Introduction
Esophageal cancer is the eighth most common cancer and sixth leading cause of cancer associated death worldwide. The 5 year survival rate for esophageal cancer patients is very poor and accounts for only 12.3%. Besides environmental risk factors, genetic factors might play an important role in the esophageal cancer carcinogenesis.

Methods:
We conducted a hospital based case–control study to evaluate the genetic effects of functional single nucleotide polymorphisms (SNPs): interleukin 9 (IL9) rs31563 C>T, IL9 rs31564 G>T, IL10 rs1800872 T>G, IL12A rs2243115 T>G, IL12B rs3212227 T>G and IL13 rs1800925 C>T on the development of esophageal cancer. A total of 380 esophageal squamous cell carcinoma (ESCC) cases and 380 controls were recruited for this study. The genotypes were determined using a custom-by-design 48-Plex SNPscan™ Kit.

Results:
The IL10 rs1800872 T>G polymorphism was associated with an increased risk of ESCC. However, there were no significant links with the other five SNPs. Stratified analyses indicated no significant risk of ESCC associated with the IL10 rs1800872 T>G polymorphism evident among any subgroups.

Conclusion:
These findings indicated that functional polymorphism IL10 rs1800872 T>G might contribute to ESCC susceptibility. However, our results were obtained with a limited sample size, so that the power of our analysis was low. Future larger studies with more rigorous study designs of other ethnic populations are required to confirm the current findings.

Keywords: IL10 - polymorphisms - esophageal cancer - molecular epidemiology - Chinese
DNA was isolated from whole blood with the QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) (Gu et al., 2012). Sample DNA (10 ng) were amplified by PCR according to the manufacturer’s recommendations. The SNP genotyping work was performed using a custom-by-design 48-Plex SNPscan™ Kit (Genesky Biotechnologies Inc., Shanghai, China) as previously described (Chen et al., 2012). This kit was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which was based on double ligation and multiplex fluorescence PCR. For quality control, repeated analyses were done for 4% of randomly selected samples with high DNA quality.

### Materials and Methods

**Ethical approval of the study protocol**

This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). All subjects provided written informed consent to be included in the study.

**Study subjects**

Three-hundred and eighty subjects with esophageal cancer were consecutively recruited from the Affiliated People’s Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and November 2009. All cases of esophageal cancer were diagnosed as ESCC by pathologic means. The exclusion criteria were patients who previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. The controls were patients without cancer frequency-matched to the cases with regard to age (±5 years) and sex recruited from the two hospitals mentioned above during the same time period. Most of the controls were admitted to the hospitals for the treatment of trauma.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2-mL samples of venous blood were collected from each subject. Individuals who smoked one cigarette per day for >1 year were defined as “smokers”. Subjects who consumed ≥3 alcoholic drinks a week for >6 months were considered to be “alcohol drinkers”.

**Isolation of DNA and genotyping by a custom-by-design 48-Plex SNPscan™ Kit**

Blood samples were collected from patients using Vacutainers and transferred to tubes lined with ethylenediamine tetra-acetic acid (EDTA). Genomic DNA was isolated from whole blood with the QIAamp...
higher in ESCC patients than in control subjects \( (P = 0.014) \). The primary information for six genotyped SNPs was in Table 2. For the six SNPs, the genotyping was successful ranging from 94.87% to 97.11% in all 760 samples. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) in our controls was similar to MAF for Chinese in database for all six SNPs (Table 2). The observed genotype frequencies for these six polymorphisms in the controls were all in HWE (Table 2).

Associations between six polymorphisms and risk of ESCC

The genotype distributions of \( IL9 \) rs31563 C>T, \( IL9 \) rs31564 G>T, \( IL10 \) rs1800872 T>G, \( IL12A \) rs2243115 T>G, \( IL12B \) rs3212227 T>G and \( IL13 \) rs1800925 C>T in the cases and the controls are shown in Table 3. In the
single locus analyses, the genotype frequencies of \( \text{IL10} \) rs1800872 T>G were 45.5% (TT), 45.8% (TG), and 8.7% (GG) in the case patients and 52.3% (TT), 38.6% (TG), and 9.0% (GG) in the control subjects, and the difference was not statistically significant (\( P = 0.146 \)). When the \( \text{IL10} \) rs1800872 TT homozygote genotype was used as the reference group, the TG genotype was associated with a significantly increased risk for ESCC (TG vs. TT: \( OR = 1.50, 95\% \ CI 1.00–2.21, P = 0.049 \)). When the \( \text{IL10} \) rs1800872 TT homozygote genotype was used as the reference group, the TT/GG genotype was not associated with the risk for ESCC (GG vs. TT: \( OR = 0.73, 95\% \ CI 0.47–1.14, P = 0.157 \)). In the recessive model, when the \( \text{IL10} \) rs1800872 TT/TG genotypes were used as the reference group, the IL10 homozygote genotype was not associated with the risk for ESCC (OR = 0.95, 95% CI 0.70–1.29, \( P = 0.875 \)).

In the dominant model, the \( \text{IL10} \) rs1800872 TG/TT genotype (TG/TT vs. TT: \( OR = 1.32, 95\% \ CI 1.08–1.62, P = 0.0007 \)) and the dominant model (TG/TT vs. TT: adjusted \( OR = 1.18, 95\% \ CI 1.03–1.35, P = 0.016 \)) were associated with a significantly increased risk of ESCC compared to the TT genotype. Logistic regression analyses revealed that the five polymorphisms were not associated with the risk of ESCC.

