**Relationship of oestrogen receptor alpha gene polymorphisms with risk for benign prostatic hyperplasia and prostate cancer in Chinese men**

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**Abstract**

The relationship of oestrogen receptor with benign prostatic hyperplasia (BPH) and prostate cancer (PC) is not clear at present. This study aimed to investigate the molecular mechanism underlying the occurrence and development of BPH and prostate.

Two hundred forty-four PC cases, 260 BPH patients, and 222 healthy men were recruited from Han people in China, and the oestrogen receptor alpha (ESR\(_a\)) gene polymorphism (rs2234693 [PvuII] and rs9340799 [XbaI]) on intron 1 was determined. The relationship of gene polymorphism with PC and BPH was evaluated with Logistic regression, and the linkage disequilibrium and haplotyping were assessed with SHEsis software.

The risk for PC in BPH patients with PvuII C allele was higher (OR = 1.437, 95% CI: 1.110–1.859), but the differentiation degree of cancer cells was relatively better in PC patients with PvuII C allele (OR = 0.419, 95% CI: 0.285–0.616), and most of them were circumscribed (OR = 0.706, 95% CI: 0.485–1.02). There was significant linkage disequilibrium between PvuII and XbaI. The genotype TTAG not only induced BPH (OR = 6.260, 95% CI: 1.407–27.852), but increased the risk for PC (OR = 6.696, 95% CI: 1.504–29.801). However, the genotype TTAG in BPH patients had no relationship with the risk for PC (P = 0.05).

Furthermore, men with haplotype TG were more likely to suffer PC (OR = 9.168, 95% CI: 2.393–35.119), but men with haplotype TA and enlarged prostate had a low risk for PC (OR = 0.708, 95% CI: 0.551–0.912).

These results show the relationship between ESR\(_a\) gene polymorphism and susceptibility to PC and BPH in Chinese men, and the ethnic and regional difference as well.

**Abbreviations:** BPH = benign prostatic hyperplasia. CI = confidence interval. ESR = oestrogen receptor. OR = odd ratio. PC = prostate cancer. PSA = prostate-specific antigen. SNP = single nucleotide polymorphisms.

**Keywords:** benign prostatic hyperplasia, gene polymorphisms, oestrogen receptor

**1. Introduction**

Prostate cancer (PC) is a common malignancy of male urinary system and its incidence varies significantly between ethnic groups and regions worldwide. The statistics from American Association for Cancer Research showed there were 241,740 new cases of PC and 28,170 patients died of PC in 2012. Among male tumors, PC is the second most common and its incidence in African Americans (241/100,000) is far higher than in Caucasians (149/100,000). In Asia, its incidence is relatively low (21/100,000 in Shanghai, China in 2005). However, the incidence of PC is increasing over year. Though a variety of studies have been conducted to investigate the etiology of PC, the molecular mechanism underlying its pathogenesis is still poorly understood.

Considering the similarity in the incidence between PC and benign prostatic hyperplasia (BPH) in the field of morbidity physiology, BPH may be an alarm signal of PC in the early period\(^{[1–3]}\). However, BPH and PC are 2 absolutely separate diseases, and epidemiological studies fail to show the significant relationship between BPH and PC either.\(^{[4,5]}\) Generally, PC is derived from BPH.\(^{[6]}\) Thus, to deeply investigate the epidemiological features of BPH will be helpful for the illustration of the pathogenesis of PC and BPH.

