Combined effect of polymorphisms in type III 5-α reductase and androgen receptor gene with the risk of benign prostatic hyperplasia in Korea

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We evaluated whether type III 5-alpha reductase (SRD5A3; steroid reductase 5-alpha 3) polymorphism was associated with susceptibility of benign prostate hyperplasia (BPH) and the combined effects in BPH risk between the type of short tandem repeat (STR) in SRD5A3 and the length of trinucleotide (CAG) repeats in androgen receptor (AR) gene. We compared the length of AC repeats in STR region of SRD5A3 gene and a CAG repeat in AR in 188 BPH patients who underwent transurethral resection of prostate (TURP) and 98 controls by polymerase chain reaction-based methods. We defined short type was less than 21 copies of AC repeats. The odds ratio for BPH between the men with at least one of short type and with both large types of STR in SRD5A3 gene was 3.10 (95% confidence interval [CI], 1.87–5.16; P = 0.000). And BPH was 2.35 times more likely to occur in with less than 23 copies of CAG repeats than men equal or greater than 23 copies in AR gene (95% CI, 1.18–2.36; P = 0.016). The men with the large type of STR and ≥ 23 copies of CAG repeats have 5.3 times BPH risk compared to the reference group with the at least one of the short type of STR and < 23 copies (P < 0.000). In conclusion, these results suggest that shorter AC repeats of SRD5A3 gene and shorter CAG repeats of AR gene were associated with an increased risk for BPH. However, the interaction between above two factors was not affected in risk of BPH.

Keywords: Genetic polymorphism, 3-Oxo-5-alpha-steroid 4-dehydrogenase, Androgen receptors, Benign prostate hyperplasia

INTRODUCTION

Benign prostate hyperplasia (BPH) is a very common disease among aging men and prevalence rate increase in men over the age of fifty (McVary, 2006) and it has been estimated that actively managed cost for BPH is 12.2 million annually in United States (Vuichoud and Loughlin, 2015). In Korea, according to recent statistics from the Health Insurance Review and Assessment Service, 1,021,222 people were registered as patients with BPH in 2014. It is the fourth most common diagnosis in older men who has lower urinary tract symptoms (LUTS) that consist of bother-some, impairment of psychological and functional well-being, and interference with daily life activities (Coyne et al., 2009).

Prostate development requires the presence of testicular androgens. 5-alpha reductase converts testosterone to dihydrotestoster-one (DHT) that is most potent androgen and affected in prostate growth and could bind to intracytoplasmic androgen receptor. And then complex of AR with DHT enhances activation of androgen response elements (Andriole et al., 2004; Steers, 2001). Recently, type III 5α-reductase was reported and was overexpressed in prostate cancer (Uemura et al., 2008). Type I 5α-reductase (SRD5A1) and type II 5α-reductase (SRD5A2) were well known and those inhibitors were clinically used as medication for alopecia and BPH (Cindolo et al., 2013; Sudduth and Koronkowski, 1993). However, the roles of type III 5-alpha reductase (SRD5A3) in BPH patients are unknown. We evaluated whether SRD5A3 polymorphism was associated with susceptibility of BPH or not and the combined effects in BPH risk between the type of short tandem repeat (STR) in SRD5A3 and the length of CAG repeats in AR gene in patients with BPH.

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MATERIALS AND METHODS

Study populations
From July 2014 to June 2016, BPH patients who had a histological confirmed diagnosis after transurethral resection of prostate at Chungnam National University Hospital were involved in this study. After approval from the Institutional Review Board, informed consent was obtained from the study participants. Genomic DNA was obtained from 188 patients with BPH (mean age, 69.7 ± 8.1 years) and from 98 healthy controls (mean age, 63.7 ± 8.1 years). The healthy controls were selected by age over 50 years old with less than 10 points of International Prostate Symptoms Scores and without evidences of prostate cancer using a prostate-specific antigen and digital rectal examinations.

