td>
<td>98.90%</td>
<td>97.48%</td>
</tr>
</tbody>
</table>

Abbreviations: MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium.

### Table 2: Distribution of selected demographic variables and risk factors in NSCLC cases and controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 1,003)</th>
<th>Controls (n = 901)</th>
<th>p^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58.76 ± 9.92</td>
<td>59.43 ± 9.67</td>
<td>0.139</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.292</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;59</td>
<td>465 (46.36%)</td>
<td>396 (43.95%)</td>
<td></td>
</tr>
<tr>
<td>≥59</td>
<td>538 (53.64%)</td>
<td>505 (56.05%)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>520 (51.84%)</td>
<td>552 (61.27%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>483 (48.16%)</td>
<td>349 (38.73%)</td>
<td></td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never</td>
<td>634 (63.21%)</td>
<td>717 (79.58%)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>369 (36.79%)</td>
<td>184 (20.42%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never</td>
<td>789 (78.66%)</td>
<td>804 (89.23%)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>214 (21.34%)</td>
<td>97 (10.77%)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;24</td>
<td>672 (67.00%)</td>
<td>497 (55.16%)</td>
<td></td>
</tr>
<tr>
<td>≥24</td>
<td>331 (33.00%)</td>
<td>404 (44.84%)</td>
<td></td>
</tr>
<tr>
<td>Type of NSCLC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>145 (14.46%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-SCC</td>
<td>858 (85.54%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: ^two-sided \( \chi^2 \) test and Student’s t-test. Abbreviations: BMI: body mass index; NSCLC: non-small-cell lung cancer; SCC: squamous cell carcinoma.

### Table 3: The frequencies of BTLA tagging polymorphisms in different NSCLC subgroups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>NSCLC (n = 1,003)</th>
<th>SCC (n = 145)</th>
<th>Non-SCC (n = 858)</th>
<th>Controls (n = 901)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2171513 G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>654 (66.06%)</td>
<td>106 (73.61%)</td>
<td>548 (64.78%)</td>
<td>582 (65.03%)</td>
</tr>
<tr>
<td>GA</td>
<td>297 (30.00%)</td>
<td>35 (24.31%)</td>
<td>262 (30.97%)</td>
<td>276 (30.84%)</td>
</tr>
<tr>
<td>AA</td>
<td>39 (3.94%)</td>
<td>3 (2.08%)</td>
<td>36 (4.26%)</td>
<td>37 (4.13%)</td>
</tr>
<tr>
<td>A allele</td>
<td>375 (18.94%)</td>
<td>41 (14.24%)</td>
<td>334 (19.74%)</td>
<td>350 (19.55%)</td>
</tr>
<tr>
<td>rs3112270 A&gt;G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>527 (53.39%)</td>
<td>74 (51.39%)</td>
<td>453 (53.74%)</td>
<td>435 (48.60%)</td>
</tr>
<tr>
<td>AG</td>
<td>392 (39.72%)</td>
<td>61 (42.36%)</td>
<td>331 (39.26%)</td>
<td>388 (43.35%)</td>
</tr>
<tr>
<td>GG</td>
<td>68 (6.89%)</td>
<td>9 (6.25%)</td>
<td>59 (7.00%)</td>
<td>72 (8.04%)</td>
</tr>
<tr>
<td>G allele</td>
<td>528 (26.75%)</td>
<td>79 (27.43%)</td>
<td>449 (26.63%)</td>
<td>532 (29.72%)</td>
</tr>
<tr>
<td>rs1982809 G&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>566 (57.29%)</td>
<td>77 (53.47%)</td>
<td>489 (57.94%)</td>
<td>471 (52.63%)</td>
</tr>
<tr>
<td>GA</td>
<td>351 (35.53%)</td>
<td>54 (37.50%)</td>
<td>357 (39.25%)</td>
<td>361 (40.34%)</td>
</tr>
<tr>
<td>AA</td>
<td>71 (7.19%)</td>
<td>13 (9.03%)</td>
<td>58 (6.87%)</td>
<td>63 (7.04%)</td>
</tr>
<tr>
<td>A allele</td>
<td>493 (24.95%)</td>
<td>80 (27.78%)</td>
<td>413 (24.47%)</td>
<td>487 (27.21%)</td>
</tr>
<tr>
<td>rs16859629 T&gt;C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>835 (85.82%)</td>
<td>115 (82.14%)</td>
<td>720 (86.43%)</td>
<td>746 (84.48%)</td>
</tr>
<tr>
<td>TC</td>
<td>131 (13.46%)</td>
<td>23 (16.43%)</td>
<td>108 (12.97%)</td>
<td>132 (14.95%)</td>
</tr>
<tr>
<td>CC</td>
<td>7 (0.72%)</td>
<td>2 (1.43%)</td>
<td>5 (0.60%)</td>
<td>5 (0.57%)</td>
</tr>
<tr>
<td>C allele</td>
<td>145 (7.45%)</td>
<td>27 (9.64%)</td>
<td>118 (13.71%)</td>
<td>142 (8.04%)</td>
</tr>
</tbody>
</table>