In recent years, studies have reported that oestrogen and its receptor play important roles in the etiology of PC and BPH.\(^{[7–9]}\) About 30% of oestrogen in males is directly released by the sertoli cells of the testes, and 70% is as a result of conversion of androgen released by the adrenal gland and testes under the catalysis of aromatase. Thereafter, studies indicate that, age brings a gradual diminution of testes which causes a decrease of blood testosterone, but the oestrogen remains at the same level over age, leading to the increase in the ratio of oestrogen to androgen. Thus, oestrogen has been regarded as a major pathogenic factor of BPH and PC.\(^{[10–12]}\) Oestrogen regulates and controls the growth and proliferation of prostatic cells by binding to the specific intranuclear receptor, oestrogen receptor (ESR).\(^{[13]}\) ESR is one of the members of nuclear factor superfamily. After binding, the receptor is activated. ESR is divided into 2 types: ESR-\(_\alpha\) and ESR-\(_\beta\). ESR-\(_\alpha\) locates in the gap
of epithelial and basal membrane of the prostate, and ESR-β locates between epithelial gaps of the prostate. Immunohistochemistry shows that, though ESRRα is not expressed in epithelium of the normal prostate, it is strongly expressed in the prostate cells of BPH, PC tissues, LNCaP, and JCA-1 cells, which implies that ESRRα has essential relationship with the occurrence of BPH and PC. Thus, to investigate the oestrogen acceptor may be helpful to elucidate the molecular mechanism of BPH and PC to a certain extent.

At present, the association of ESRRα gene polymorphism with the risk for PC and BPH has not been reported in Chinese patients. In the present study, PCR-RFLP was employed to detect the single nucleotide polymorphisms (SNP) of ESRRα gene (rs2234693 [PvuII] and rs9340799 [XbaI]) at intron 1, and their relationship with PC and BPH was further evaluated. Our results showed that, the unit point gene or allelic genes, composite genes had a relationship with the occurrence and development of PC and BPH in Chinese patients. Therefore, our findings explain the differences of PC and BPH among ethnics and regions to a certain extent and provide evidence for the molecular mechanism underlying the pathogenesis of PC and BPH.

2. Methods

2.1. Subjects

A total of 244 PC patients, 260 BPH patients, and 222 healthy men were recruited between January 2012 and December 2014 in Zhejiang province and the peripheral blood was sampled. The age ranged from 47 to 90 years (median: 71.77 years) in PC patients, from 52 to 89 years (median: 71.28 years) in BPH patients, and from 48 to 83 years (median: 66.61 years) in healthy men. The diagnosis of PC and BPH was all confirmed by pathological results of the resected specimen (Table 1). According to TNM staging system developed by the American Joint Committee on Cancer Staging, PC was classified as nonmetastatic and metastatic PC. The pathological grade of PC was evaluated with Gleason score: 2 to 6, intermediarily or well differentiated adenocarcinoma; 7 to 10, poorly differentiated adenocarcinoma. For healthy men, the serum prostate-specific antigen (PSA) was <4.0 μg/mL, prostate was normal as shown by ultrasound examination and there were no clinical manifestations of PC and BPH.

The whole study was approved and authorized by the Ethical Committee of Luqiao Division in Affiliated Taizhou Hospital of Wenzhou Medical College, Taizhou City. Written informed consent was obtained from each subject before recruitment.

2.2. Genotyping

Whole Blood Genome Extraction Kit (Fastagen Biotech, Shanghai, China) was employed to extract genomic DNA from peripheral blood, which was then restored at 4°C. Following primers were used: PvuII: Forward: 5'-CTG CCA CCC TAT CTC TAT CTT TTC TCT TTC CTA TTC TTC TCC-3', Reverse: 5'-TCT TTC TCT GCC ACC CTC GG CAG TCG ATT ATC TGA-3'; XbaI: Forward: 5'-CTG CCA CCC TAT CTC TAT CTT TTC TCT CTA TTC TCC-3', Reverse: 5'-TCT TTC TCT GCC ACC CTC GG CAG TCG ATT ATC TGA-3'. The reaction mixture of PCR included 5 μL of 10× Ex Taq Buffer, 4 μL of 25mM MgCl2, 4.0 μL of 2.5 mM dNTP, 0.5 μL of 10mM primers, 80 to 200 ng of DNA template, 0.2 μL of 5U/μL Ex Taq DNA polymerases (Ftemenes), and double distilled water (total volume: 50μL). The conditions of PCR were as follows: pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing for 45 seconds, 72°C for 60 seconds, and a final extension at 72°C for 5 minutes; the concentration and purity of PCR products were determined after 2.0% agarose gel electrophoresis. Then, 10 μL of PCR products was digested with restriction enzymes for 4 hours, followed by 2.0% agarose gel electrophoresis. Sequencing verification of genotype results will be done by randomization. For quality control, samples (100) were randomly selected for validation by genotyping and sequencing. The variation of T and C bases occurs in PvuII, while the variation of A and G bases occurs in XbaI. In the present study, only wild homozygous genes

