The correlation between Aromatase (CYP19A1) and Bladder cancer in Chinese Han Population

CURRENT STATUS: UNDER REVIEW

Shufang Zhang
Haikou people's Hospital

Chong Zhang
People's Hospital of Hainan Province

Xiaohong Wen
Haikou people's hospital

Shunlan Wang
Haikou People's hospital

Haowei He
haikou people's hospital

Linlin Zheng
haikou people's hospital

Mei Chen
haikou peolpe's hospital

Fei Wang
People's Hospital of Hainan Province

Corresponding Author
feiwang1211@163.com
ORCiD: https://orcid.org/0000-0002-6258-6671

DOI: 10.21203/rs.3.rs-21779/v1

SUBJECT AREAS
Urology & Nephrology  Cancer Biology

KEYWORDS
Bladder cancer, Aromatase (CYP19A1), polymorphism
Abstract

Background

Several P450 metabolic enzymes genes have been discovered that affect the genetic susceptibility of bladder cancer. While, there is no report about CYP19A1 gene polymorphism on the susceptibility of bladder cancer. So, we performed a case-control study to investigate the association between polymorphisms in CYP19A1 gene and the susceptibility to bladder cancer in Chinese Han population.

Materials:

Five SNPs were selected and genotyped in 217 patients and 550 healthy controls. Odds ratio (OR) and 95% confidence intervals (CI) were calculated by logistic regression adjusted for age and gender.

Results

We found that CYP19A1 rs4646 “A” allele and rs17601876 “GA” genotype added the risk of bladder cancer (rs4646: OR = 1.28, 95%CI: 1.01 - 1.62, p = 0.043; rs17601876: OR= 1.64, 95%CI: 1.17 - 2.29, p = 0.004 ), while with rs6493487 “G” and rs1062033 “G” allele in CYP19A1 gene reduced the risk of bladder cancer (rs6493487: OR = 0.68, 95%CI: 0.51 - 0.89, p = 0.005; rs1062033: OR = 0.36, 95%CI: 0.28 - 0.47, p = 9.09E-16). WHO grade stratified analysis shown that rs6493487 (OR =0.42, 95%CI: 0.23 - 0.76, p = 0.004) and rs17601876 (OR =0.44, 95%CI: 0.23 - 0.85, p = 0.015) reduced the risk of bladder cancer with WHO III-IV, when compared with the bladder cancer with WHO I-II. Age stratified analysis shown that rs4646 “A”, rs6493487 “G”, and rs1062033 “G” allele were associated with bladder cancer risk.

Conclusion

Our results suggested that the Aromatase CYP19A1 gene has potential roles in the genetic susceptibility to bladder cancer.

Introduction

Bladder cancer, also known as urothelial cancer of the bladder, is the most common malignancy affecting the urinary system. Most cases occur in people over 60 years, the incidence of bladder cancer increases with age, the incidence of bladder cancer in males is significantly higher than that in females (1, 2). Currently, smoke and occupational exposure (aromatic compounds: benzidine and
beta-naphthylamine) have been identified as the most common environmental pathogens of bladder cancer (3–5). But still can't explain that some people will never get bladder cancer even if they are exposed to specific chemicals. Therefore, more and more people realized that the evolution of bladder cancer is a multi-gene, multi-factor participation process, which is the result of complex genetic and environmental factors.

In the human body, a variety of metabolic enzymes play an important role in the activation and inactivation of exogenous chemicals, thus, the genetic polymorphism of metabolic enzymes affects the occurrence of bladder cancer in individuals exposed to pathogenic factors. For example, one meta-analysis on CYP2E1 gene and bladder cancer, found that CYP2E1 gene polymorphisms might be a protective factor against bladder cancer in Asian people (6). In research of Turkish population revealed that the GSTT1 null genotype increased the risk of bladder cancer with OR of 3.08 (7). Two SNPs (rs2472299 and rs2198843) on CYP1A1 gene has been identified that increased the risk of bladder cancer (8). On the study of Spanish population provides strong evidence for the role of common CYP1B1 variants as risk factors for BC (9). In addition, the P540 genes that affect the risk of bladder cancer include CYP2D6 (10), CYP2C9 (11), and so on. While, there is no report about CYP19A1 gene polymorphism on the susceptibility of bladder cancer.