Abbreviations: MAF: minor allele frequency; HWE: Hardy–Weinberg equilibrium.
aimed at exploring the relationship between *BTLA* tagging SNPs and the risk of NSCLC.

## 2. Materials and Methods

### 2.1. Subject

The present study involved 1,003 NSCLC patients and 901 healthy controls. Those patients were continuously recruited from Fujian Medical University Union Hospital, Fuzhou, China, from October 2014 to January 2018, and the diagnosis was confirmed by postoperative pathology. The major inclusion criteria for NSCLC patients were (a) the case firstly to be diagnosed, (b) the individuals with no history of other cancers, (c) without any autoimmune disease, and (d) did not receive any chemo- or radiotherapy prior to enrollment. In the same period, the controls were recruited in the Affiliated Union Hospital of Fujian Medical University (Fuzhou, China) and the Affiliated People’s Hospital of Jiangsu University (Zhenjiang, China), and the individuals without any cancer history, mainly healthy, checking individuals, matched with the NSCLC patients by age. The information collected from the patients included age, sex, drinking, smoking history, height, and weight. Furthermore, body mass index (BMI) of more than 24 kg/m² was considered as

### 2.2. Materials and Methods

#### 2.2.1. Genotyping

The association of *BTLA* tagging SNPs with the risk of NSCLC was assessed by a logistic regression model. The *BTLA* tagging SNPs were genotyped using the TaqMan method with a LightCycler instrument (Roche, Mannheim, Germany). The genotyping was performed in a 96-well format with 20 μl of DNA in each well. The PCR conditions were as follows: 95°C for 15 minutes, 45 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 2 minutes. The alleles were identified using the LightCycler software version 3.5 (Roche). The association of *BTLA* tagging SNPs with the risk of NSCLC was assessed by a logistic regression model. The *BTLA* tagging SNPs were genotyped using the TaqMan method with a LightCycler instrument (Roche, Mannheim, Germany). The genotyping was performed in a 96-well format with 20 μl of DNA in each well. The PCR conditions were as follows: 95°C for 15 minutes, 45 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 2 minutes. The alleles were identified using the LightCycler software version 3.5 (Roche).
Table 5: Stratified analyses between BTLA rs1982809 G>A polymorphism and NSCLC risk by sex, age, smoking status, alcohol consumption, and BMI.

<table>
<thead>
<tr>
<th>Variable</th>
<th>BTLA rs1982809 G&gt;A (case/control)</th>
<th>Adjusted OR (95% CI); P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>299/289</td>
<td>177/228</td>
</tr>
<tr>
<td>Female</td>
<td>267/182</td>
<td>174/133</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;59</td>
<td>254/203</td>
<td>173/162</td>
</tr>
<tr>
<td>≥59</td>
<td>312/268</td>
<td>178/199</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>355/371</td>
<td>220/285</td>
</tr>
<tr>
<td>Ever</td>
<td>211/100</td>
<td>131/76</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>443/408</td>
<td>280/332</td>
</tr>
<tr>
<td>Ever</td>
<td>123/63</td>
<td>71/29</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>368/257</td>
<td>245/201</td>
</tr>
<tr>
<td>≥24</td>
<td>198/214</td>
<td>106/160</td>
</tr>
</tbody>
</table>

Notes: *the genotyping was successful in 1,003 (98.50%) NSCLC cases, and 901 (99.33%) controls for BTLA rs1982809 G>A. *Adjusted for age, sex, smoking status, alcohol consumption, and BMI (besides stratified factors accordingly) in a logistic regression model. Abbreviations: BMI: body mass index; NSCLC: non-small-cell lung cancer.