Analysis of the length of AC repeats in SRD5A3 gene and CAG repeats in AR gene
For the analysis of STR, genomic DNAs were isolated from 1-mL blood sample using by Genomic DNA Prep Kit (Cat. No. SGD61-S120, Solgent Co., Daejeon, Korea) according to the manufacturer’s instruction. The qualities of isolated genomic DNA samples were tested using 1% agarose gel electrophoresis and their quantities were measured by NanoDrop (Thermo Fisher Scientific, Waltham, MA USA). The polymerase chain reaction (PCR) products for the loci containing the STR region (SRD5A3 gene, NCBI reference sequence: NM_024592.4) were obtained using a mixture containing 10 pmol from each primer: 5’- GAT GAG ACT TCT CCA AGC TG-3’ (forward) and 5’- CAA CCA ACA GTT ATT GAG CAC -3’ (reverse), 10X h-Taq buffer, 10 mM dNTP(T), h-Taq DNA polymerase (Solgent Co.), and 200-ng genomic DNA isolated from blood sample. And we also obtained the PCR products for the loci containing the CAG repeats (AR gene, National Center for Biotechnology Information [NCBI] reference sequence: BC132975.1) using each primer: 5’- TCC ACC TAC CGA GGA GCT-3’ (forward) and 5’- TGT GAA GGT TGC TGT TCC TCA TC-3’ (reverse). After the initial denaturation of the reaction mixture at 95°C for 3 min, amplification was achieved by 35 cycles at 95°C for 20 sec, 58°C for 40 sec and 72°C for 60 sec, and a final extension at 72°C for 5 min. After purifying the PCR product using PCR Purification Kit (Solgent Co.), sequences of the STR region were confirmed by the direct sequencing analysis. For the Genescan analysis of STR, AC repeat loci were amplified by the fluorescent dye-tagged forward primer and reverse primer. After the purification of amplified PCR product, 2-ng PCR products were mixed with Hi-Di TM Formamide (Applied Biosystems, Foster City, CA, USA) and POP-4 TM Polymer (Applied Biosystems) size marker. The reaction mix were incubated at 96°C, 2 min and 4°C, 3 min, and then analyzed by ABI 3100 genetic analyzer (Applied Biosystems). The obtained data of samples were analyzed by GeneMapper 4.0 (Applied Biosystems) program. According to the values analyzed by GeneMapper 4.0 and nucleotide sequence, the repeat numbers of STR for each sample were estimated as previously reported (Park et al., 2013).

Statistical analysis
Statistical analysis was performed to identify an association between the BPH status and the length of STR of SRD5A3, the length of CAG repeats in the AR gene, and in combination. The odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated using a chi-square test and binary logistic regression. Age adjustment was incorporated into the logistic regression. A P-value < 0.05 was considered statistically significant. All the tests in this article were implemented using IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA).

RESULTS
We defined that a short type of allele had less than 21 copies of AC repeats in the STR region of SRD5A3 gene. Short and short type of STR in SRD5A3 gene was 1.89 times more likely to occur in BPH patients than in controls (OR, 1.89; 95% CI, 0.98–3.64; P = 0.058). The men who had at least one of short type have a 3.1 times of BPH risk compared with controls (OR, 3.10; 95% CI, 1.87–5.16; P = 0.000) (Table 1).

We divided into two groups as the cutoff value was 22 copies of

| Type of STR | BPH | Control | OR (95% CI) | P-value* |
|-------------|-----|---------|-------------|----------|
| SS          | 45 (23.9) | 14 (14.3) | 1.00 (reference) | -        |
| SL/LS       | 74 (39.4) | 21 (21.4) | 0.91 (0.42–1.97) | 0.815    |
| LL          | 69 (36.7) | 63 (64.3) | 2.94 (1.47–5.85) | 0.002    |
| SS          | 45 (23.9) | 14 (14.3) | 1.00 (reference) | -        |
| SL & LL     | 143 (76.1) | 84 (85.7) | 1.89 (0.98–3.64) | 0.058    |
| SS & SL     | 119 (63.3) | 35 (35.7) | 1.00 (reference) | -        |
| LL          | 69 (36.7) | 63 (64.3) | 3.10 (1.87–5.16) | 0.000    |

Values are presented as number (%). STR, short tandem repeats; OR, odds ratio; CI, confidence interval; SS, short and short type; SL, short and long type; LS, long and short type; LL, long and long type (short type was defined as numbers of AC repeats of STR less than 21 copies).

