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- α reductase (*SRD5A3*; steroid reductase 5- α 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- α -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 bothersome, 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- α reductase converts testosterone to dihydrotestosterone

(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 5- α -reductase was reported and was overexpressed in prostate cancer (Uemura et al., 2008). Type I 5- α -reductase (*SRD5A1*) and type II 5- α -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- α 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'-GTA GAT GAG ACT TCT CCA AGC TG-3' (forward) and 5'-CAA CAA 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 AAG 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

Table 1. Case-control genotype of *SRD5A3* association with benign prostate hyperplasia (BPH)

Type of STR	BPH	Control	OR (95% CI)	<i>P</i> -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

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.

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 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)	<i>P</i> -value	Age-adjusted	
					OR' (95% CI)	<i>P</i> -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 (55.1)	2.35 (1.18-2.36)	0.016	1.85 (1.09–3.13)	0.022

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; 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 <i>SRD5A3</i>	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.

STR, short tandem repeats; 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 *SRD5A3* gene may induce the dysfunction of *SRD5A3* to be converted testosterone to DHT (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 power-

ful 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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