2.2. Selection of BTLA Tagging SNPs. The four candidate SNPs were ascertained by applying Genome Variation Server data [18, 19, 21] (http://gvs.gs.washington.edu/GVS147/). We gave priority to represent the main linkage disequilibrium blocks. The following main criteria were used: (a) minor allele frequency ≥ 0.05, (b) linkage disequilibrium of r² < 0.8 between the SNPs, (c) with the 5-kb extent upstream and downstream of the gene regions, and (d) the genotyping value ≥ 95% in the CHB cohort. Four BTLA tagging SNPs (rs1982809, rs16859629, rs2171513, and rs3112270) were finally selected to decipher the association between the BTLA polymorphisms and NSCLC risk. The results are shown in Table 1.

2.3. DNA Extraction and Genotyping. Around 2 mL of the peripheral blood samples of the subjects (early in the morning, empty stomach) was collected and placed in a vacuum ethylene diamine tetra-acetic acid anticoagulant tube. Genomic DNA was carefully extracted from the collected samples with the help of DNA blood mini kit (Promega, Madison, USA) by following manufacturer’s guidelines. The BTLA rs1982809, rs16859629, rs2171513, and rs3112270 genotypes were assessed by the SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) as per manufacturer’s guidelines. For the qualitative assessment, 76 samples (4%) were randomly selected and subsequently tested by another laboratory technician. The genotypes of BTLA were well confirmed repeatedly.

2.4. PCR-Specific Experimental Procedures

2.4.1. DNA Cleavage. The DNA was diluted to a concentration of 30 to 50 ng/μL. From this 4 μL, DNA was transferred to a 96-well plate followed by the addition of 2.5 μL 4X DNA buffer. Thereafter, the total volume was made up to 10 μL by the addition of sterile ddH₂O. The contents were mixed well and incubated at 98°C for 5 min. Afterwards, the plates were immediately placed in ice.
2.4.2. PCR Reaction. A 10 μL of the premixture prepared was centrifuged at 3000 rpm for 30 seconds. After 4 cycles of the PCR program, the mixture was subjected to 70°C warm bath.

2.4.3. Multiple Fluorescence PCR Reactions. A 19 μL PCR premixture was added to each hole. This was followed thorough mixing and centrifugation at 3000 rpm for 30 seconds. The reaction was subjected to 34 cycles of the PCR program followed by a 4°C warm bath.

2.4.4. Reading of PCR Results. The GeneMapper 4.2 software (Applied Biosysin, USA) was employed for the genotype analysis of the original sequence data.

2.5. Statistical Analysis. For the selected BTLA genotypes in controls, the internet software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) was employed to assess if the genotype frequency distribution conforms to Hardy–Weinberg equilibrium (HWE) or not. Mean ± standard deviation (SD) was adopted for continuous variables. The t-test was applied to calculate the difference between NSCLC and healthy subjects. The chi-square ($\chi^2$) or Fisher test was utilized to compare the distributions of the BTLA SNPs in related categorical variables between the two groups, including age, gender, tobacco use, drinking, BMI, and genotype frequency. Multivariate logistic regression was used to examine the adjusted odds ratio (OR) and 95% confidence intervals (CIs) in evaluating the relationship between selective BTLA SNPs and susceptibility to NSCLC. All data were analyzed by the SAS 9.4 (windows version; SAS Institute Inc., Cary, NC) and SPSS 23 software (windows version; International Business Machines Corp.). Only $P < 0.05$ was considered as a statistically significance.

3. Results

3.1. Baseline Characteristics. A total of 1,003 NSCLC cases and 901 controls were recruited in this study. The risk factors that may influence the development of NSCLC are listed in Table 2. Although a good match was observed between the age of the NSCLC patients and the control group ($P = 0.139$), significant differences were observed in sex, smoking, alcohol consumption, and BMI between the two groups ($P < 0.001$). The genotyping rate of BTLA rs2171513, rs3112270, rs1982809, and rs16859629 was higher than 95% (99.00%, 98.84%, 98.90%, and 97.48%, respectively) as shown in Table 1. Minor allele frequencies (MAFs) in the...
control were similar to those of the Chinese populations, and the genotype frequency distributions were in accordance with the HWE.

Collectively, the results suggest that the genotypes in BTLA rs2171513 and rs16859629 mutations may be associated with the development of NSCLC.

