NAD(P)H: quinone oxidoreductase 1 gene rs1800566 polymorphism increases the risk of cervical cancer in a Chinese Han sample

A STROBE-complaint case-control study

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Abstract
Recently, 2 studies from Thai and American investigated the relationship between NAD(P)H: quinone oxidoreductase 1 (NQO1) gene rs1800566 polymorphism and cervical cancer risk and generated contrary results. However, no Chinese reports have addressed this relationship until now. To explore the association between NQO1 gene rs1800566 polymorphism with cervical cancer, we performed a study in a Chinese Han sample.

Using an unmatched case-control design, we enrolled 450 cervical cancer patients and 568 controls in the Central Hospital of Wuhan from January 2010 to December 2016. The genotypes were determined by sequencing polymerase chain reaction product. Hardy-Weinberg equilibrium was assessed using the Chi-square test. The univariate and multi-variate logistic regression with odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the association between the NQO1 gene rs1800566 polymorphism and cervical cancer susceptibility.

The Chi-square test indicated that significant allele and genotype distributions differences were observed between case group and control group (P<.001). The logistic regression indicated that TT genotype was associated with higher risk of cervical cancer compared with those with the CT or CC genotype (TT vs CC: OR=2.82, 95%CI: 1.91–4.17, P<.001; TT vs CT: OR=2.02, 95%CI: 1.36–3.01, P<.001). The effects of NQO1 show dominant model (TT/CT vs CC: OR=1.67, 95%CI: 1.30–2.15, P<.001) and recessive model (TT vs. CT/CC: OR=2.43, 95%CI: 1.68–3.52, P<.001). The significant relationship between NQO1 rs1800566 polymorphism and cervical cancer risk was also found in stratified analyses. The cross-over analysis indicated that there are potential interactions between genetic factors and human papillomavirus infection/ contraceptive oral use for the risk of cervical cancer.

NQO1 gene rs1800566 polymorphism is associated with elevated risk of cervical cancer in Chinese Han women. The interactions between rs1800566 polymorphism and human papillomavirus infection/ contraceptive oral use further reinforce this association.

Abbreviations: BMI = body mass index, CI = confidence interval, HPV = human papillomavirus, NQO1 = NAD(P)H: quinone oxidoreductase 1, OR = odds ratio, SNP = single nucleotide polymorphism.

Keywords: case-control, cervical cancer, gene polymorphism, NAD(P)H: quinone oxidoreductase 1

1. Introduction
Cervical cancer is the second common malignant tumor in women, and is also the third major cause of cancer death in women in developing countries. [1] Cervical intraepithelial neoplasia is a precancerous lesion of cervical cancer. It is a general term for a series of cervical squamous epithelial lesions, and it can develop into squamous invasive carcinoma. [2] The occurrence and development of cervical cancer is a rather complex biological process, which is related to many factors. [3,4] Human papillomavirus (HPV) infection is the main risk factor for cervical cancer. [5] However, some HPV-infected women do not progress to cervical cancer, which indicated some other factors may be involved in the process of cervical cancer. Previous studies also identified some others risk factors such as socio-demographic and behavioral factors. [6–9] Genetic variation may also contribute to the occurrence of cervical cancer.

Gene polymorphism plays an important role in a variety of pathophysiological processes, including cancer. Among them, single nucleotide polymorphisms (SNPs) is the most common genetic variation of human genes, and is considered to be the new generation of genetic markers. It has been shown that the SNP polymorphism of multiple genes is closely related to the genetic
susceptibility due to the regulation of DNA mismatch repairs, cell cycle, metabolism and immunity.\textsuperscript{[10,11]} NAD(P)H: quinone oxidoreductase 1 (NQO1), also known as DT-diaphorase, is a cytoplasmic flavoenzyme encoded by a gene located on chromosome 16q22.\textsuperscript{[15,11]} NQO1 uses NADH or NADPH as substrates to directly reduce quinones to hydroquinone. Functions of NQO1 include xenobiotic detoxification, superoxide scavenging and the maintenance of endogenous antioxidant vitamins.\textsuperscript{[12,13]} It is conceivable that NQO1 plays an important role in protection normal cells against oxidative injury and carcinogenesis. Paradoxically, despite the cellular functions of this cell protector, the antioxidant role of NQO1 was suggested by evidence that the disruption of the NQO1 gene or genetic polymorphism increased the risk of chemical-induced toxicity and cancers.\textsuperscript{[14]} Two studies also explored the association between NQO1 gene polymorphism and cervical cancer. However, the results remain inconsistent because of the differences in study population, and sample size. One study from Thailand population achieved a negative association between NAOQ1 rs1800566 and cervical cancer.\textsuperscript{[15]} Another study identified a positive association. In the present study, we explored the association between NQO1 gene rs1800566 polymorphism with cervical cancer in a Chinese Han sample.

