KJU
Korean Journal of Urology

www.kjurology.org
http://dx.doi.org/10.4111/kju.2013.54.6.404

Basic and Translational Research

Is the Expression of Androgen Receptor Protein Associated With the Length of AC Repeats in the Type III 5-α Reductase Gene in Prostate Cancer Patients?

Jong Mok Park, Ki Hak Song, Jae Sung Lim, Jin Woo Kim[1], Chong Koo Sul

Department of Urology, Chungnam National University School of Medicine, Daejeon, Korea, [1]Department of Cancer Biology, Wake Forest University, Winston-Salem, NC, USA

Purpose: Type III 5-α reductase (SRD5A3; steroid 5-α reductase 3) may be associated with the progression of prostate cancer (PCa). The aim of our study was to determine whether the length of AC repeats in the *SRD5A3* gene is associated with the risk of PCa and the expression of androgen receptor (AR) protein in Korean men.

Materials and Methods: We compared the length of AC repeats in the short tandem repeat (STR) region of the *SRD5A3* gene in 68 PCa patients and 81 control subjects by genotyping. A total of 55 patients in the PCa group underwent radical prostatectomy. We evaluated the expression of AR protein by using Western blotting and tested the association between the type of AC repeats in the *SRD5A3* gene and AR protein expression and clinical and pathologic parameters.

Results: The short type of STR had less than 21 copies of AC repeats in the *SRD5A3* gene. The SS type (short and short type) of STR of the *SRD5A3* gene was 2.2 times as likely to occur in PCa patients as in controls (odds ratio, 2.21; 95% confidence interval, 1.14 to 4.31; p=0.019). However, AC repeats of the *SRD5A3* gene were not associated with AR protein expression or clinical or pathologic parameters in PCa samples.

Conclusions: These results suggest that the short AC repeats of *SRD5A3* polymorphism are associated with an increased risk of PCa. *SRD5A3* polymorphism may contribute to a genetic predisposition for PCa.

Keywords: *3-Oxo-5-alpha-Steroid 4-Dehydrogenase; Androgen receptors; Genetic polymorphism; Prostatic neoplasms*

Article History:
received 8 March, 2013
accepted 21 April, 2013

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Corresponding Author:
Ki Hak Song
Department of Urology, Chungnam National University Hospital, Chungnam National University School of Medicine, 282 Munhwa-ro, Jung-gu, Daejeon 301-721, Korea
TEL: +82-42-280-7777
FAX: +82-42-257-0966
E-mail: urosong@cnu.ac.kr

INTRODUCTION

Prostate cancer (PCa) is an important disease for public health care in the United States (US), Europe, and Korea because it is first in prevalence and second in mortality among men's cancer [1]. The incidence rate of PCa has markedly increased over the past 10 years in Korea [2]. Much evidence suggests that the risk factors for PCa include genetic background, such as race, family history, and variants of specific genes. African American men have a higher prevalence rate of PCa than do Caucasian or Hispanic men in the US [1]. In contrast, the incidence and mortality rates for Hispanic men are one third lower than for non-Hispanic Caucasians [3]. Environmental factors also play an important role with genetic factors in the initiation and development of PCa [4,5].

Concerning molecular modification in the prostate, the most important factor in the development and progression of PCa is activation of the androgen receptor (AR) signaling pathway. PCa growth is usually androgen dependent and androgen deprivation therapy is a standard therapy in patients with advanced and metastatic PCa [6]. In this pathway, 5-α reductase converts testosterone to dihydrotestosterone (DHT), which can bind to the AR. The com-

© The Korean Urological Association, 2013

plex of the AR with DHT enhances activation of androgen response elements (AREs) [7].

Recently, type III 5-α reductase (SRD5A3) was identified and overexpression of this gene in PCa was reported [8]. Type I 5-α reductase (SRD5A1) and type II 5-α reductase (SRD5A2) have been extensively studied, and those inhibitors are in clinical use for alopecia and benign prostatic hyperplasia [9,10]. However, few reports are available on the function and roles of SRD5A3 in PCa. The aim of this study was to evaluate the association between *SRD5A3* gene polymorphisms and PCa risk and the expression of AR protein in Korean men.