3.3. Association of BTLA Polymorphisms with Susceptibility in Different NSCLC Subgroups. The results showed that AA genotype of BTLA rs2171513 polymorphism might be considered as a susceptibility factor for the development of squamous cell carcinoma (SCC) (AA/AG: OR = 0.67, 95%CI = 0.45-0.99, and P = 0.044) (Table 4). Adjusting the risk factors (age, sex, smoking, drinking, and BMI status), the BTLA rs2171513 polymorphism was not relevant to the occurrence of SCC. Nonetheless, using TT and TT+TC genotypes as references, the results showed that the CC genotype of BTLA rs16859629 polymorphisms may promote the development of SCC (CC vs. TT; adjusted OR = 9.55, 95%CI = 1.32-68.66, and P = 0.025).

In nonsquamous cell carcinoma (non-SCC), using AA genotype as reference, it was found that GG and GA+GG genotypes of BTLA rs2171513 polymorphism might be associated with the development of SCC (CC vs. TT+TC: adjusted OR = 3.25, 95%CI = 1.02-10.41, and P = 0.046). Adjusting the risk factors (age, sex, smoking, drinking, and BMI status), the genotypes of BTLA rs3112270 polymorphism were not closely correlated with NSCLC susceptibility, whereas the GA genotype of BTLA rs1982809 SNPs still decreased the susceptibility of NSCLC patients (GA: adjusted OR = 0.81, 95%CI = 0.66-0.99, and P = 0.043; Table 4).
Meanwhile, it was also found that the GA and GA+AA genotypes of BTLA rs1982809 polymorphism also decreased the occurrence of non-SCC with reference to GG genotype (GA vs. GG: OR = 0.79, 95%CI = 0.65-0.97, and P = 0.022; AA/GA vs. GG: OR = 0.81, 95%CI = 0.67-0.98, and P = 0.026). Adjusting the related risk factors, it is concluded that the BTLA rs3112270 polymorphism did not exhibit the tendency to change the risk to non-SCC. However, BTLA rs1982809 polymorphism was a protective factor.
rs1982809 decreased the incidence of NSCLC (GA vs. GG: $\geq$ phism in the subgroup analyses are depicted in Table 5. In
the case-control study is less than 0.03, it is classi fi 
(\textit{BTLA}
shown in Table 9 and Figure 1. No signi
Trs16859629Grs1982809Grs2171513Ars3112270 haplotype, the haplo-
95
P
(Tables 6
rs2171513, rs3112270, and rs16859629 polymorphisms were
The SHESIS software online (http://
haplotype analysis. If the probability of
analysis.bio-x.cn/myAnalysis.php) was used to perform the
59-year subgroup, it was found that the variants of
BTLA
susceptibility to NSCLC. However, our
previously reported that BTLA rs1982809 might act as a potential bio-
marker in predicting the multiple organ dysfunction syn-
drome. In another study, rs1982809 polymorphism of the
\textit{BTLA} was found to be associated with the risk of kidney can-
cancer [17]. Similarly, in our previous study, we report that the
gene variation in \textit{BTLA} rs1982809 increased the susceptibility to EGJA in ever smoking subjects [18]. In contrary, Cao
et al. concluded that the distribution of genotype in \textit{BTLA}
rs1982809 was not different from the ESCC and the control
group [19]. In short, the previous results were ambiguous,
even contradictory. Considering that genetic variations
played different roles in different cancers, 1,904 participants
were enrolled to conduct a more precise evaluation. In this
study, we showed that the G to A changes of \textit{BTLA}
rs1982809 genotype reduced the overall risk of NSCLC, espe-
cially the non-SCC, BMI $\geq$ 24 kg/m$^2$, $\geq$59 year, and never drinking subgroups. These facts indicated that \textit{BTLA}
rs1982809 polymorphism could play a critical role in the sus-
ceptibility to NSCLC. However, our findings should be inter-
preted with cautions. Studies with larger sample sizes are
required to further validate the effects of this locus on
NSCLC.

The \textit{BTLA} rs16859629 SNP, which is located in the
intron variant sequence, plays an important role in alterna-
tive splicing [29]. It has been reported that SNP located in
the intron regions may affect the susceptibility to several
human diseases [30–32]. When adjusted for including 5

![Figure 1: There was no significant linkage disequilibrium between \textit{BTLA} polymorphisms ($r^2 < 0.8$).](image-url)

of the susceptibility to non-SCC (GA: adjusted OR = 0.79, 95%CI = 0.64-0.97, and $P = 0.026$; AA+GA: adjusted OR = 0.81, 95%CI = 0.66-0.99, and $P = 0.037$).