Cytochrome P450 19A1 (CYP19A1), also known as aromatase, encoded by the CYP19A1 gene, has a key role in estrogen biosynthesis, irreversible step in the synthesis of estrogens through mediating the conversion of androstenedione and testosterone to estrone and estradiol. Moreover, several studies have shown that expression of aromatase is correlated with tumorigenesis in prostate cancer (12), bladder cancer (13), etc. Thus, the CYP19A1 polymorphism may affect changes in estrogen levels and is associated with risk of bladder cancer.

So, we selected five SNPs on CYP19A1 gene to assess the association between CYP19A1 gene polymorphisms and bladder cancer in Chinese Han population from Hainan Province.

Materials And Methods
This study was approved by the Research Ethics Committee of the Haikou people’s Hospital in Haikou, and the study was performed in accordance with the World Medical Association Declaration of
A case-control study was performed to determine the association between polymorphisms of aromatase (CYP19A1) gene and BC. 217 BC cases (age 64.40 ± 10.99 years) and 550 age-matched healthy controls (age 63.92 ± 6.62 years) were enrolled from Haikou people’s Hospital. All BC cases were diagnosed by histological confirmation and were from outpatients of the Department of Urology. Also, we recruited controls from adult health examinations. The case group and the control group were all Han nationality and were living Haikou and surrounding areas, and there was no difference in the distribution of the land. Written informed consent was obtained from all study subjects before a questionnaire interview and biological specimen collection.

SNP selection and genotyping
By reviewing CYP19A1 and urinary system tumor related literature, we selected five SNPs on CYP19A1 gene (rs4646, rs6493487, rs1062033, rs17601876, and rs3751599) according to the principle that the minimum allele frequency was greater than 0.05, which were associated with tumor. The primers for related sites were designed according to Agena online software.

5 ml venous blood was taken and whole genome DNA was extracted. The single nucleotide polymorphisms rs4646, rs6493487, rs1062033, rs17601876, and rs3751599 were detected in the case group and the control group by MassARRAY time flight mass spectrometry array SNP genotyping platform, which consistent with the method of the previously published article (14-16).

Statistical analysis
Demographic characteristics were counted. The Hardy-Weinberg equilibrium (HWE) was calculated by χ² test (17). Five genetic models were used to evaluate the association between gene polymorphisms and breast cancer risk. Odds ratios (ORs) and its corresponding 95%CI were estimated using an logistic regression model with adjustments for age and gender through the PLINK software (18). Risk analysis of CYP19A1 and bladder cancer was also performed in different genetic models according the age stratification.

Results
Table 1 shows the basic information of the 767 participants, a total of 217 bladder cancer (64.40 ± 10.99 years) and 550 controls (63.92 ± 6.62) including in our research, and the mean age of the case
group and the control group is matched \( (p = 0.549) \). Of 217 patients with bladder cancer, 68 were classified as WHO I-II and 86 as WHO III-IV, and 63 patients unknown.

Table 2 shows the basic information of five gene polymorphisms on the CYP19A1 gene, including gene, position, alleles, SNP function annotations and HWE results. The results of the HWE showed that the genotype frequency distributions of CYP19A1 in the control groups was in line with genetic balance (all \( p > 0.05 \)), which showed that all of the 5 SNPs were at equilibrium and were representative.