MATERIALS AND METHODS

1. Study populations

From January 2011 to June 2012, PCa patients with a histologically confirmed diagnosis after transrectal ultrasonography (TRUS)-guided biopsy at Chungnam National University Hospital were enrolled in this study. After approval from the Institutional Review Board, informed consent was obtained from the study participants. The healthy controls were selected by the criteria of age over 50 years without evidence of PCa by prostate-specific antigen (PSA) testing, digital rectal examination, and TRUS. A total of 68 PCa patients (mean age, 69.9±7.4 years; prostate volume, 38.3±14.7) and 81 healthy control subjects (mean age, 60.7±10.6 years; prostate volume, 26.3±5.6; PSA, 1.9±0.7 ng/mL) were studied. The number of patients with clinical low-stage (T1c, 6 cases; T2, 47 cases) and high-stage (T3, 14 cases; T4, 1 case) cancer was 53 (77.9%) and 15 (22.1%), respectively.

2. Analysis of short tandem repeats in the *SRD5A3* gene

For the analysis of short tandem repeats (STRs), genomic DNAs were isolated from 1-mL blood samples by use of the Genomic DNA Prep Kit (catalog no. SGD61-S120, SolGent Co., Daejeon, Korea) according to the manufacturer's instructions. The qualities of isolated genomic DNA samples were tested by using 1% agarose gel electrophoresis and their quantities were measured by spectrophotometer.

The polymerase chain reaction (PCR) products for the loci containing the STR region (*SRD5A3* gene, National Center for Biotechnology Information reference sequence: NM_024592.4) were obtained by 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 the blood sample. After the initial denaturation of the reaction mixture at 95°C for 3 minutes, amplification was achieved by 35 cycles at 95°C for 20 seconds, 58°C for 40 seconds, and 72°C for 60 seconds and a final extension at 72°C for 5 minutes. After purifying the PCR product by use of the PCR Purification Kit (SolGent Co.), sequences of the STR region were confirmed by direct sequencing analysis. For the Genescan analysis of STRs, AC repeat loci were amplified by the fluorescent dye-tagged forward primer and reverse primer. After the purification of amplified PCR product, 2 ng of the PCR products was mixed with Hi-Di Formamide (Applied Biosystems, Foster City, CA, USA) and POP-4 Polymer (Applied Biosystems) size marker. The reaction mix was incubated at 96°C for 2 min and 4°C for 3 minutes and then analyzed by use of the ABI3100 genetic analyzer (Applied Biosystems). The obtained data were analyzed by use of GeneMapper 4.0 (Applied Biosystems). According to the values analyzed by GeneMapper 4.0 and the nucleotide sequence, the repeat number of STRs for each sample was estimated by use of the equation shown in Fig. 1.

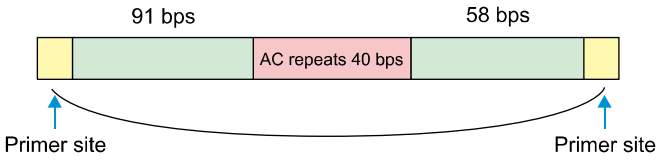

FIG. 1. Scheme of calculation for numbers of AC copies in *SRD5A3* gene. For examples, if the value analyzed by GeneMapper program was 189.03, short tandem repeat was the 20 repeats as follow an equation; [(189.03)−(91+58)]/2=20.015 (20 repeats).

3. Western blotting of AR

A total of 55 patients in the PCa group underwent radical prostatectomy, and expression of AR protein was measured by Western blotting. Frozen prostate tissue obtained from prostatectomy was pulverized by using a precooled mortar and pestle in liquid nitrogen and homogenized at 4°C in 1×radioimmunoprecipitation assay buffer (Sigma-Aldrich Co., St. Louis, MO, USA; ready-to-use solution containing 150 mM NaCl, 1.0% IGEPAL CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris, pH 8.0). Homogenates were centrifuged at 13,000×g for 20 minutes at 4°C, and the supernatant was either used immediately or stored at -70°C. Total protein was measured by using a Bradford dye-binding protein assay kit according to the manufacturer's instructions (Thermo Scientific, Pittsburgh, PA, USA). An aliquot of the supernatant was kept for protein determination, and Laemmli sample buffer (Bio-Rad Laboratories Inc., Hercules, CA, USA) containing β-mercaptoethanol was added to the remainder. The samples were boiled for 5 minutes, and then the equivalent of 50 μg total protein was loaded onto a 10% polyacrylamide gel. Samples were separated in an electrophoresis module assembly (Mini-Protean TetraCell, Bio-Rad Laboratories Inc.) under constant voltage. After electrophoresis, the proteins were transferred to polyvinylidene fluoride membranes by using a trans-blot semi-dry transfer cell (Mini-Protean TetraCell) for 2 hours at 4°C. Nonspecific binding sites were blocked by incubation in 1×phosphate buffered saline (PBS) containing 5% skim milk and 0.1% Tween 20 (PBSTM) for 1 hour. The membrane was incubated overnight at 4°C with anti-AR antibody (N-20, Santa Cruz Biotechnology Inc., Santa Cruz,