| Table 1  | Clinical and demographic characteristics of participants at baseline. |
|----------|-----------------------------------------------------------------------|
|          | PC (n = 244) BPH (n = 260) Controls (n = 222) | PC vs controls | BPH vs controls |
| Ages at diagnosis, y | 71.89±8.03 72.29±7.86 66.61±7.70 | <0.001 | <0.001 |
| <60 | 22 (9.0%) 22 (8.5%) 28 (12.6%) | | |
| 60-69 | 66 (27.1%) 84 (32.3%) 108 (46.8%) | | |
| 70-79 | 112 (45.9%) 118 (45.4%) 76 (34.2%) | | |
| ≥80 | 44 (18.0%) 36 (13.8%) 10 (4.5%) | | |
| Smoking status | | | |
| Nonsmoker | 160 (65.6%) 154 (59.2%) 144 (64.9%) | 0.969 (0.662–1.420) 0.872 | 1.271 (0.877–1.840) 0.204 |
| Smoker | 84 (34.4%) 106 (40.8%) 78 (35.1%) | | |
| Drinking status | | | |
| No drinking | 120 (49.2%) 124 (47.7%) 113 (50.9%) | 1.071 (0.745–1.541) 0.711 | 1.137 (0.795–1.627) 0.480 |
| Drinking | 124 (50.8%) 136 (52.3%) 109 (49.1%) | | |
| Gleason score | | | |
| <7 | 86 (35.2%) | | |
| ≥7 | 158 (64.8%) | | |
| TNM stage | | | |
| Localized | 146 (59.8%) | | |
| Aggressive | 98 (40.2%) | | |

BPH = benign prostatic hyperplasia, CI = odd ratio, OR = confidence interval, PC = prostate cancer, TNM = tumor node metastasis.

Data are expressed as mean± standard deviation (SD), and compared using unpaired t test.

χ² test.
were used as reference genotypes (PvuII TT and XbaI AA) for comparison.

2.3. Statistical analysis

SPSS version 13.0 was used for statistical analysis, and Hardy–Weinberg equilibrium was used to evaluate the reliability of collected information. Logistic regression was employed to analyze the frequency of genotype and alleles, and then the odd ratio (OR) and 95% confidence interval (CI) were calculated after adjustment for age. All tests of significance were based on a two-tailed test.

The frequencies of each ESRα genotype in the collected case group and control group accorded with Hardy–Weinberg equilibrium (P > 0.05). Genotyping showed that the frequency of TT, CT, and CC genotypes of PvuII was 38.5% (94/244), 41.8% (102/244), and 19.7% (48/244), respectively, in PC patients, and 46.2% (120/244), 43.1% (112/260), and 10.8% (28/260), respectively, in BPH patients, and 41.4%, 43.2%, and 15.3%, respectively, in healthy controls (Table 2).

The relationship of each genotype of ESRα gene with pathological grades and clinical stages was further evaluated. Results showed that, compared with wild homozygous TT genotype of PvuII, mutant CC and CT genotypes of C allele were seldom found in poorly differentiated adenocarcinoma patients with the OR of 0.388 (0.204–0.740) and 0.211 (0.098–0.454), respectively, after adjustment for age (Table 3). Compared with T allele, C allele was also seldom observed in poorly differentiated PC patients (OR = 0.419, 95% CI: 0.283–0.616, P < 0.001). Additionally, the comparison between mutant CC and TT genotypes of PvuII showed patients with mutant CC genotype seldom developed metastasis to other sites (OR = 0.499, 95% CI: 0.278–0.894, P = 0.020). However, there was no relationship of XbaI SNP with pathological grades and clinical stages (P > 0.05).