2. Materials and methods

2.1. Study sample and data collection

Using an unmatched case-control design, we enrolled 450 cervical cancer patients in the Central Hospital of Wuhan from January 2010 to December 2016. The control sample were from the health examination center of our hospital during the same period. All cervical patients were confirmed by pathology biopsy. The included subjects must meet the following criteria: the case group are not diagnosed with other cancer types except cervical cancer. The cases and controls do not have severe cardiovascular diseases, infectious disease, severe liver and kidney dysfunction, and other autoimmune diseases. We used the Quanto 1.2.4 software to calculate the sample size under the following conditions: \( \alpha = 0.05, \beta = 0.10 \), expected odds ratios (OR) = 1.8. The calculated sample size is 356 in the control group and case group. Our study meets the requirement of sample size. The collected data consisted of age, body mass index (BMI), smoking, HPV infection, history of contraceptive oral use. The clinicopathologic characteristics of the cervical cancer patients were extracted from medical records. According to previous studies and our available data, we included age, BMI, smoking, tumor stage, histological grade, and lymph node metastasis. This study was conducted with prior approval of Ethics committee of The Central Hospital of Wuhan. Sample collection was accordance with ethic’s criteria of national human genome research. Written informed consent with the use of archived DNA samples for research purposes was obtained from all participant’s parents or their guardians.

2.2. SNP Genotyping

Peripheral venous blood was collected from every fasting participant in the morning, then dealt by EDTA and stored in \(-80^\circ\text{C}\). The DNA extraction kit (Tiangen, China) was used to extract the genomic DNA from blood samples. Primers for rs1800566 and rs7830 were designed by Primer Premier 5.0 and synthesized by Sangon Biotech (Shanghai, China). Primer sequence of rs1800566 were F: 5’- GCC TCC TTA TCA GAG TG TGT C -3’ and R: 5’- ACA GTG GTG TCT CAT CCC A -3’. Reaction conditions for polymerase chain reaction were initial degeneration at 94°C for 5 minutes, 32 cycles of degeneration at 94°C for 60s, annealing at 54°C for 45s, extension at 72°C for 50s, and followed by final extension at 72°C for 10minutes. Polymerase chain reaction products were genotyped by Sanger sequencing method (Sangon Biotech, Shanghai, China).

2.3. Statistical analysis

The continuous variable was expressed as mean \pm standard deviation (BMI), and the \( t \)-test was used for comparison between case group and control group. The category variables were expressed using the count and percent, and the chi-square was used to compare the difference between 2 groups. Hardy-Weinberg equilibrium was assessed using the Chi-square test. The univariate and multi-variate logistic regression were used to explore the association between genotype and cervical cancer in several common genetic mode (TT vs TC, TT vs CC, TT/TC vs CC, TT vs TC/CC), followed by the calculation of crude and adjusted ORs with 95% confidence interval (CIs). The adjusted variable included age, BMI, smoking, HPV infection, and contraceptive oral use. We also performed the subgroup analysis in the age (<60 vs \( \geq \)60 (old people)), BMI (<24 vs \( \geq \)24 (overweight)), smoking (Yes vs No), HPV infection (Yes vs No) and contraceptive oral use (Yes vs No) to explore the interaction effect between gene and behavioral factors.\textsuperscript{[17,18]} Finally, we analyzed the relationship between gene polymorphism and clinical parameters in case group. All analyses were performed using the SPSS 23.0 (SPSS Inc., Chicago) \( P < .05 \) was considered statistically significant.