We analysis the influence of CYP19A1 gene polymorphisms on the risk of bladder cancer by logistic regression (Table 3), found that CYP19A1 rs4646 A allele and AA-AC genotype promoted the risk of bladder cancer in the allele model \( (OR = 1.28, 95\%CI: 1.01-1.62, p = 0.043) \) and dominant model \( (OR = 1.59, 95\%CI: 1.15-2.20, p = 0.005) \). While, individuals with rs6493487 G allele and rs1062033 G allele in CYP19A1 gene had 32% and 64% reduction in risk of bladder cancer compared with individuals with reference allele in the allele model \( (rs6493487: OR = 0.68, 95\%CI: 0.51-0.89, p = 0.005; rs1062033: OR = 0.36, 95\%CI: 0.28-0.47, p = 9.09E-16) \). In the dominant model, individuals carrying the GA-AA genotype of CYP19A1 rs17601876 have a 39% higher risk of developing bladder cancer than GG genotype individuals \( (OR = 1.39, 95\%CI: 1.01-1.93, p = 0.045) \), and in the genotype model, individuals carrying the GA genotype of CYP19A1 rs17601876 have a 64% higher risk of developing bladder cancer than GG genotype individuals \( (OR = 1.64, 95\%CI: 1.17-2.29, p = 0.004) \), which have a risk effect against disease.

Then stratified analysis was conducted according to age (Table 4), and it was found that in the allele model, rs4646 A allele increased the risk of bladder cancer \( (OR = 1.42, 95\%CI: 1.03-1.95, p = 0.033) \) and rs6493487 G allele decreased the risk of bladder cancer \( (OR = 0.60, 95\%CI: 0.41-0.89, p = 0.011) \) in less than 65 years old group; while in more than 65 years old group, we did not find significant results. In less than 65 years old group and more than 65 years old group, we also found that rs1062033 G allele decreased the risk of bladder cancer in the allele model \( (\leq 65 \text{ years}: OR = 0.38, 95\%CI: 0.28-0.54, p = 1.13E-08; > 65 \text{ years}: OR = 0.35, 95\%CI: 0.23-0.52, p = 7.88E-08) \).

Then stratified analysis was conducted according to WHO grade (Table 4), we found that rs6493487
(OR = 0.42, 95%CI: 0.23–0.76, p = 0.004) and rs17601876 (OR = 0.44, 95%CI: 0.23–0.85, p = 0.015) reduced the risk of bladder cancer with WHO III-IV, when compared with the bladder cancer with WHO I-II.

**Discussion**

**Clinical Implications**

Several P450 metabolic enzymes genes have been discovered that affect the genetic susceptibility of bladder cancer. While, there is no report about CYP19A1 gene polymorphism on the susceptibility of bladder cancer. We performed a case-control study to investigate the association between polymorphisms in CYP19A1 gene and the susceptibility to bladder cancer. The result revealed that CYP19A1 rs4646 “A” allele and rs17601876 “GA” genotype increased the risk of bladder, while, CYP19A1 rs6493487 “G” allele and rs1062033 “G” allele reduced the risk of bladder cancer.

Aromatase is the last step-limiting enzyme in the synthesis of estrogen. Its quantity and activity directly determine the content of androgen and estrogen in the body. It belongs to the cytochrome P450 family and has the function of catalyzing the synthesis of estrogen. The aromatase is encoded by the CYP19A1 gene, the structure and activity of the aromatase protein can be affected by genetic variation, which can reduce, inactivate, or increase the enzyme activity. In 95 patients with breast cancer who received letrozole, patients with the CYP19A1 rs4646 A allele were found to be less effective than those with the C allele (19). In a study on the adverse metabolic effects of thiazides on hypertensive patients, CYP19A1 rs6493487 G allele association with glucose response to hydrochlorothiazide in meta-analysis of African-Americans (20). In our research, we found that CYP19A1 rs4646 “A” allele promoted the risk of bladder cancer, and rs4646 located on 3’UTR. SNPs located in the 3’-UTRs of genes may affect the expression of the gene by reinforcing, weakening or disrupting the miRNA-mRNA interaction, and thereby confer the individual’s disease risk (21). Nguyen DP et al. found that Aromatase expression was significantly associated with bladder cancer tumor stage, and Cox regression analysis demonstrated that aromatase expression was associated with a more than 2-fold risk of cancer recurrence (HR = 2.37) (13). After this, Shulin Wu et al. also found that in 88 bladder cancer cases examined, high aromatase expression was present in 37.5% and 73.9% of
Tumor epithelium (TE) and tumor related stroma (TS) respectively, and high aromatase expression in TS was significantly associated with poorer overall survival (22). Those remind us aromatase (CYP19A1) plays an important role in the development of bladder cancer. And the risk allele of rs4646 may influence the expression of aromatase (CYP19A1), and increased the risk of bladder cancer. However, its specific molecular mechanism is unknown, and our subsequent further research will clearly define the mechanism and provide a basis for early diagnosis of bladder cancer.