Considering the distinct linkage disequilibrium between PvuII and XbaI of ESRα in control group (D* = 0.958, r² = 0.398),
composite and haplotype genes were further analyzed (Table 4). Results showed that, compared with healthy men (0.9%), the frequency of TTAGI was significantly higher in PC patients (5.7%, OR=6.696, 95% CI: 1.504–29.801, P=0.004) and BPH patients (5.4%, OR=6.260, 95% CI: 1.407–27.832, P=0.006), which indicates that TTAGI increases the risk for PC and BPH. The frequency of TG haplotype in PC patients was 4.7% compared with healthy controls (OR, 3.372 [1.316–8.570], P=0.006). Similarly, compared with TT genotype, BPH patients with heterozygous mutant CT genotype of PvuII had a higher risk for PC (OR=2.199, 95% CI: 1.283–3.770, P=0.004). Moreover, the frequency of CCAA haplotype in PC group was 7.4%, which was higher than in BPH group (2.3%), indicating that it has a high risk for PC (OR, 3.372 [1.316–8.641]). Analysis with healthy controls (OR=9.168, 95% CI: 2.393–35.119, P<0.001).

### Table 4
Multi-genotypes and haplotype frequencies for ESR-α polymorphisms in PC patients, BPH patients and healthy controls.

| Multi-genotypes | Cases (freq) | Controls (freq) | OR (95% CI) | P value | Cases (freq) | Controls (freq) | OR (95% CI) | P value |
|-----------------|-------------|----------------|-------------|---------|-------------|----------------|-------------|---------|
| **Prostate cancer vs controls** | | | | | | | | |
| CCGS | 8 (3.3%) | 10 (4.5%) | 0.719 (0.278–2.855) | 0.493 | 222 | 260 | - | - |
| CCAG | 22 (9.0%) | 16 (7.2%) | 1.276 (0.652–2.497) | 0.476 | 16 (6.2%) | 16 (6.2%) | 0.844 (0.412–1.730) | 0.643 |
| CCAA | 18 (7.4%) | 8 (3.6%) | 2.131 (0.907–5.002) | 0.076 | 8 (3.6%) | 8 (3.6%) | 0.632 (0.216–1.850) | 0.398 |
| CTXX | 4 (1.6%) | 0 (0.0%) | - | 0.055 | 0 (0.0%) | 0 (0.0%) | - | - |
| CTAAG | 48 (19.7%) | 52 (23.4%) | 0.801 (0.514–1.247) | 0.325 | 46 (17.7%) | 52 (23.4%) | 0.703 (0.450–1.096) | 0.119 |
| CTAAG | 50 (20.5%) | 44 (19.8%) | 1.043 (0.663–1.641) | 0.857 | 64 (24.6%) | 44 (19.8%) | 1.321 (0.856–2.039) | 0.208 |
| TTGG | 0 (0.0%) | 0 (0.0%) | - | 2 (8.8%) | 0 (0.0%) | 2 (8.8%) | - | - |
| TTAG | 14 (5.7%) | 2 (0.9%) | 6.696 (1.504–29.801) | 0.004 | 14 (5.4%) | 2 (0.9%) | 6.260 (1.407–27.852) | 0.006 |
| TTAAG | 80 (32.8%) | 90 (40.5%) | 0.715 (0.430–1.045) | 0.082 | 104 (40.0%) | 90 (40.5%) | 0.978 (0.679–1.409) | 0.004 |
| **BPH vs controls** | | | | | | | | |

Haplotypes

| Haplotype | Cases (freq) | Controls (freq) | OR (95% CI) |
|-----------|-------------|----------------|-------------|
| TG | 22.91 (0.047) | 2.37 (0.005) | 9.168 (2.393) |
| TA | 267.09 (0.547) | 327.87 (0.631) | 0.708 (0.551) |
| CG | 85.09 (0.174) | 71.87 (0.138) | 1.317 (0.936) |
| CA | 112.91 (0.231) | 96.13 (0.185) | 1.327 (0.978) |