CA, USA) at a dilution of 1:500. After washing with 1×PBSTM, the membrane was incubated for 1 hour at room temperature with goat antirabbit IgG-horseradish peroxidase (Santa Cruz Biotechnology Inc.) diluted to 1:2000 and washed four times for 15 minutes each in 1× PBSTM. Membranes were also probed with antiactin monoclonal antibody (1:1000, Santa Cruz Biotechnology Inc.) as an internal control. The responses were identified at 110 kDa. Immunoreactive proteins were detected and visualized by use of a chemiluminescence reagent (Dogen, Seoul, Korea) and the scanned films were quantified by using a gel documentation system. We defined overexpression as a value greater than 50 percent of the ratio (expression of AR/β-actin).

4. Statistical analysis

The data were analyzed statistically and expressed as the mean (SD); groups were compared by using the chi-square test, and a p-value <0.05 was considered statistically significant. The associations between the STR of *SRD5A3* genotypes and risk for PCa and expression of AR protein within patients with PCa were analyzed by using IBM SPSS ver. 20.0 (IBM Co., Armonk, NY, USA). Binary logistic regression tests were used to calculate the p-values and 95% confidence intervals (95% CIs) for the odds ratios (ORs).

RESULTS

Patients with PCa had similar patterns of frequency in *SRD5A3* STR genotype compared with control samples. There was a statistical trend that 42.6% of patients with PCa had the 20, 20 genotype compared with 28.4% of control samples (Fig. 2; chi-square=2.97, df=1, p=0.084). We defined a short type of allele as having less than 21 copies of AC repeats in the STR region of the *SRD5A3* gene. The SS type (short and short type) STR of the *SRD5A3* gene was 2.2 times as likely to occur in PCa patients as in controls (Table 1; OR, 2.21; 95% CI, 1.14 to 4.31; p=0.019).

We obtained 55 tumor tissues samples from prostatectomy to evaluate the expression of AR protein and an association with *SRD5A3* polymorphism. However, AC repeats of the *SRD5A3* polymorphism were not associated with AR protein expression in patients with PCa who underwent retropubic radical prostatectomy (Table 2; OR, 2.46; 95% CI, 0.73 to 8.31; p=0.146). We also analyzed the association between the type of STR in SRD5A3 and clinical and pathologic parameters such as stage, Gleason score, and surgical margin. There were no statistically significant associations for any parameter (clinical stage: OR, 1.41; 95% CI, 0.43 to 4.61; pathologic stage: OR, 1.64; 95% CI, 0.44 to 6.08; Gleason score: OR, 1.81; 95% CI, 0.52 to 6.35; surgical margin: OR, 2.40; 95% CI, 0.53 to 10.93).

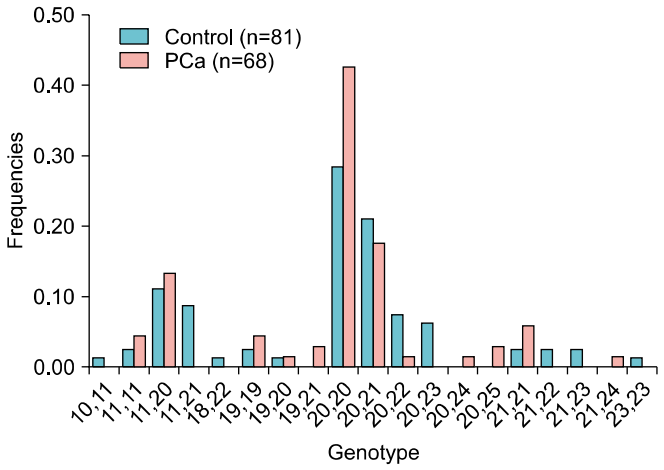

FIG. 2. The frequencies of SRD5A3 short tandem repeat between control and patients with prostate cancer. 42.6% of patients with prostate cancer (PCa) had the 20,20 genotype compared to 28.4% of control (χ^2=2.97, df=1, p=0.084).

DISCUSSION

5-Alpha-Reductase has three isoenzymes: SRD5A1, SRD5A2, and SRD5A3. SRD5A1 and SRD5A2 are well known to be associated with the development and progression of PCa [11,12]. SRD5A1 is expressed primarily in the skin and liver and, to a lesser extent, in the prostate, whereas SRD5A2 is expressed predominantly in prostate

TABLE 1. Case-control genotype of *SRD5A3* association with prostate cancer

Type of STR	Prostate cancer	Control	OR (95% CI)
SS	45 (66.2)	38 (46.9)	1.00 (reference)
SL	18 (26.5)	36 (44.5)	1.66 (0.49–5.65)
LL	5 (7.3)	7 (8.6)	2.75 (0.22–33.65)
SL & LL	23 (33.8)	43 (53.1)	2.21 (1.14–4.31)

Values are presented as number (%). Short type was defined as number of AC repeats of STR less than 21 copies.
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.

