INTRODUCTION

Bladder cancer (BC) ranks the 9th among all cancers globally and about 430,000 new BC cases are diagnosed annually, resulting in 165,000 deaths every year. Nearly a quarter of BC patients are muscle-invasive with a high propensity for rapid growth and metastasis. Three-quarters of all BC cases occur in men. Nearly 75% of BC patients are non-muscle-invasive and 25% are muscle-invasive or metastatic. The risk factors of BC may include smoking, gender, and occupational exposure to polycyclic aromatic hydrocarbons.
However, BC patients may be partly threatened by the above risk factors, suggesting some other factors such as genetic causes may involve in the pathogenesis of BC. A host of GWASs have identified novel loci for BC patients. Leptin (LEP), an adipocyte-originated hormone, mediates food consumption and adjusts immune and inflammatory reactions via its receptor. LEP is dominantly produced by fat cells. LEP is critical for weight control and is probably associated with the carcinogenic process. LEP activates JAK/STAT and AKT pathways to facilitate the proliferation and invasion of endometrial cancer cells in humans. LEP can regulate the division and movement of prostate cancer cells. LEP is related to the occurrence and survival of BC. In addition, the LEP receptor is expressed abnormally in BC tissues and probably takes part in the initiation of BC. LEP rs2167270 G > A polymorphism positioned at 5′-untranslated region is associated with LEP mRNA translation and with LEP levels. Recently, the association between LEP G19A polymorphism and risk of different cancers has been explored, which, however, presents contradictory findings. Moreover, the connection between LEP G19A polymorphism and BC risk has not addressed in China. Therefore, we carried out this case-control article to verify whether this polymorphism confers susceptibility to BC in Chinese people.

2 MATERIALS AND METHODS

2.1 Subjects

Totally 355 BC patients were enrolled from the Affiliated Huai’an No.1 People’s Hospital of Nanjing Medical University from July 2014 to January 2019. At the same time, 435 healthy controls receiving physical examination from this hospital were involved. The controls were matched with the BC patients in terms of sex and age. The detailed selection criteria for BC patients were shown in our previous study. The diagnosis of BC was based on clinical symptoms, auxiliary examination, and pathological findings. The exclusion criteria were history of malignancy, chronic diseases, and reception of chemo- or radiotherapy. The detailed clinical characteristics of BC patients were extracted from their medical records. All subjects completed a written informed consent. This research was performed as per the 1964 Declaration of Helsinki and was approved by the Ethnic Committee of the Hospital.

2.2 Genotyping

LEP G19A polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). Genomic DNA was isolated with a QIAamp DNA blood mini kit. The primers were 5′-CCC GCGAGGTGCACACTG-3′ (forward) and 3′-AGGAGGAAAGGACGCGCC-5′ (reverse). To examine the quality control of genotyping, we chose 10% representative DNA samples and tested in a blind manner. The concordance of the genotypes was 100%.

2.3 Statistical analysis

Clinical and demographic data were examined with Student’s t test or chi-square test (χ²). Based on the Hardy-Weinberg equilibrium (HWE) test, the expected and experimental genotype frequencies of LEP G19A polymorphism in the control group were compared via the chi-square test. The association of this polymorphism with BC risk was investigated through logistic regression with odds ratio (OR) and 95% confidence interval (CI). P < .05 implied significance. All statistical analyses were carried out on SPSS 22.0 (SPSS Inc.).

| Variable                  | Cases (n = 355) | Controls (n = 435) | P  |
|---------------------------|-----------------|--------------------|----|
| Age (y)                   | 60.81 ± 10.57   | 61.25 ± 9.73       | .541 |
| Sex                       |                 |                    |    |
| Male                      | 303 (85.4%)     | 363 (83.4%)        | .464 |
| Female                    | 52 (14.6%)      | 72 (16.6%)         |    |
| Smoking                   |                 |                    |    |
| Yes                       | 251 (70.7%)     | 180 (41.4%)        | <.001 |
| No                        | 104 (29.3%)     | 255 (58.6%)        |    |
| Drinking                  |                 |                    |    |
| Yes                       | 233 (65.6%)     | 173 (39.8%)        | <.001 |
| No                        | 122 (34.4%)     | 262 (60.2%)        |    |
| Tumor grade               |                 |                    |    |
| High(G2 + G3)             | 227 (63.9%)     |                    |    |
| Low(G1)                   | 128 (36.1%)     |                    |    |
| Tumor size (cm)           |                 |                    |    |
| <3                        | 263 (74.1%)     |                    |    |
| ≥3                        | 92 (25.9%)      |                    |    |
| TNM stage                 |                 |                    |    |
| I                         | 78 (22.0%)      |                    |    |
| II                        | 100 (28.2%)     |                    |    |
| III                       | 104 (29.3%)     |                    |    |
| IV                        | 73 (20.6%)      |                    |    |
| Tumor node metastasis     |                 |                    |    |
| Yes                       | 108 (30.4%)     |                    |    |
| No                        | 247 (69.6%)     |                    |    |
| Distant metastasis        |                 |                    |    |
| M0                        | 332 (93.5%)     |                    |    |
| M1                        | 23 (6.5%)       |                    |    |
| Histology                 |                 |                    |    |
| Papillary                 | 291 (82.0%)     |                    |    |
| Nonpapillary              | 64 (18.0%)      |                    |    |