**Table 5**
Frequencies of ESR-α genotypes, alleles, and haplotypes in BPH and PC patients.

| SNPs | PC (freq) | BPH (freq) | OR (95% CI) | P value |
|------|-----------|------------|-------------|---------|
| Prull | TT | 94 (38.5%) | 120 (46.2%) | 1.00 (ref) |
| CT | 102 (41.8%) | 112 (43.1%) | 2.199 (1.283–3.770) | 0.004 |
| CC | 48 (19.7%) | 28 (10.8%) | 1.172 (0.800–1.716) | 0.415 |
| T | 200 (59.4%) | 352 (67.7%) | 1.00 (ref) |
| C | 198 (40.6%) | 168 (32.3%) | 1.437 (1.110–1.859) | 0.006 |
| Xbal | AA | 148 (60.7%) | 174 (69.0%) | 1.00 (ref) |
| AG | 84 (34.4%) | 76 (29.0%) | 1.473 (0.614–3.533) | 0.385 |
| GG | 12 (4.9%) | 10 (4.0%) | 1.303 (0.891–1.906) | 0.172 |
| A | 380 (84.8%) | 424 (81.5%) | 1.00 (ref) |
| G | 108 (241%) | 96 (18.5%) | 1.266 (0.932–1.727) | 0.131 |
| Multi-genotypes | CCGS | 8 (3.3%) | 6 (2.3%) | 1.435 (0.491–4.197) |
| CCAG | 22 (9.0%) | 16 (6.2%) | 1.511 (0.744–2.951) |
| CCAA | 18 (7.4%) | 6 (2.3%) | 3.372 (1.316–8.841) |
| CTXX | 4 (1.6%) | 2 (0.8%) | 2.150 (0.390–11.845) |
| CTAAG | 48 (19.7%) | 46 (17.7%) | 1.139 (0.728–1.878) |
| CTAAG | 50 (20.5%) | 64 (24.6%) | 0.789 (0.519–1.201) |
| TTGG | 0 (0.0%) | 2 (0.8%) | - |
| TTAG | 14 (5.7%) | 14 (5.4%) | 1.070 (0.499–2.318) |
| TTAAG | 80 (32.8%) | 104 (40.0%) | 0.752 (0.508–1.054) |
| TTGG | 22.91 (0.047) | 24.13 (0.046) | 1.012 (0.564–1.818) |
| TA | 267.09 (0.547) | 327.87 (0.631) | 0.708 (0.551–0.912) |
| CG | 85.09 (0.174) | 71.87 (0.138) | 1.317 (0.936–1.853) |
| CA | 112.91 (0.231) | 96.13 (0.185) | 1.327 (0.978–1.801) |

**BPH** = benign prostatic hyperplasia, **CI** = confidence interval, **OR** = odd ratio, **ESR** = oestrogen receptor. **PvII** = Polymorphism at codon 701 of the ERα gene.
SHEsis showed that BHP patients with TA haplotype had a lower risk for PCI, and the risk was 0.7 times higher than that in patients without TA haplotype.