3. Results

3.1. General characteristics of study sample

The general characteristics of study sample were presented in the Table 1. The present study consisted of 450 cervical cancer patients and 568 health controls. No significant differences were found in age (\( P = .747 \)), BMI (\( P = .906 \)), and smoking (\( P = .079 \)) between cases group and control group. Compared with control group, the case group tend to have HPV infection (94.0% vs 11.8%, \( P < .001 \)). The rate of contraceptive oral use was higher in the case group than that in the control group (30.9% vs 18.5%, \( P < .001 \)). Among 450 cases included in the study, 247 patients belong to stage I/II (54.9%), and 203 patients belong to stage III/ IV (45.1%). For differentiation type, 48.9% of patients were lowly differentiated, and 51.1% were moderately/highly differentiated. Among 450 cervical cancer patients, 404 (89.8%) patients were adenocarcinoma, 32 patients (7.1%) were squamous cell carcinoma, and 14 (3.1%) were other types of cancer. Besides, 39.8% of patients (\( n = 179 \)) had lymph node metastasis.

3.2. Association between NQO1 gene rs18600566 polymorphism and cervical cancer risk

The Table 2 presented the allele and genotype distributions of NQO1 gene rs18600566 polymorphism in the control group and case group. The Hardy-Weinberg equilibrium among controls
show no significance. Significant allele and genotype distributions differences were observed between 2 groups (P < .001). The results indicated that the TT genotype was associated with higher risk of cervical cancer compared with those with the CT or CC genotype (TT vs CC: OR = 2.82, 95% CI: 1.91–4.17, P < .001; TT vs CT: OR = 2.02, 95% CI: 1.36–3.01, P < .001); the effects of NQO1 show dominant model (TT/CT vs CC: OR = 1.67, 95% CI: 1.30–2.15, P < .001) and recessive model (TT vs CT/CC: OR = 2.43, 95% CI: 1.68–3.52, P < .001). Compared with C allele, the T allele increased the risk of cervical cancer (OR = 1.69, 95% CI: 1.40–2.04, P < .001). However, the effects of NQO1 only presented a recessive model (TT vs CT/CC: aOR = 3.86, 95% CI: 1.96–7.65, P < .001) and homoyzgote model (adjOR = 3.89, 95% CI: 1.89–8.02, P < .001) after adjusting age, BMI, smoking, HPV infection and contraceptive oral use.

The stratified analyses were performed based on BMI, age, smoking, HPV infection, contraceptive oral use. The significant relationship between NQO1 rs1800566 polymorphism and cervical cancer risk was also found in different stratified analyses (Table 3).

### 3.3. Interactive effect of gene and behavioral factors for the risk of cervical cancer

We performed the cross-over analysis to explore the interactive effects of gene factor and behavioral factors (HPV infection and contraceptive oral use). For people without HPV infection, the TT genotype was associated with cervical cancer risk and the CT genotype was not associated with cervical cancer compared to people without HPV infection carrying CC genotype. For those with HPV infection carrying TT or CT genotype were significantly associated with an elevated risk of cervical cancer as compared to non-HPV infection carrying CC genotype (TT+HPV infection vs CC+non-HPV infection: OR = 6.19 (95% CI:2.02–7.37), P < .001; CT+HPV infection vs CC+non-HPV infection: OR = 5.78(95% CI: 4.96–6.61), P < .001); For contraceptive oral use, both the carrying TT genotype and contraceptive oral use were associated with the risk of cervical, respectively. The combination of TT genotype and contraceptive oral use have higher risk of cervical compared to people carrying TT genotype only (TT+contraceptive oral use vs CC genotype only: OR = 4.29(95%CI: 2.16–8.53), P < .001). For people without contraceptive oral use, the CT genotype was not associated with the risk of cervical cancer as compare to the CC genotype. For people with contraceptive oral use, however, the CT genotype was significantly associated with the risk of cervical as compared to those without contraceptive oral use carrying CC genotype (CT+contraceptive oral use vs CC genotype only: OR = 2.79 (95%CI: 1.77–4.39), P < .001). This suggested that there are potential interactions between genetic factors and environment factors in the cervical cancer.