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

The distributions of age, sex, smoking, and alcohol status as well as the clinical information of BC patients are summarized in Table 1. The 355 BC patients (303 males, 52 females) were aged 60.81 ± 10.57 years old, and the 435 controls (363 males, 72 females) were aged 61.25 ± 9.73 years old. No significant differences between BC patients and controls were observed in sex (P = .464) or age (P = .541). The incidence rates of smoking and drinking among BC patients were considerably higher than controls (both P < .001). The tumor size, distant metastasis, histology, and tumor node metastasis (TNM) of BC patients were listed in Table 1.

### Results

#### Table 2: Genotype frequencies of LEP G19A polymorphisms in cases and controls

| Models         | Genotype | Case (n = 355)a | Control (n = 435)b | OR (95% CI) | P-value | bOR (95% CI) | bP-value |
|----------------|----------|-----------------|--------------------|-------------|---------|-------------|----------|
| rs2167270      | Co-dominant GG | 228 (64.6%)     | 242 (55.9%)        | 1.00 (Reference) | —       | —           | —        |
|                | Heterozygote GA  | 114 (32.3%)     | 162 (37.4%)        | 0.75 (0.55-1.01) | .057    | 0.75 (0.56-1.01) | .062     |
|                | Homozygote AA   | 11 (3.1%)       | 29 (6.7%)          | 0.40 (0.20-0.83) | .013    | 0.40 (0.20-0.83) | .013     |
| Dominant       | GG         | 228 (64.6%)     | 242 (55.9%)        | 1.00 (Reference) | —       | —           | —        |
|                | AA + GA     | 125 (35.4%)     | 191 (44.0%)        | 0.70 (0.52-0.93) | .014    | 0.70 (0.52-0.93) | .015     |
| Recessive      | GA + GG    | 342 (96.9%)     | 404 (93.4%)        | 1.00 (Reference) | —       | —           | —        |
| Allele         | G          | 570 (80.7%)     | 646 (74.6%)        | 1.00 (Reference) | —       | —           | —        |
|                | A          | 136 (19.3%)     | 220 (25.4%)        | 0.70 (0.55-0.89) | .004    |             |          |

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

*aThe genotyping was successful in 353 cases and 433 controls for rs2167270.

**Table 3: Stratified analyses between LEP G19A polymorphisms and the risk of bladder cancer**

| Variable | (Case/Control) | GG | GA | AA | GA vs GG | AA vs GG | AA vs GG + GA | AA + GA vs GG |
|----------|----------------|----|----|----|----------|----------|---------------|---------------|
| Sex      | Male           | 199/204 | 92/133 | 10/24 | 0.71 (0.51-0.99); .041 | 0.43 (0.20-0.92); .029 | 0.48 (0.23-1.03); .058 | 0.67 (0.49-0.91); .003 |
|          | Female         | 29/38   | 22/29 | 1/5  | 0.99 (0.48-2.07); .987 | 0.26 (0.02-2.37); .233 | 0.26 (0.03-2.32); .229 | 0.89 (0.43-1.82); .742 |
| Smoking  | Yes           | 160/106 | 80/60 | 9/13 | 0.88 (0.58-1.34); .558 | 0.46 (0.19-1.11); .084 | 0.48 (0.20-1.15); .998 | 0.81 (0.54-1.20); .289 |
|          | No            | 68/136 | 34/102 | 2/16 | 0.67 (0.41-1.08); .101 | 0.25 (0.06-1.12); .070 | 0.29 (0.07-1.29); .105 | 0.64 (0.40-1.02); .059 |
| Alcohol  | Yes           | 142/99  | 82/63 | 8/10 | 0.91 (0.60-1.38); .648 | 0.56 (0.21-1.46); .235 | 0.58 (0.22-1.50); .260 | 0.86 (0.58-1.28); .460 |
|          | No            | 86/143 | 32/99 | 3/19 | 0.54 (0.33-0.87); .011 | 0.26 (0.08-0.91); .036 | 0.32 (0.09-1.12); .074 | 0.49 (0.31-0.78); .003 |
| Age (y)  | <60            | 106/103 | 47/76 | 1/8  | 0.60 (0.38-0.95); .028 | 0.12 (0.02-0.99); .049 | 0.15 (0.02-1.18); .071 | 0.56 (0.36-0.87); .010 |
|          | ≥60           | 122/139 | 67/86 | 10/21 | 0.89 (0.59-1.33); .561 | 0.54 (0.25-1.20); .130 | 0.57 (0.26-1.23); .153 | 0.80 (0.56-1.20); .307 |