rs17601876 “GA” genotype increased the risk of bladder. In rectal cancer, GG genotype of rs17601876 increased the mortality risk (HR = 2.6) (23). Besides that rs6493487 G allele and rs1062033 G allele in CYP19A1 gene reduced the risk of bladder cancer. These sites are located in the intron region of the CYP19A1 gene. Introns can increase transcript levels by affecting the rate of transcription, nuclear export, and transcript stability. Moreover, introns can also increase the efficiency of mRNA translation (24). However, the specific mechanism of rs17601876, rs6493487 and rs1062033 on the CYP19A1 gene affecting bladder cancer is unclear.

Study Limitations
In summary, our research report that CYP19A1 rs4646 and rs17601876 increased the risk of bladder, and CYP19A1 rs6493487 and rs1062033 reduced the risk of bladder cancer. The interaction between genes and the environment and the molecular mechanisms of genes in bladder cancer have not been studied, which will be the focus of our follow-up research.

Conclusion
In summary, our research report that CYP19A1 rs4646 and rs17601876 increased the risk of bladder, and CYP19A1 rs6493487 and rs1062033 reduced the risk of bladder cancer. The interaction between genes and the environment and the molecular mechanisms of genes in bladder cancer have not been studied, which will be the focus of our follow-up research.

Declarations
Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable
Availability of data and material

Data sharing not applicable to this article as no data-sets were generated or analyzed during the current study.

Competing interests

The authors declare that they have no competing interests.

Funding

Hainan natural fund innovation research team project[2017CXTD010. National natural science foundation of China: 81760465. Key r&d projects in hainan province: ZDYF2019163

Authors’ contributions

Shufang Zhang and Chong Zhang contributed equally to this work. All authors read and approved the manuscript.

Acknowledge

Conflict of interest

The authors declare no conflict of interest.

References

1. Bladder cancer. diagnosis and management of bladder cancer: (c) NICE (2015)
   Bladder cancer: diagnosis and management of bladder cancer. BJU Int. 2017;120(6):755–65.
2. Martinez Rodriguez RH, Buisan Rueda O, Ibarz L. Bladder cancer: Present and future. Med Clin (Barc). 2017;149(10):449–55.
3. Cohen SM, Shirai T, Steineck G. Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol Suppl. 2000(205):105–15.
4. Kiriluk KJ, Prasad SM, Patel AR, Steinberg GD, Smith ND. Bladder cancer risk from occupational and environmental exposures. Urol Oncol. 2012;30(2):199–211.
5. Pavanello S, Carta A, Mastrangelo G, Campisi M, Arici C, Porru S. Relationship between Telomere Length, Genetic Traits and Environmental/Occupational Exposures
in Bladder Cancer Risk by Structural Equation Modelling. Int J Environ Res Public Health. 2017;15(1).

6. Yin X, Xiong W, Wang Y, Tang W, Xi W, Qian S, et al. Association of CYP2E1 gene polymorphisms with bladder cancer risk: A systematic review and meta-analysis. Med (Baltim). 2018;97(39):e11910.

7. Berber U, Yilmaz I, Yilmaz O, Haholu A, Kucukodaci Z, Ates F, et al. CYP1A1 (Ile462Val), CYP1B1 (Ala119Ser and Val432Leu), GSTM1 (null), and GSTT1 (null) polymorphisms and bladder cancer risk in a Turkish population. Asian Pac J Cancer Prev. 2013;14(6):3925–9.

8. Grando JP, Kuasne H, Losi-Guembarovski R, Sant’ana Rodrigues I, Matsuda HM, Fuganti PE, et al. Association between polymorphisms in the biometabolism genes CYP1A1, GSTM1, GSTT1 and GSTP1 in bladder cancer. Clinical experimental medicine. 2009;9(1):21–8.

