INTRODUCTION

Esophageal cancer (EC) is the 8th dominant malignant tumor and the 6th primary cause of cancer-associated mortality worldwide. There were about 442,000 cases and 440,000 deaths of EC worldwide in 2013. It is histologically divided into esophageal squamous cell carcinoma and esophageal adenocarcinoma mainly. Most EC patients require effective treatments, including chemotherapy, chemo-radiotherapy, or surgical operation. Risk factors for EC are drinking, smoking, salted plants and nitrosamines, thermally treated foods, silica fibers from millet bran, vitamin and mineral insufficiency. Nevertheless, these risk factors only partly account for its frequent occurrence, indicating other factors such as genetic factors may also associate with the risk of EC.

The 5′-AMP-activated protein kinase (AMPK), encoded by AMP-activated protein kinase catalytic subunit alpha-1 gene (PRKAA1), plays a pivotal role in carcinogenesis, owing to its involvement in cell growth, cell cycle modulation, rapamycin-pathway restriction, and energy metabolism. PRKAA1 gene is located at 5p13.1. Rs13361707 C/T polymorphism is positioned in the first intron of PRKAA1 gene. Up to date, research discovered that this polymorphism was linked with the risk of gastric cancers. However, the existing findings were conflicting. Besides, two Chinese studies investigating the relationship between EC risk and rs13361707 C/T polymorphism in PRKAA1 gene yielded no positive results. Thus, the aims of this study were to explore the connection between this variant in PRKAA1 gene and EC susceptibility in Chinese individuals. In addition, we aimed to explore the relationship between PRKAA1 rs13361707 C/T polymorphism and clinical features of EC patients.
2 | MATERIALS AND METHODS

2.1 | Subjects

Totally, 814 EC patients and 961 healthy controls were recruited from our hospital. The inclusion criteria were as follows: (a) EC patients were pathologically diagnosed; (b) EC patients underwent operation for the first time. Exclusion criteria included: (a) EC patients receiving chemotherapy, radiotherapy and other treatments, or with history of esophageal diseases; (b) Patients with a second primary tumor or tumor of unclear origin; (c) Patients with incomplete clinical records. The controls were those undergoing complete physical examination in the same hospital. All subjects provided written informed consent. Approval was acquired from the review board of the tested Hospital. The Helsinki declaration was obeyed throughout.

2.2 | DNA extraction and genotyping

Peripheral bloods of the subjects were sampled by vacutainer tubes with EDTA, centrifuged, and maintained at ~80°C. Genomic DNAs from the bloods were extracted using a Puregene DNA purification kit (Gentra) and genotyped by MALDI-TOFMS on a MassARRAY system (Sequenom). Ended genotyping reactions were guided onto a 384-well spectroCHIP instrument (Sequenom) on a MassARRAY nanodispenser (Sequenom) and analyzed by MALDI-TOFMS. About 10% of the samples were randomly chosen for genotyping again, and the accordance rate was 100%.12

2.3 | Statistical methods

Hardy-Weinberg equilibrium (HWE) test in the control group was analyzed by the goodness-of-fit chi-square ($\chi^2$) test. Categorical data were assessed by chi-square test and displayed as frequencies (percentages). Continuous data were exhibited as mean ± standard deviation. Differences in categorical data and continuous data were tested by chi-square test and one-way ANOVA test or an independent samples t test, respectively.13 Relationship between PRKAA1 rs13361707 C/T polymorphism and EC risk was assessed by logistic regression with multiple genetic models adjusted by age and gender. The false-positive report probability (FPRP) of significant results was assessed in this study.13,14 P < .05 stood for significant level. Statistical analyses were accomplished on SPSS17.0 (SPSS Inc).