### 3.4. Correlation between NQO1 gene rs1800566 polymorphism and the clinical parameters of cervical cancer

We then assessed the relationship between NQO1 rs1800566 polymorphism and the clinicopathological characteristics of cervical cancer patients. As presented in the Table 5, the rs1800566 CT genotype was more frequent in patients with TNM stage III/IV. The NQO1 rs1800566 polymorphism may

### Table 1

**Comparison of general characteristic between case and control group.**

| Factors                  | Case group (n=450) | Control group (n=568) | χ²/t | P   |
|--------------------------|-------------------|-----------------------|-----|-----|
| Age                      |                   |                       |     |     |
| < 60                     | 276 (61.3%)       | 354 (62.3%)           | 0.104 | .747 |
| ≥60                      | 174 (38.7%)       | 214 (37.7%)           |     |     |
| BMI                      | 23.7 ± 3.1        | 23.7 ± 3.0            | 0.119 | .906 |
| Smoking                  |                   |                       | 3.089 | .709 |
| Yes                      | 86 (19.1%)        | 85 (15.0%)            |     |     |
| No                       | 364 (80.9%)       | 463 (85.0%)           |     |     |
| HPV infection            |                   |                       | 679.621 | < .001 |
| Yes                      | 423 (94.0%)       | 67 (11.8%)            |     |     |
| No                       | 27 (6.0%)         | 501 (88.2%)           |     |     |
| Contraceptive oral use   |                   |                       | 23.0848 | < .001 |
| Yes                      | 139 (30.9%)       | 103 (18.5%)           |     |     |
| No                       | 311 (69.1%)       | 463 (81.5%)           |     |     |
| TNM stage                |                   |                       |     |     |
| I                        | 247 (54.9%)       |                       |     |     |
| II/IV                    | 203 (45.1%)       |                       |     |     |
| Differentiation          |                   |                       |     |     |
| Low                      | 220 (48.9%)       |                       |     |     |
| Moderate/High            | 230 (51.1%)       |                       |     |     |
| Histology                |                   |                       |     |     |
| Adenocarcinoma           | 404 (89.8%)       |                       |     |     |
| Squamous cell carcinoma  | 32 (7.1%)         |                       |     |     |
| Others                   | 14 (3.1%)         |                       |     |     |
| Lymph node metastasis    |                   |                       |     |     |
| Yes                      | 179 (39.8%)       |                       |     |     |
| No                       | 271 (60.2%)       |                       |     |     |
| rs1800566                 |                   |                       | 28.718 | < .001 |
| CC                       | 185 (41.1%)       | 306 (53.9%)           |     |     |
| CT                       | 178 (39.6%)       | 211 (37.1%)           |     |     |
| TT                       | 87 (19.3%)        | 51 (9.0%)             |     |     |

BMI = body mass index.
be associated with TNM stage ($P = .034$). The T allele also show significant association with differentiation grade as compared to the C allele ($P = .027$). No significant association between TT or CT genotype and other clinicopathological characteristics of cervical cancer.

### 4. Discussion

In the present study, our results indicated that NQO1 rs1800566 polymorphism was associated with elevated risk of cervical cancer in a Chinese Han sample. The subgroup analyses suggested that this significant effect seems not be affected by BMI, age, smoking, HPV infection, and contraceptive oral use. The coexistence of TT/CT genotype and HPV infection or contraceptive oral use will increase the risk of cervical cancer. Furthermore, the SNP was significantly related to TNM stage III/IV in cervical cancer patients.