* P-value was compared to reference.
the length of CAG repeats in AR gene. The men who had a less than 22 copies CAG repeats of AR gene had a 1.5 times of BPH risk but there was no statistical significance. However, if cutoff value was 23 copies of the length of CAG repeats in AR gene, the men who had a less than 23 copies CAG repeats of AR gene had a 2.35 times of BPH risk compared with controls (OR, 2.35; 95% CI, 1.18–2.36; P = 0.016) (Table 2).

We calculated age-adjusted P-value to be compared SS and SL type with LL type of STR in SRD5A3 gene and cutoff value was 23 copies of the length of CAG repeats in AR gene according to strong statistical evidences from Tables 1 and 2. The age adjustment did not affect the statistical significance of two variables in the multivariate logistic regression model (Table 3).

In Table 4, we examined the combined effects of the type of STR and the length of CAG repeats. The men who have the large type of STR and ≥23 copies of CAG repeats have 5.3 times BPH risk compared to the reference group of men who have the at least one of the short type of STR and <23 copies of CAG repeats (P < 0.000). Similarly, the men who have the large type of STR and <23 copies of CAG repeats have 2.2 times BPH risk compared to the reference group (P = 0.027). The upward trend of BPH risk along the risk groups is observed (P < 0.000).

**DISCUSSION**

The development of histologic BPH needs androgens and aging changes. In prostate tissue, testosterone is irreversibly converted to DHT by 5 alpha-steroid reductase (SRD5A) that have a major roles to make various secretory proteins to associate with prostate development and growth (Zhu and Imperato-McGinley, 2009). 5-Alpha reductase has three isoenzymes to be identified as SRD5A1, SRD5A2, and SRD5A3 (Thomas et al., 2005; Uemura et al., 2008). SRD5A1, SRD5A2 are well known and developed inhibitors to decreased prostate volume and prevent alopecia and widely used therapeutic agents for BPH (Sudduth and Koronkowski, 1993). The SRD5A3 gene is located in chromosome 4q12. SRD5A3 had been called SRD5A2L, SRD5A2L1, 3-oxo-5-alpha-

| Table 2. Case-control genotype of CAG repeats in androgen receptor gene association with benign prostate hyperplasia (BPH) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Length of CAG repeats | BPH | Control | OR (95% CI) | P-value |
| < 22 | 82 (43.6) | 33 (33.7) | 1.00 (reference) | |
| ≥ 22 | 106 (56.4) | 65 (66.3) | 1.52 (0.92–2.53) | 0.105 |
| < 23 | 113 (60.1) | 44 (44.9) | 1.00 (reference) | |
| ≥ 23 | 75 (39.9) | 54 (55.1) | 2.35 (1.18–2.36) | 0.016 |

Values are presented as number (%). OR, odds ratio; CI, confidence interval.

| Table 3. Age adjusted case-control genotype of type of STR in SRD5A3 and the length of CAG repeats in AR gene association with benign prostate hyperplasia (BPH) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Factor | BPH | Control | OR (95% CI) | P-value | Age-adjusted |
| | | | | | OR (95% CI) | P-value |
| SRD5A3 | | | | | |
| SS & SL | 119 (63.3) | 35 (35.7) | 1.00 (reference) | | |
| LL | 69 (36.7) | 63 (64.3) | 3.10 (1.87-5.16) | < 0.000 | 3.64 (2.09–6.34) | < 0.000 |
| Length of CAG repeats | | | | | |
| < 23 | 113 (60.1) | 44 (44.9) | 1.00 (reference) | | |
| ≥ 23 | 75 (39.9) | 54 (56.1) | 2.35 (1.18–2.36) | 0.016 | 1.85 (1.09–3.13) | 0.022 |

Values are presented as number (%). OR, odds ratio; CI, confidence interval; SS, short and short type; SL, short and long type; LL, long and long type (short type was defined as numbers of AC repeats of STR less than 21 copies).