9. Salinas-Sanchez AS, Donate-Moreno MJ, Lopez-Garrido MP, Gimenez-Bachs JM, Escribano J. Role of CYP1B1 gene polymorphisms in bladder cancer susceptibility. J Urol. 2012;187(2):700–6.

10. Sobti RC, Al-Badran Al, Sharma S, Sharma SK, Krishan A, Mohan H. Genetic polymorphisms of CYP2D6, GSTM1, and GSTT1 genes and bladder cancer risk in North India. Cancer Genet Cytogenet. 2005;156(1):68–73.

11. Fortuny J, Kogevinas M, Garcia-Closas M, Real FX, Tardon A, Garcia-Closas R, et al. Use of analgesics and nonsteroidal anti-inflammatory drugs, genetic predisposition, and bladder cancer risk in Spain. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2006;15(9):1696–702.

12. Grindstad T, Skjefstad K, Andersen S, Ness N, Nordby Y, Al-Saad S, et al. Estrogen
receptors alpha and beta and aromatase as independent predictors for prostate
cancer outcome. Scientific reports. 2016;6:33114.

13. Nguyen DP, O'Malley P, Al Hussein Al Awamlh B, Furrer MA, Mongan NP, Robinson BD, 
et al. Association of Aromatase With Bladder Cancer Stage and Long-Term Survival:
New Insights Into the Hormonal Paradigm in Bladder Cancer. Clin Genitourin Cancer.
2017;15(2):256 - 62.e1.

14. Thomas RK, Baker AC, Debiasi RM, Winckler W, Laframboise T, Lin WM, et al. High-
throughput oncogene mutation profiling in human cancer. Nat Genet.
2007;39(3):347-51.

15. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY
iPLEX platform. Current protocols in human genetics. 2009;Chap. 2:Unit 2.12..

16. Guo T, Hao H, Zhou L, Zhou F, Yu D. Association of SNPs in the TIMP-2 gene and large
artery atherosclerotic stroke in southern Chinese Han population. Oncotarget.
2018;9(4):4698–706.

17. Adamec C, [EXAMPLE, OF THE USE OF THE NONPARAMETRIC TEST. TEST X2 FOR
COMPARISON OF 2 INDEPENDENT EXAMPLES]. Ceskoslovenske zdravotnictvi.
1964;12:613–9.

18. Bland JM, Altman DG. Statistics notes. The odds ratio. BMJ. 2000;320(7247):1468.

19. Garcia-Casado Z, Guerrero-Zotano A, Llombart-Cussac A, Calatrava A, Fernandez-
Serra A, Ruiz-Simon A, et al. A polymorphism at the 3'-UTR region of the aromatase
gene defines a subgroup of postmenopausal breast cancer patients with poor
response to neoadjuvant letrozole. BMC Cancer. 2010;10:36.

20. Del-Aguila JL, Beitelishees AL, Cooper-Dehoff RM, Chapman AB, Gums JG, Bailey K, et
al. Genome-wide association analyses suggest NELL1 influences adverse metabolic
response to HCTZ in African Americans. Pharmacogenomics J. 2014;14(1):35–40.
21. Kertesz M, Iovino N, Unnerstall U, Gaul U, Segal E. The role of site accessibility in microRNA target recognition. Nat Genet. 2007;39(10):1278-84.

22. Wu S, Ye J, Wang Z, Lin SX, Lu M, Liang Y, et al. Expression of aromatase in tumor related stroma is associated with human bladder cancer progression. Cancer Biol Ther. 2018;19(3):175-80.

23. Slattery ML, Lundgreen A, Herrick JS, Kadlubar S, Caan BJ, Potter JD, et al. Variation in the CYP19A1 gene and risk of colon and rectal cancer. Cancer Causes Control. 2011;22(7):955-63.

24. Shaul O. How introns enhance gene expression. The international journal of biochemistry & cell biology. 2017;91(Pt B):145 – 55.