To date, 2 studies from other samples have explored the relationship between NQO1 gene and cervical cancer risk with inconsistent results. Jira et al performed a case-control study including 56 cervical patients and 32 controls and did find any significantly higher frequency genotype of rs1800566 in cancer samples. This is a study with small size. Using a family-based association test, Hu et al explored the relationship between NQO1 rs1800566 polymorphism and cervical cancer in Caucasian sample, and they found this SNP C allele was associated with the risk of cervical cancer, especially in women infected with type-26/type-18 related HPVs. They also admitted that they may not have sufficient power in subgroup analyses. This result is definitely different from our finding. We identified the T allele was a risk genotype for cervical cancer. This inconsistent result may be attributed to the differences of sample size and sample setting. Because another study from Japanese study with 131 cervical cancer patients also found the TT

### Table 3

Subgroup-analyses between NQO1/ rs1800566 gene polymorphism and the risk of cervical cancer.

| Variables | CC | CT | TT | TT versus CC | TT versus CT | TT/CT versus CC | TT versus CT/CC | T versus C |
|-----------|----|----|----|-------------|-------------|----------------|----------------|-----------|
| BMI ≥24   | 104/190 96/125 55/30 | 3.35 (2.02–5.55) | <0.001 | 2.39 (1.42–4.01) | 0.001 | 1.53 (1.04–2.25) | 0.033 | 2.85 (1.58–2.15) | 0.001 | 1.85 (1.45–2.36) | <0.001 |
| BMI <24   | 81/116 82/86 32/21 | 2.18 (1.17–4.05) | 0.012 | 1.60 (0.85–2.99) | 0.142 | 1.78 (1.26–2.47) | 0.001 | 2.80 (1.40–4.93) | <0.001 | 1.49 (1.11–1.99) | 0.007 |
| Age (≤60) | 102/184 119/136 55/34 | 2.92 (1.79–4.77) | <0.001 | 1.85 (1.13–3.03) | 0.014 | 1.45 (0.97–2.17) | 0.068 | 2.34 (1.48–3.71) | <0.001 | 1.75 (1.39–2.22) | <0.001 |
| Age >60   | 83/122 59/75 32/17 | 2.77 (1.44–5.31) | 0.002 | 2.39 (1.21–4.72) | 0.002 | 1.85 (1.34–2.55) | 0.011 | 2.61 (1.40–4.89) | 0.003 | 1.60 (1.17–2.18) | 0.003 |
| Smoking   | Yes | 28/54 27/24 31/7 | 8.54 (3.34–21.80) | <0.001 | 3.94 (1.47–10.57) | 0.005 | 3.61 (1.92–6.78) | <0.001 | 6.28 (2.58–15.29) | <0.001 | 3.72 (2.33–5.95) | <0.001 |
| No        | 157/252 151/187 58/44 | 2.04 (1.31–3.18) | 0.001 | 1.58 (1.01–2.47) | 0.046 | 1.44 (1.00–1.99) | 0.009 | 1.81 (1.19–2.76) | 0.006 | 1.42 (1.16–1.75) | 0.001 |
| HPV infection | Yes | 174/33 173/30 79/4 | 3.75 (1.28–10.93) | 0.010 | 3.49 (1.19–10.23) | 0.016 | 1.38 (0.82–3.33) | 0.213 | 4.07 (1.69–9.60) | 0.002 | 1.60 (1.07–2.39) | 0.021 |
| No        | 11/273 8/181 8/47   | 4.22 (1.61–11.05) | 0.001 | 2.38 (1.37–10.80) | 0.007 | 1.74 (0.79–3.83) | 0.167 | 3.62 (1.28–10.23) | 0.015 | 2.13 (1.22–3.71) | 0.006 |
| Contraceptive oral use | Yes | 52/50 58/40 29/13 | 2.14 (1.00–4.59) | 0.047 | 1.54 (0.71–3.20) | 0.270 | 1.62 (1.22–2.17) | 0.001 | 2.56 (1.66–3.97) | <0.001 | 7.95 (5.62–11.25) | <0.001 |
| No        | 133/254 120/171 58/38 | 2.91 (1.84–4.62) | <0.001 | 2.18 (1.36–3.48) | 0.001 | 1.58 (0.84–2.65) | 0.084 | 1.82 (0.90–3.72) | 0.097 | 2.00 (1.60–2.50) | 0.219 |