4. Discussion

Oestrogen receptor plays an important role in accommodating the diseases relevant with hormone. Therefore, to study the oestrogen receptor gene polymorphism will be helpful for the elucidation of molecular mechanism underlying the occurrence and development of diseases related to this hormone. At present, though a variety of cancers (such as endometrial carcinoma and mammary cancer) have been reported to have relationship with the polymorphisms of PvuII and XbaI of ESRa in Chinese people, the correlation of their polymorphisms with the pathogenesis of PC is still poorly understood.[27,28] The present study was undertaken to investigate the relationship of SNP of ESRa with PC and BPH in Han Chinese men recruited from East China. Our results showed that there is no distinct relationship between PvuII and XbaI sites and risk of PC or BPH. At present, no study has been conducted to investigate the relationship between PvuII polymorphism and BPH, although results from experiments about the relationship of PvuII polymorphism with PC are in accordant with those in Japanese and USA men.[29,30] A study on Iran men showed that the C allele (CT or CC) of PvuII increased the risk for PC. Compared with wild homoygous TT genotype, mutant CT and CC increased the risk for PC by 3.12 and 4.73 times, respectively.[31] Our results indicated that, for healthy men with C allele of PvuII, the risk for PC and BPH was not increased. However, in BHP patients, the risk for PC increased by 1.4 times in the presence of C allele of PvuII. This indicates that C allele of PvuII may be a predisposing factor of PC. However, our results were inconsistent with that from a Japanese study.[29] Their results showed that, compared with the healthy men with CC genotype of PvuII, the risk for PC increased by 3.44 times in healthy men with TT genotype of PvuII. They also found that, compared with CC genotype, TT genotype will increase the risk for PC,[23] which was consistent with our finding. Even if in Chinese men with C allele or CT or CC genotype suffering PC, the differentiation degree of cancer cells was higher, and the cancer cells seldom metastasized into other sites, which indicates that the prognosis of patients with C allele is better. As to XbaI, our results showed no relationship with the risk for PC, which was similar to the findings from a Japanese study,[25,29] but not with those from the European and USA studies. In a study on black and white people in the United States, Hernandez et al.[30] found that the risk for PC in American black people with AG genotype and G allele (AG+GG) increased by 2.25 and 2.14 times, respectively. However, a recent study on Iran men showed that the risk for PC in men with AG genotype increased by 4.36 times as compared with those with AA genotype.[31] The results on PvuII and XbaI from different regions and ethnic groups are quite different, which may reflect the influence of genetic backgrounds, diet habits, life style, and even sunlight exposure on the risk for BPH and PC.[12]

However, it is still not clear how PvuII and XbaI influence the occurrence and development of PC and BPH. PvuII and XbaI are the 2 most common sites of polymorphism, and all the polymorphisms occur in the intron 1 which contains promoter, enhancer, and other important regulatory sequences, and thus its polymorphisms may affect the expression and function of ESRa.[13] T allele of PvuII changes to T genes, and then other sites, B-myb will bind to myb transcription factor on the gene order, increasing the transcription of downstream report gene. Therefore, C allele may increase the transcriptional activity of ESRa to a large extent. It is not clear whether XbaI has a separate effect on oestrogen receptor. Of note, the distance between XbaI and PvuII is only 30 bp, which exists strong linkage disequilibrium and may have a negative effect on the function of PvuII, or regulate and control target genes by forming composite genes with PvuII or haplotype.

Thus, the synergistic action or inhibitory action expressed by the above 2 sites in the form of composite genes or haplotype was further investigated in this study. Results showed that, haplotype TG or CA increased the risk for PC in healthy men, but haplotype TA decreased the risk for PC in both healthy men and BPH patients. Additionally, haplotype CG decreased the risk for BPH. Analysis of composite genes indicated that TTAG increased the risk for both BPH and PC. However, in case of BPH, the composite genes had no influence on the risk for PC. CCAG genes increased the risk for PC in BPH patients.

In control group, healthy men were recruited. However, in the Japanese studies,[24,30] both BPH patients and healthy men were included in control group, which may bias the results, especially the relationship between BPH and PC. Therefore, our study not only illustrated the mechanism of the occurrence and development of PC which provides a method to screen the subjects with high risk for PC, but also provided evidence on the relationship between BPH and PC to a certain extent, which may assist the early detection and diagnosis of PC and BPH. However, there were limitations in this study: the sample size was small, shortage of gene sites, and horizontal analysis on different ethnic people and zones. In our future studies, we will investigate the SNPs in subjects with different genetic background and retrospectively analyze studies on this issue to clarify the influence of ESRa gene polymorphisms on the occurrence and development of PC and BPH. During the transformation from BPH to malignance, there are prostatic intraepithelial neoplasm and proliferative inflammatory atrophy, which might be the keys to inducing canceration. However, due to sample sizes and difficulties in clinical diagnosis, these samples were not analyzed. Future studies will focus on these cases.

In summary, our study shows the close relationship between ESRa gene polymorphism and risk for PC and BPH in Chinese men, which varies between ethnic groups.

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