The genotype frequencies of \textit{BTLA} rs1982809 polymor-
phism in the subgroup analyses are depicted in Table 5. In
$\geq$59-year subgroup, it was found that the variants of \textit{BTLA}
rs1982809 decreased the incidence of NSCLC (GA vs. GG: $P = 0.036$). In never alcohol and BMI $\geq 24$ kg/m$^2$ subgroup,
it was found that similar genotype variants of \textit{BTLA}
rs1982809 might be a protective factor of NSCLC (never smoking subgroup: GA vs. GG: $P = 0.013$; AA/GA vs. GG: $P = 0.016$; BMI $\geq 24$ kg/m$^2$: GA vs. GG: $P = 0.030$; AA/GA vs. GG: $P = 0.041$). Additionally, it was found that \textit{BTLA}
rs2171513, rs3112270, and rs16859629 polymorphisms were
not associated with the morbidity of NSCLC in subgroups
(Tables 6–8).

3.4. SNP Haplotypes. The SHESIS software online (http://
analysis.bio-x.cn/myAnalysis.php) was used to perform the
haplotype analysis. If the probability of \textit{BTLA} haploid
in the case-control study is less than 0.03, it is classified as
others. In the end, six subgroups were built. The results are
shown in Table 9 and Figure 1. No significant linkage dise-
equilibrium (LD) relationship was observed among the
\textit{BTLA} rs16859629, rs1982809, rs2171513, and rs3112270
($r^2 < 0.8$). It was found that compared to \textit{BTLA}
T:rs16859629G,rs1982809G,rs2171513A,rs3112270 haplotype, the haplo-
type \textit{BTLA} T:rs16859629A,rs1982809A,rs2171513G,rs3112270 signifi-
cantly reduced the susceptibility to NSCLC (OR = 0.66, 95%CI = 0.659-0.930, and $P = 0.005$).

4. Discussion

The pathogenesis of LC is overly complex. It is believed that
LC might be a disease driven by multiple genes [22]. Epide-
miological studies have proved the correlation of the etiology
of LC and the gene-environment interaction [23, 24]. Recently,
immune checkpoint inhibitors have attracted remarkable attention in cancer treatment. For instance, PD-
1 and CTLA-4 immunity inhibitors have achieved certain
efficacy in the treatment of advanced LC [25]. Nonetheless,
the cause of LC is still largely unclear. A previous study had
reported that overexpression of \textit{BTLA} could predict a poor
prognosis in NSCLC patients [12].

In the present case-control study, the potential relation-
ship between the \textit{BTLA} rs1982809 G>A, rs16859629 T>C,
rs2171513 G>A, and rs3112270 A>G SNPs and susceptibility
to NSCLC was explored [23, 26]. It was found that \textit{BTLA}
rs1982809 polymorphism might reduce the risk of overall
NSCLC. But candidate locus of \textit{BTLA} rs2171513,
rs3112270, and rs16859629 SNPs could not affect the suscep-
tibility to NSCLC. In NSCLC subgroup analysis, \textit{BTLA}
rs16859629 SNPs could increase the risk of SCC. However,
\textit{BTLA} rs1982809 SNPs might reduce susceptibility to non-
SCC. In addition, \textit{BTLA} rs1982809 SNPs could reduce the
susceptibility to NSCLC in the BMI $\geq 24$ kg/m$^2$, $\geq$59 year,
and never drinking subgroups. To the best of our knowledge,
the present study for the first time reports the relationship
between \textit{BTLA} SNPs and NSCLC susceptibility in the Chi-
nese population.