BMI = body mass index, NQO1 = NADPH quinone oxidoreductase 1.
genotype was associated with the risk of cervical squamous cell carcinoma but not for other types. It could be because of the ethnic factors. The frequency of T allele is 0.217 in Caucasians and 0.398 in Japan. Further research is needed to clarify this association and possible mechanism. But the present study with large sample size proved more stronger evidence for the associated between NQO1 rs1800566 polymorphism and cervical cancer. Previous studies suggested the effect of NQO1 genotypes on cervical carcinogenesis varies in association with smoking behaviors. However, our study indicated the relationship between NQO1 rs1800566 polymorphism and cervical cancer was independent of smoking behavior. We achieved positive relationship between rs1800566 and cervical cancer in both smokers and non-smokers. In the univariate analysis, we also found no significant association between smoking and cervical cancer. Instead of this, we found that contraceptive oral use and HPV infection were associated with cervical cancer. Therefore, we further performed a cross-over analysis for exploring the potential gene-environment interaction. Our results suggested the combined effects of NQO1 gene rs1800566 polymorphism and contraceptive oral use/HPV infection elevated the risk of cervical cancer. Previous study found no association between oral contraceptive use and breast cancer but increased risk for cervical cancer in a large prospective cohort. Our study confirms that and also found positive interaction between the rs1800566 polymorphism and contraceptive oral use. This may be related to the fact that rs1800566 can affect the activity of some exogenous substance because The NQO1 gene (rs1800566) TT can affect cancer development through the reduction cytotoxic agents containing the quinone moiety into hydroquinone in lung, bladder and colorectal cancer patients. Further studies are required. It is well known that HPV infection is a major risk factor of cervical cancer. The wild-type NAO1 can partially inhibits P53 degradation mediated by the HPV E6 protein while this does not happen for rs1800566 variants. Therefore, the interaction between rs1800566 and HPV infection can be attributed to effect of inactive NQO1 gene on p53 degradation and increased the risk of cervical cancer development.

We also found the SNP polymorphism was correlated with and TNM stage differentiation grade, which indicated this SNP may affect the progression of cervical cancer. Ma et al reported NQO1 expression was higher in SCCs of the cervix than in the normal cervical epithelia, and closely related to the differentiation, clinical stage and lymph node metastasis. Zhang et al demonstrated that NQO1 overexpression inhibits the proliferation and induces apoptosis of HCC cell by activating the AMPK/PGC-1α pathway. Additionally, Chen et al had proven that NQO1 could potentiate NSCLC cell proliferation by enhancing cellular glucometabolic, and NQO1 depletion triggered metabolic shift of TCA cycle independent of PDHX and PDK. These studies indicated that NQO1 was oncogene, and the high expression level of NQO1 is associated with multiple malignant tumors progression including cervical cancer. For the validation of this relationship between rs1800566 and progression of cervical cancer, the follow-up data is required.

Several study limitations should be addressed. First, the gene-environment interaction is various, we only performed 2 factors related to cervical cancer. Some other behavior factors should be considered in the future study such as parity and condom use. Second, considering the role of NQO1 gene in the many solid tumors, we assumed the NQO1 gene may affect the progression of cervical cancer. The follow-up data about the relationship between rs1800566 polymorphism and prognosis should be analyzed. Third, the present study explores the association between rs1800566 polymorphism and cervical cancer from epidemiology view and we could not provide data in vivo and vitro. Finally, selection bias may exist for case-control design.

5. Conclusions
In conclusion, NQO1 gene rs1800566 polymorphism is associated with elevated risk of cervical cancer in Chinese Han women. There is an interaction between rs1800566 polymorphism and some behavioral factors. A study with larger sample size is needed for the future studies and the molecular mechanism should be given attention.

Author contributions
LL conceived and designed the research; YSS ZJN analyzed the data; YSS created all tables; YSS drafted the manuscript; ZJJ and LL made critical revision of the manuscript; all authors read and approved the final manuscript.

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