Note: Bold values are statistically significant (P < .05).
3.2 | Relationship of LEP G19A polymorphism with BC risk

The genotype distributions of LEP G19A polymorphism among the BC patients and controls were compared (Table 2). The genotype distribution of this polymorphism in controls obeyed HWE (P > .05; Table 2). AA or AA + GA genotype was linked with a lower risk of BC (AA vs GG: OR, 0.40, 95% CI, 0.20-0.83, P = .013; AA + GA vs GG: adjusted OR, 0.70, 95% CI, 0.52-0.93, P = .014; AA vs GA + GG: adjusted OR, 0.45, 95% CI, 0.22-0.91, P = .027). This association still held true after adjustment for age and gender. Furthermore, A allele carriers were less susceptible to BC (A vs G: OR 0.70, 95% CI, 0.55-0.89, P = .004). Stratified analyses showed the risk of BC was considerably lower in non-drinkers, women, and non-smokers, but not in terms of age (Table 3).

3.3 | Relationship of LEP G19A polymorphism with clinical data of BC

Eventually, the link of LEP G19A polymorphism with the clinical data of BC patients was explored (Table 4). Data demonstrated that this polymorphism was correlated largely with tumor size (<3 cm) (AA vs GG: OR 0.22, 95% CI, 0.06-0.74, P = .008), distant metastasis (AA vs GG: OR 0.11, 95% CI, 0.03-0.48, P = .001), and tumor node metastasis (AA vs GG: OR 0.22, 95% CI, 0.06-0.79, P = .012), but not with TNM stage or histology of BC.

4 | DISCUSSION

Herein, we found that LEP G19A polymorphism resulted in a less risk of BC. Stratified analyses yielded remarkable correlation in the subgroups of females, non-smokers, and non-drinkers. Also, this polymorphism was related to tumor size, distant metastasis, and TNM in BC patients.

Skibola et al.9 reported for the first time that LEP G19A polymorphism was related to a smaller risk of non-Hodgkin's lymphoma (NHL). Later, the positive finding for NHL was replicated in another Caucasian population.17 However, this association was not revealed in a recent Chinese study.18 A large Australian case-control article with 774 esophageal cancer cases and 1352 controls obtained no significant results regarding LEP G19A polymorphism.19 Qiu et al.20 from China confirmed the negative findings with esophageal cancer. However, in another Chinese study, LEP G19A polymorphism was positively associated with risk of esophagogastric junction adenocarcinoma.21 Other studies from Germany,22 USA,23 and Mexico24 observed inconsistent results for colorectal cancer (CRC). The LEP G19A polymorphism was related to CRC risk only in the study from Mexico.24 As for urinary system cancers, no significant results were
reported about the correlation between LEP G19A polymorphism and prostate cancer risk in studies from USA\textsuperscript{23} and Finland.\textsuperscript{26} Up to date, no genetic study investigated this polymorphism in BC populations. As we know, we were the first to test the link of LEP G19A polymorphism with BC risk and found a positive result. In addition, positive findings were obtained among females, non-smokers, and non-drinkers, suggesting individuals exposed to these risk factors are more prone to BC.

Nevertheless, this study has some limitations. Firstly, selection bias may lead to spurious findings. Secondly, the sample size was small. Thirdly, we only explored one locus in LEP or LEPR gene.\textsuperscript{30} Fourthly, gene-environment interactions cannot be tested due to lack of relevant data. Last, functions of the LEP G19A polymorphism should be addressed.

In conclusion, LEP G19A polymorphism is related to a decreased risk of BC in Chinese Han people. This finding should be validated by larger-size fine-mapping research with function analysis.

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ETHICAL APPROVAL
All experiments involving human subjects were conducted as per the ethical standards of the institutional and/or national research committee as well as the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

INFORMED CONSENT
Informed consent was obtained from all individuals.

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