3 | RESULTS

3.1 | Information of subjects

The baseline characteristics of all individuals are presented in Table S1, including the demographics and environmental risk factors. The controls and cases were matched in age, sex, and smoking. However, the proportion of drinkers in EC cases was significantly higher than that in controls. The type of EC was mostly (96.1%) squamous cell carcinoma. We also included the clinical parameters, TNM stage, pathological grading, histology, and distant metastasis of EC in Table S1.

3.2 | PRKAA1 rs13361707 C/T polymorphism and EC susceptibility

Genotype distributions of the target polymorphism in the two groups are shown in Table 1 and Figure S1. A significant discrepancy was observed between groups. We found CC genotype or C allele carriers showed a decreased risk for EC patients (CC vs TT: OR, 0.64, 95% CI, 0.49-0.83; C vs T: 0.80, 0.70-0.91; both $P = .001$). The significant associations still held true after adjusting for age and sex.

Next, stratified analyses by age, gender, drinking, and smoking were evaluated. Data revealed that a protective role of rs13361707 C/T polymorphism in EC susceptibility was strengthened in the subgroups of males, smokers, drinkers, and individuals at age ≥ 60 years (Table 2).

3.3 | Relationship of clinical features of EC with PRKAA1 rs13361707 C/T polymorphism

CC or TC + CC genotype was involved in avoidance of EC patients from differentiation deterioration, distant metastasis, and squamous cell carcinoma, indicating the rs13361707 C/T polymorphism participated in the pathological grading, distant metastasis, and histology of EC (Table 3).

3.4 | False-positive report probability results

We preset 0.2 as the FPRP threshold. As shown in Table S2, the significant findings for the rs13361707 polymorphism remained noteworthy at the prior probability of .1 in the dominant, recessive, homozygote, and allele models.

4 | DISCUSSION

In this study, rs13361707 C/T polymorphism of PRKAA1 gene was related with a lower risk of EC in this tested Chinese Han population. Subgroup analyses observed this significant association in males, smokers, drinkers, and those aged ≥ 60 years. Furthermore, rs13361707 C/T polymorphism was linked with pathological grading, distant metastasis, and squamous cell carcinoma.

A host of studies have evaluated the relationship between rs13361707 C/T polymorphism of PRKAA1 gene and the risk of
several cancers. Slattery et al firstly probed into the connection of this polymorphism with rectal cancer (91 cases, 999 controls) and colon cancer (1574 cases, 1940 controls) in two case-control studies, but reported no positive findings. The remaining genetic studies regarding this polymorphism focused on gastric cancer (GC). A genome-wide relationship study in Chinese descents identified non-cardia GC for this polymorphism. The positive results of this study were replicated by a case-control study with large sample size from Korea. Kim et al confirmed the relationship between rs13361707 C/T polymorphism and GC susceptibility. A subsequent Chinese study also observed positive findings for GC patients and showed rs13361707 C/T polymorphism was a protective factor for GC. A study from Europe with mixed Caucasian populations obtained negative results for the association between this SNP and GC risk. However, a recent meta-analysis combining all included studies showed no relationship between this SNP and GC risk in Asians. A study of 1340 breast cancer cases and 2536 controls in Caucasians obtained no association between this polymorphism and

### Table 1: Genotype frequencies of PRKAA1 gene polymorphism in cases and controls

| Models          | Genotype | Case (n, %) | Control (n, %) | OR (95% CI) | P-value | *OR (95% CI) | *P-value |
|-----------------|----------|------------|---------------|-------------|---------|--------------|---------|
| rs13361707      | Co-dominant | TT         | 226 (27.8%)   | 214 (22.3%) | 1.00    | -            | -       |
|                 | Heterozygote | TC         | 405 (49.8%)   | 476 (49.5%) | 0.81    | 0.64-1.01    | .070    |
|                 | Homozygote | CC         | 183 (22.4%)   | 271 (28.2%) | 0.64    | 0.49-0.83    | .001    |
|                 | Dominant   | TT         | 226 (27.8%)   | 214 (22.3%) | 1.00    | -            | -       |
|                 |            | CC + TC    | 588 (72.2%)   | 747 (77.7%) | 0.75    | 0.60-0.93    | .008    |
|                 | Recessive  | TC + TT    | 631 (77.6%)   | 690 (71.8%) | 1.00    | -            | -       |
|                 | Allele     | T          | 857 (52.6%)   | 904 (47.0%) | 1.00    | 0.70-0.91    | .001    |

Note: Bold values are statistically significant (P < .05).