Tables

Table 1 The information of all participants

| Variable          | Cases (217) | Control (550) | p value |
|-------------------|-------------|---------------|---------|
| Age               | Mean ± SD, years | 64.40 ±10.99  | 63.92 ± 6.62 | 0.549 |
|                   | >65         | 103(47%)      | 197(36%) |
|                   | ≤65         | 114(53%)      | 353(64%) |
| Gender            | Male        | 175(81%)      | 379(69%) |
|                   | Female      | 42(19%)       | 171(31%) |
| WHO grade         | I-II        | 68(31%)       | 86(40%) |

* p< 0.05 indicates statistical significance
Table 2 The information of five gene polymorphisms on the CYP19A1 gene

| SNP      | Chromosome | Position     | Alleles | Gene    | MAF-Ci |
|----------|------------|--------------|---------|---------|--------|
| rs4646   | 15         | 51210647     | G/T     | CYP19A1 | 0.34   |
| rs6493487| 15         | 51221532     | A/G     | CYP19A1 | 0.20   |
| rs1062033| 15         | 51255741     | C/G     | CYP19A1 | 0.23   |
| rs17601876| 15       | 51261712     | A/G     | CYP19A1 | 0.34   |
| rs3751599| 15         | 51281336     | C/T     | CYP19A1 | 0.07   |

MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium

*p < 0.05 indicates statistical significance

Table 3 Risk analysis of CYP19A1 and bladder carcinoma in different genetic models by logistic regression analysis

| SNP      | Model            | Allele/Genotype | Case | Control | OR (95%CI) |
|----------|------------------|-----------------|------|---------|------------|
| rs4646   | Allele model     | C               | 285  | 779     | 1          |
|          |                  | A               | 149  | 319     | 1.28 (1.01 - 1.59) |
|          | Genotype model   | CC              | 85   | 276     | 1          |
|          |                  | AC              | 115  | 227     | 1.68 (1.20 - 2.35) |
|          |                  | AA              | 17   | 46      | 1.17 (0.63 - 2.15) |
|          | Dominant model   | CC              | 85   | 276     | 1          |
|          |                  | AC-AA           | 132  | 273     | 1.59 (1.15 - 2.20) |
|          | Recessive model  | CC-AC           | 200  | 503     | 1          |
| SNP          | Allele model | Genotype model | Dominant model | Recessive model | Log-additive model |
|--------------|--------------|----------------|----------------|----------------|--------------------|
| rs6493487    | A            | AA: 17, 46    | AA: 138, 289   | AA: 138, 289   | 0.68 (0.51 - 0.89) |
|              |              | G: 85, 300    |                | AG-GG: 71, 258 | 0.56 (0.40 - 0.77) |
|              |              |                |                |                | 0.35 (0.26 - 0.45) |
| rs1062033    | C            | CC: 129, 158  | CC: 129, 158   | CC: 129, 158   | 0.36 (0.28 - 0.47) |
|              |              | G: 99, 496    |                | CG-GG: 87, 391 | 0.27 (0.19 - 0.38) |
|              |              |                |                |                | 0.32 (0.23 - 0.42) |
|              |              |                |                |                | 0.14 (0.07 - 0.23) |
| rs17601876   | G            | GG: 82, 252   | GG: 82, 252    | GG: 82, 252    | 0.25 (0.13 - 0.38) |
|              |              | A: 147, 366   |                |                | 0.55 (0.28 - 0.57) |
|              |              |                |                |                | 1.03 (0.81 - 1.28) |
|              |              |                |                |                | 1.64 (1.17 - 2.28) |
|              |              |                |                |                | 0.55 (0.28 - 0.57) |
### Table 4 Risk analysis of CYP19A1 and bladder carcinoma in different genetic models according the age stratification

| SNP      | Model                | Allele/Genotype | Age > 65 years | OR (95%CI)       |
|----------|----------------------|-----------------|----------------|------------------|
|          |                      |                 | Case | Control |                   |
| rs4646   | Allele model         | C               | 135  | 264     | 1 (1.01 - 1.03)   |
|          |                      | A               | 71   | 130     | 1.07 (0.75 - 1.53) |
|          | Genotype model       | CC              | 41   | 93      | 1 (1.03 - 1.03)   |
|          |                      | AC              | 53   | 78      | 1.64 (0.97 - 2.79) |
|          |                      | AA              | 9    | 26      | 0.87 (0.36 - 2.10) |
|          | Dominant model       | CC              | 41   | 93      | 1 (1.01 - 1.03)   |
|          |                      | AC-AA           | 62   | 104     | 1.46 (0.88 - 2.42) |