### Discussion

In this hospital-based case-control study of ESCC, we investigated the associations of \( \text{IL9} \) rs31563 C>T, \( \text{IL9} \) rs31564 G>T, \( \text{IL12A} \) rs2243115 T>G, \( \text{IL12B} \) rs3212227 T>G and \( \text{IL13} \) rs1800925 C>T with risk of ESCC in a high risk Chinese population. Our multivariable logistic analysis revealed that \( \text{IL10} \) rs1800872 TT genotype had an increased risk of ESCC.

Previous studies suggested that inflammatory cytokine gene SNPs such as \( \text{IL10} \) rs1800872 polymorphism is associated with smoking-related cancers (Oh et al., 2010). \( \text{IL10} \) gene is located on chromosome 1 (1q31-1q32), comprising five exons (Eskdale et al., 1997). Within the \( \text{IL10} \) gene promoter region, rs800872 (-592 T>G), were reported to be associated with different \( \text{IL10} \) expression.

\( \text{IL10} \) has been shown to regulate the differentiation and proliferation of several immune cells (Couper et al., 2008). \( \text{IL10} \) plays a key role in tumor development and metastasis. \( \text{IL10} \) has both tumor-promoting and tumor-inhibiting properties. Higher serum and peri-tumoral levels had been reported in many malignancies (Jebreel et al., 2007). To date, lines of research have investigated the contributions of \( \text{IL10} \) gene polymorphisms to the predisposition to different cancer types, such as oral, stomach, liver, breast, ovarian, cervical, prostate, and so on (Howell et al., 2007). However, no positive association was found between \( \text{IL10} \) rs1800872 TT and ESCC till now. We found \( \text{IL10} \) rs1800872 TT variant homozygote rather than \( \text{IL10} \) rs1800872 TT homozygote was associated with ESCC risk.

### Table 4.

Stratified Analyses Between \( \text{IL10} \): rs1800872 T>G Polymorphism and ESCC Risk by Sex, Age, Smoking Status and Alcohol Consumption

<table>
<thead>
<tr>
<th>Variable</th>
<th>( \text{IL10}: \text{rs1800872 T&gt;G (case/control)} )</th>
<th>Adjusted OR ( ^{\text{a}} ) (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT</td>
<td>TG</td>
<td>GG</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
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<td>Male</td>
<td>116/131</td>
<td>115/98</td>
<td>20/23</td>
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<td>Female</td>
<td>46/60</td>
<td>48/43</td>
<td>11/10</td>
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<td>59/57</td>
<td>61/45</td>
<td>13/9</td>
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<tr>
<td>≥60</td>
<td>103/134</td>
<td>102/96</td>
<td>18/24</td>
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<tr>
<td>Smoking status</td>
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<td>Never</td>
<td>92/128</td>
<td>93/96</td>
<td>19/18</td>
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<tr>
<td>Ever</td>
<td>70/63</td>
<td>70/45</td>
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<tr>
<td>Never</td>
<td>105/136</td>
<td>109/101</td>
<td>19/21</td>
</tr>
<tr>
<td>Ever</td>
<td>57/55</td>
<td>54/40</td>
<td>12/12</td>
</tr>
</tbody>
</table>

\( ^{a} \)The genotyping was successful in 356 (93.7%) ESCC cases, and 365 (96.1%) controls for \( \text{IL10}: \text{rs1800872 T>G} \); \( ^{b} \)Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model.
polymorphism was not associated with risk of ESCC among smoking or non-smoking subgroups. This might because our sample size was relatively small; the numbers of GG genotypes and GA genotypes in subgroups were not large enough.

There was no significantly association between the other five SNPs and ESCC risk in our population. These findings were consistent with some previous researches. The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of \( IL10 \) rs1800872 \( G \) was 0.284 among 380 control subjects, which is consistent with that of Chinese Han population (0.267) in SNP DataBase, but significantly lower than that of Sub-Saharan African (0.525) population and European (0.792) population (http://www.ncbi.nlm.nih.gov/SNP).

Considering \( IL10 \) rs1800872 \( T>G \) mutant alleles in the control group, OR, ESCC samples and control samples, the power of our analysis (\( \alpha = 0.05 \)) was 0.482 in 356 ESCC cases and 365 controls with adjusted OR 1.36 for \( IL10 \) rs1800872 \( T>G \).

Several limitations need to be addressed. The patients and controls were enrolled from hospitals and may not represent the general population, inherited biases may occur. The polymorphisms investigated in our study were chosen based on their functional considerations, and may not give a comprehensive view about genetic variability in \( IL10 \). Further fine-mapping studies in the susceptible region of the variants are needed. The moderate sample size limited the statistical power of our study. Further studies are warranted to confirm our findings, particularly the gene-environment interaction are warranted to clarify esophageal carcinogenesis genetic mechanism. Detailed information on cancer metastasis and survival information were not available till now, which restricted us from further analyses on the role of \( IL10 \) polymorphisms in ESCC progression and prognosis.

In conclusion, our study provides evidence that functional \( IL10 \) rs1800872 \( T>G \) polymorphism may contribute to the risk of ESCC. However, our results were obtained with a limited sample size, the power of our analysis was low, and therefore allowed us to draw just preliminary conclusions. Future larger studies with more rigorous study designs of other ethnic populations and tissue-specific biological characterization are required to confirm current findings.

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References