\textit{BTLA} rs1982809, as a locus in the 3′-untranslated region
(UTR), has been reported to exhibit an association with the
development of some malignancies. The 3′-UTR plays a cru-
ial role in the regulation of mRNA expression [27, 28]. Kar-
abon et al. reported that \textit{BTLA} could exert its effects on T-
lymphocytes by affecting mRNA expression levels due to T
substitutions of rs1982809 [16]. Additionally, it was also
reported that \textit{BTLA} rs1982809 might act as a potential bio-
marker in predicting the multiple organ dysfunction syn-
drome. In another study, rs1982809 polymorphism of the
\textit{BTLA} was found to be associated with the risk of kidney can-
cer [17]. Similarly, in our previous study, we report that the
gene variation in \textit{BTLA} rs1982809 increased the susceptibility
to EGJA in ever smoking subjects [18]. In contrary, Cao
et al. concluded that the distribution of genotype in \textit{BTLA}
rs1982809 was not different from the ESCC and the control
group [19]. In short, the previous results were ambiguous,
even contradictory. Considering that genetic variations
played different roles in different cancers, 1,904 participants
were enrolled to conduct a more precise evaluation. In this
study, we showed that the G to A changes of \textit{BTLA}
rs1982809 genotype reduced the overall risk of NSCLC, espe-
cially the non-SCC, BMI $\geq 24$ kg/m$^2$, $\geq$59 year, and never drinking subgroups. These facts indicated that \textit{BTLA}
rs1982809 polymorphism could play a critical role in the sus-
ceptibility to NSCLC. However, our findings should be inter-
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required to further validate the effects of this locus on
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The \textit{BTLA} rs16859629 SNP, which is located in the
intron variant sequence, plays an important role in alterna-
tive splicing [29]. It has been reported that SNP located in
the intron regions may affect the susceptibility to several
human diseases [30–32]. When adjusted for including 5
covariates by regression analysis, it was found that BTLA rs16859629 SNP increased the risk of SCC. In two recent studies, the genotype distribution of BTLA rs16859629 showed no statistically significant differences between EGJA cases and controls and the same conclusion was made for susceptibility to ESCC [18, 19]. Interestingly, the present study first explored that BTLA rs16859629 SNP could promote the susceptibility to cancer. However, considering that the number of SCC was relatively small, the results might not be convincing enough. In the future, a larger case-control group study should be performed to unveil the susceptibility relationship between BTLA rs16859629 and SCC. Previous studies have shown that the genetics of the SNP locus in the BTLA rs16859629, rs1982809, rs2171513, and rs3112270 may not be random [18]. In the present study, it was found that haplotype type of the four candidate SNP loci may play an important role in heredity. It was found that the haplotype BTLA $T_{rs16859629A_{rs1982809G_{rs2171513G_{rs3112270}}}}$ could significantly change the susceptibility to NSCLC, affecting 18.70% of the normal population. Interestingly, compared to the study by Tang et al. [18], it was found that with alteration of rs1982809, the effect of this single locus on haplotype significantly reversed the association with cancer. When BTLA rs1982809 was allele A, it decreased the risk of NSCLC. This is in contrary to the findings of Tang et al. [18]. However, our findings need to be further verified in the future studies. In addition, stepwise analysis of the SPSS 23 software was used to analyze the correlation of age, sex, smoking, drinking, BMI, and the four candidate SNPs. We found that the BTLA rs1982809 among the four candidate SNPs may be a key factor affecting the susceptibility to NSCLC. The detailed results are shown in supplementary document 1.

Despite some interesting findings, our study suffers from the following potential limitations. Firstly, our sample is only from two hospitals, the Affiliated Hospital of Fujian Medical University and Jiangsu University. Although the four selected SNPs were consistent with HWE and there is no significant difference between MAF and database of Chinese Han populations, the bias might still be unavoidable. Secondly, the sample sizes of some subgroups are relatively small, especially in the SCC group. As such, our conclusions may not be sufficient to testify the real relationship of BTLA polymorphisms with susceptibility to SCC. The replication of the study with larger sample sizes is required to further validate our findings. Thirdly, only four functional loci in BTLA gene were selected in our study, and, therefore, other polymorphisms of BTLA should not be ignored. Finally, a functional study for these identified SNPs was not performed.

5. Conclusions

Despite some shortcomings, our findings preliminarily suggest that BTLA rs1982809 and rs16859629 SNPs may contribute to the risk of NSCLC. However, a thorough study with larger samples should be worth to elucidate the potential molecular functions of these BTLA polymorphisms.

Data Availability

The data used to support the findings of this study are available from the corresponding author according to reasonable request.

Conflicts of Interest

The authors declare that they have no potential financial conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Weifeng Tang and Shuchen Chen contributed to the conception and design; Shuchen Chen participated in the administrative support; Jusi Wang, Rui Cao, Zhan Chen, and Qiang Zhang participated in the provision of study materials or patients; Jusi Wang, Tingyu Chen, and Chengxiong You contributed to the collection and assembly of data; data analysis and interpretation were done by Jusi Wang and Weifeng Tang; manuscript writing was done by Jusi Wang; Weifeng Tang and Shuchen Chen participated in writing—review and editing.

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Supplementary Materials

Please see the document 1 about stepwise analysis of BTLA rs16859629, rs1982809, rs2171513, and rs3112270. (Supplementary Materials)

References