- **p** < 0.05 indicates statistical significance

| Model                | Allele model | Genotype model | Dominant model | Recessive model | Log-additive model |
|----------------------|--------------|----------------|----------------|-----------------|-------------------|
| SNP                  |              |                |                |                 |                   |
| rs3751599            | G            | A              |                |                 |                   |
| Allele model         | 403          | 1018           | 1              |                 |                   |
| Genotype model       | GG           | GA             | AA             |                 |                   |
|                      | 186          | 74             | 26             |                 |                   |
| Dominant model       | GG           | GA-AA          | AA             |                 |                   |
|                      | 186          | 78             | 4              |                 |                   |
| Recessive model      | GG-GA        | AA             |                 |                 |                   |
|                      | 217          | 4              |                |                 |                   |
| Log-additive model   | -            | -              | -              |                 | 1.03 (0.81 - 1.03) |

- GA-AA: 135 (OR: 1.39, 95% CI: 1.01 - 1.93)
- GG-GA: 205 (OR: 1, 95% CI: 1 - 1)
- AA: 12 (OR: 0.42, 95% CI: 0.22 - 0.80)
- - (OR: 1.03, 95% CI: 0.81 - 1.32)
- - (OR: 0.98, 95% CI: 0.64 - 1.51)

- Log-additive model
- Allele model
- Genotype model
- Dominant model
- Recessive model
- Log-additive model

**p** < 0.05 indicates statistical significance.
| Allele model | rs6493487 | Allele model | rs1062033 | Allele model | rs17601876 |
|--------------|-----------|--------------|-----------|--------------|-----------|
| Recessive model | CC-AC | 94 | 171 | 1 |
| | AA | 9 | 26 | 0.67 (0.29 - 1.56) |
| Log-additive model | - | - | - | 1.13 (0.78 - 1.64) |
| Genotype model | A | 148 | 267 | 1 |
| | G | 48 | 125 | 0.69 (0.47 - 1.02) |
| Dominant model | AA | 57 | 92 | 1 |
| | AG | 34 | 83 | 0.73 (0.43 - 1.25) |
| | GG | 7 | 21 | 0.61 (0.23 - 1.59) |
| Recessive model | AA-AG | 91 | 175 | 1 |
| | GG | 7 | 21 | 0.69 (0.27 - 1.77) |
| Log-additive model | - | - | - | 0.76 (0.51 - 1.13) |
| rs1062033 | C | 164 | 227 | 1 |
| | G | 42 | 167 | 0.35 (0.23 - 0.52) |
| Genotype model | CC | 66 | 68 | 1 |
| | CG | 32 | 91 | 0.38 (0.22 - 0.65) |
| | GG | 5 | 38 | 0.13 (0.05 - 0.37) |
| Dominant model | CC | 66 | 68 | 1 |
| | CG-GG | 37 | 129 | 0.31 (0.18 - 0.51) |
| Recessive model | CC-CG | 98 | 159 | 1 |
| | GG | 5 | 38 | 0.2 (0.07 - 0.55) |
| Log-additive model | - | - | - | 0.37 (0.24 - 0.56) |
| rs17601876 | G | 135 | 251 | 1 |
| | A | 71 | 143 | 0.92 (0.65 - 1.31) |
| Genotype model | GG | 39 | 86 | 1 |
| | GA | 57 | 79 | 1.60 (0.94 - 2.71) |
| | AA | 7 | 32 | 0.50 (0.19 - 1.28) |
| Dominant model | GG | 39 | 86 | 1 |
| | GA-AA | 64 | 111 | 1.29 (0.78 - 2.15) |
| Recessive model | GG-GA | 96 | 165 | 1 |
| | AA | 7 | 32 | 0.39 (0.16 - 0.95) |
Table 5: Risk analysis of CYP19A1 and bladder carcinoma in different genetic models according to the WHO grade stratification