* The genotyping was successful in 814 cases and 961 controls for rs13361707.

* Adjust sex and age.

### Table 2: Stratified analyses between rs13361707 polymorphism and the risk of esophageal cancer

| Variable | (case/control) | TT vs TT | CC vs TT | CC vs TT + TC | CC + TC vs TT |
|----------|----------------|----------|----------|---------------|---------------|
| Sex      | Male           | 156/137  | 287/348  | 134/180       |               |
|          | Female         | 70/77    | 118/128  | 49/91         |               |
| Smoking  | Yes            | 108/113  | 208/249  | 114/139       |               |
|          | No             | 118/101  | 197/227  | 69/132        |               |
| Alcohol  | Yes            | 126/120  | 250/265  | 114/149       |               |
|          | No             | 100/94   | 155/211  | 69/122        |               |
| Age (years) | <60   | 74/79    | 163/172  | 62/93         |               |
|          | ≥60            | 152/135  | 242/304  | 121/178       |               |

Note: Bold values are statistically significant (P < .05).
breast cancer risk. Another study implied rs13361707 C/T polymorphism was unrelated to lung cancer risk. Recently, two Chinese studies observed no association between this SNP and EC susceptibility. However, a meta-analysis found a relationship between this polymorphism and EC susceptibility when the data of these two Chinese studies were combined. Our study revealed that rs13361707 C/T polymorphism in PRKAA1 gene was related to EC susceptibility, which was consistent with the findings of the above meta-analysis. Obviously, the findings of this study were different from other Chinese studies. The following points may potential factors contributing to these differences. One, the sample sizes were diverse. The study by Dong et al only enrolled 186 controls and 110 EC cases, which may yield false-negative results. Two, clinical heterogeneity is an important factor. Dai et al investigated esophageal squamous cell carcinoma, while this study explored overall EC patients. Three, distinct diets and living styles may also contribute to it. Stratified analyses further suggested the association was maintained in the subgroups of males, smokers, drinkers, and individuals at age ≥ 60 years, indicating individuals exposed to these risk factors were prone to EC. Additionally, rs13361707 C/T polymorphism was also found to be connected with the pathological grading, distant metastasis, and squamous cell carcinoma of EC patients.

The present study had potential limitations. First, the sample size was limited, which may yield false-positive findings and decrease the power of this study. Second, the design of this retrospective case-control study may result in selection biases, thereby exerting effects on the credibility of conclusions. Third, the functions of PRKAA1 rs13361707 C/T polymorphism should be studied. We should explore whether this polymorphism could affect the expression of PRKAA1 gene and protein. Fourth, we only explored one SNP of PRKAA1 gene; however, one SNP could not explain the decreased risk of EC patients fully; whether this SNP was in linkage disequilibrium with other SNPs in PRKAA1 gene should be investigated. Last, gene-environment factors interactions should be studied. As is known to all, the interaction between environment factor and genetic factor contributed to the risk of EC. Thus, genetic factor in this study could not explicate decreased susceptibility to EC patients comprehensively.

The PRKAA1 rs13361707 C/T polymorphism is related to a lower risk of EC in the tested Chinese Han population.

CONCLUSIONS

The authors declare no conflict of interest.
AUTHOR CONTRIBUTIONS
BZ conceived the entire study; JQZ analyzed the data; CL performed statistical analysis; CL and JQZ wrote the paper.

DATA AVAILABILITY STATEMENT
The relevant data could be available when the corresponding author was contacted.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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