| Table 4. The combined effects between type of STR in SRD5A3 gene and the length of CAG repeats in androgen receptor gene in patients with benign prostate hyperplasia (BPH) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Type of STR in SRD5A3 | Length of CAG repeats | BPH | Control | OR (95% CI) | P-value |
| | | | | | |
| SS & SL | < 23 | 71 (37.8) | 19 (19.4) | 1.00 (reference) | |
| | ≥ 23 | 48 (25.5) | 16 (16.3) | 1.25 (0.58-2.66) | 0.571 |
| LL | < 23 | 42 (22.3) | 25 (25.5) | 2.22 (1.10-4.52) | 0.027 |
| | ≥ 23 | 27 (14.4) | 38 (38.8) | 5.26 (2.59-10.66) | < 0.000 |

Values are presented as number (%). P_interaction > 0.05, P_trend < 0.000. OR, odds ratio; CI, confidence interval; SS, short and short type; SL, short and long type; LL, long and long type (short type was defined as numbers of AC repeats of STR less than 21 copies).
steroid 4-dehydrogenase 3, and CDG1P, but SRD5A3 was approved as the symbol in the HUGO Gene Nomenclature Committee database. Recently, Uemura et al. (2008) reported that SRD5A3 is overexpressed in hormone-refractory prostate cancer tissues and regulates growth and viability in a prostate cancer cell line. However the roles of SRD5A3 in BPH condition have been unknown, there were a few literatures in association study between polymorphisms in SRD5A and BPH. Klotsman et al. (2004) reported that polymorphisms in SRD5A2 were not associated with severity of BPH but SRD5A1. This study was evaluated CAG repeat of AR gene, single nucleotide polymorphism of SRD5A2 gene and two silent polymorphisms in SRD5A1 however not assessed combined effects each genes. Our results in association between polymorphism in SRD5A3 and BPH risk suggested that the men who had at least one of short type have a 3.1 times of BPH risk compared with the men who had both of large type (OR, 3.10; 95% CI, 1.87–5.16; P = 0.000) (Table 1). Riley and Krieger (2003) reported that longer STRs provided larger and more accessible loops for processing resulting in transcripts with shorter half-lives and induced abnormal function of protein. And we already suggest possible mechanism that longer AC repeats of AR gene had a 2.4 times of BPH risk compared with the men who had less than 23 copies CAG repeats in AR gene (Park et al., 2013). It is involved prostate proliferation and may affect with risk of BPH.

AR gene is located in X-chromosome q12 and has major roles in proliferation and developments of prostate cells. There were several association studies in CAG repeats of AR gene and BPH risk. In Brazilian study, there was no evidence for an association between AR CAG repeat length in BPH risk in a population-based sample (Biolchi et al., 2012). However, Renko et al. (2008) reported that there was an association between short AR gene CAG repeat length and a small prostate volume, which confirms a previous finding in the Finnish population. Our findings in polymorphism in AR gene was similar result with Finnish population study that if cutoff value was 23 copies of the length of CAG repeats, the men who had a less than 23 copies CAG repeats of AR gene had a 2.4 times of BPH risk compared with the men who had an equal and greater than 23 copies CAG repeats (OR, 2.35; 95% CI, 1.18–2.36; P = 0.016) (Table 2). The association of the length of CAG repeats in AR with BPH is still controversial; we suggest that the longer repeats of CAG would have stronger susceptibility with BPH. We also evaluated combined effect polymorphism in SRD5A3 and AR gene because SRD5A and AR gene play a key roles in prostate proliferation by binding AR with DHT converted from testosterone which is more powerful form of androgen. We assumed that if it may occur aberrant translation in SRD5A3 and AR genes, the functions of SRD5A3 and AR would be impaired and caused less BPH incidence, however, statistically significant results were not proven meanwhile statistical trend was shown. The main limitation of this study is the lack of age matched controls. Because most of the middle aged patients visiting the urology department had previous urinary tract infection, systemic diseases, or LUTS, it was difficult for us to enroll the age match controls for comparison in this pilot study.

In conclusion, we investigated polymorphisms of SRD5A3 gene in BPH risk and evaluated association between the length of CAG repeat of AR and polymorphisms of SRD5A3 gene. We observed that short AC repeats of SRD5A3 and a less than 23 copies of CAG repeats of AR gene were associated with an increased risk for BPH. SRD5A3 and AR polymorphisms may contribute to a genetic predisposition for BPH. However, the interaction between the length of CAG repeats in AR gene and the length of AC repeats in SRD5A3 was not affected in risk of BPH.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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