| SNP    | Model                  | Allele/Genotype | WHO III-IV | WHO I-II | OR (95%CI) |
|--------|------------------------|-----------------|------------|----------|------------|
| rs4646 | Allele model           | C               | 112        | 81       | 1          |
|        |                        | A               | 60         | 55       | 0.79 (0.5 - 1.2) |
|        | Genotype model         | CC              | 30         | 20       | 1          |
|        |                        | AC              | 52         | 41       | 0.79 (0.38 - 1.0) |
|        |                        | AA              | 4          | 7        | 0.37 (0.09 - 1.0) |
|        | Dominant model         | CC              | 30         | 20       | 1          |
|        |                        | AC-AA           | 56         | 48       | 0.72 (0.36 - 1.0) |
|        | Recessive model        | CC-AC           | 82         | 61       | 1          |
|        |                        | AA              | 4          | 7        | 0.43 (0.12 - 1.0) |
|        | Log-additive model     | -               | -          | -        | 0.68 (0.39 - 1.0) |
| rs6493487 | Allele model     | A               | 145        | 98       | 1          |
|         |                        | G               | 21         | 34       | 0.42 (0.23 - 0.62) |
|         | Genotype model         | AA              | 63         | 40       | 1          |
|         |                        | AG              | 19         | 18       | 0.6 (0.28 - 1.2) |

p < 0.05 indicates statistical significance
| rs1062033 | Allele model | G     | 143 | 114 | 1   |
|-----------|--------------|-------|-----|-----|-----|
|           | C            | 29    | 20  | 1.16 (0.62 - 2.)  |
| Genotype  | CC           | 60    | 48  | 1   |
|           | CG           | 23    | 18  | 1.19 (0.56 - 2.)  |
|           | GG           | 3     | 1   | 3.64 (0.34 - 39)  |
| Dominant  | CC           | 60    | 48  | 1   |
|           | CG-GG        | 26    | 19  | 1.29 (0.62 - 2.)  |
| Recessive | CC-CG        | 83    | 66  | 1   |
|           | GG           | 3     | 1   | 3.39 (0.32 - 35)  |
| Log-additive model | - | - | - | 1.36 (0.71 - 2.) |

| rs17601876 | Allele model | G     | 120 | 82  | 1   |
|-----------|--------------|-------|-----|-----|-----|
|           | A            | 52    | 54  | 0.66 (0.41 - 1.)  |
| Genotype  | GG           | 34    | 18  | 1   |
|           | GA           | 52    | 46  | 0.55 (0.27 - 1.)  |
|           | AA           | 0     | 4   | / |
| Dominant  | GG           | 34    | 18  | 1   |
|           | GA-AA        | 52    | 50  | 0.51 (0.25 - 1.)  |
| Recessive | GG-GA        | 86    | 64  | 1   |
|           | AA           | 0     | 4   | / |
| Log-additive model | - | - | - | 0.44 (0.23 - 0) |

| rs3751599 | Allele model | G     | 158 | 126 | 1   |
|-----------|--------------|-------|-----|-----|-----|
|           | A            | 14    | 10  | 1.12 (0.48 - 2)  |
| Genotype  | GG           | 72    | 58  | 1   |
|           | GA           | 14    | 10  | / |
|           | AA           | 0     | 0   | / |
| Dominant  | GG           | 158   | 126 | 1   |
|           | GA-AA        | 14    | 10  | 1.22 (0.5 - 3)  |
| Recessive | GG-GA        | 86    | 68  | 1   |
\begin{tabular}{c c c c c}
 & AA & 0 & 0 & /
\hline
Log-additive model & - & - & - & 1.22 (0.5 - 3)
\end{tabular}

\textit{p} < 0.05 indicates statistical significance