TABLE 2. Association between AR expression and genotype of *SRD5A3* in patients who underwent radical prostatectomy

Type of STR	Expressions of androgen receptor		OR (95% CI)
	Underexpression	Overexpression	
SS	18 (78.3)	19 (59.4)	1.00 (reference)
SL	4 (17.4)	9 (28.1)	2.13 (0.56–8.16)
LL	1 (4.3)	4 (12.5)	3.79 (0.39–37.20)
SL & LL	5 (21.7)	13 (40.6)	2.46 (0.73–8.31)

Values are presented as number (%). Short type was defined as number of AC repeats of STR less than 21 copies.
AR, androgen receptor; 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.

epithelium and other genital tissues [13]. Functional SRD5A2 is a prerequisite for normal development of the prostate and external genitalia in males, and insufficient exposure of the prostate to DHT appears to protect against the development of PCa. The *SRD5A3* gene is located in chromosome 4q12. *SRD5A3* had been called *SRD5A2L*, *SRD5A2L1*, 3-oxo-5-alpha-steroid 4-dehydrogenase 3, and CDG1P, but *SRD5A3* was approved as the symbol in the HUGO Gene Nomenclature Committee database. Recently, Uemura et al. [8] reported that SRD5A3 is overexpressed in hormone-refractory PCa tissues and regulates growth and viability in a PCa cell line. Overexpression of SRD5A3 in castration-resistant PCa suggests a potential role in cancer progression in an androgen-deprived prostate microenvironment [8]. Godoy et al. [14] mentioned that SRD5A3 protein might be a potential biomarker for malignancy, because of overexpression of SRD5A3 in tumor samples compared with their benign counterparts in breast, testis, lung, thyroid, and prostate. It has also been reported that the AR can directly bind to the negative ARE of the *SRD5A3* promoter in LNCaP cells [15]. However, an ARE region was not detected in the promoter of *SRD5A1* or that of *SRD5A2* by promoter analysis and chromatin immunoprecipitation assay. This means that AR expression may be associated with the *SRD5A3* gene. Therefore, we evaluated whether the *SRD5A3* polymorphism was associated with the risk of PCa or expression of AR protein. In this study, the shorter length AC repeat in the STR region of the *SRD5A3* gene was associated with increasing frequency of PCa (Table 1). On other hand, the men with alleles that had at least one longer AC repeat length of the *SRD5A3* gene had a lower risk of PCa.

Riley and Krieger [16] suggested that *SRD5A2*, macrophage scavenger receptor-1, and tumor necrosis factor receptor-21 are strong candidates for PCa-predisposing genes. They found that AC repeats in the STR regions were 9 times more common in mRNAs encoding membrane functions than in the total untranslated region (UTR) database and that these might be UTR signals for some mRNAs encoding membrane-targeted proteins [17]. They also suggested that longer STRs provided larger, more accessible loops for processing, which resulted in transcripts with shorter half-lives [17,18]. For some genes, however, the presence of the STR had a preserving effect, augmenting gene product activity, possibly as the result of improved mRNA transport or survival [19,20]. We thought that longer AC repeats of the *SRD5A3* gene might affect the function of *SRD5A3* to convert testosterone to DHT, which may be involved in PCa growth and affect the risk of PCa.

The importance of the AR signalling pathway cannot be overemphasized in the development and progression of PCa. As we previously mentioned, androgens play an important role in PCa development and progression. The primary androgen in the prostate tissue is DHT, which is irreversibly catalyzed from testosterone by 5-α reductase. DHT, which has much more affinity than testosterone, binds to the intracytoplasmic AR, and the complex of the steroid-receptor goes into the nucleus and enhances activation of the ARE [7]. Mitsiades et al. [21] recently reported that metastatic PCa expresses higher transcript levels of AR, SRD5A1, and SRD5A3, whereas expression of SRD5A2 and some CYP3A enzymes was decreased. Distinct patterns of dysregulated enzyme expression are involved in intratumoral androgen metabolism in PCa [21]. We evaluated the association between AR protein expression and clinical and pathologic parameters such as stage, Gleason score, and surgical margin and the length of the AC repeat in the *SRD5A3* gene but did not find any statistically significant associations. The small sample size analyzed in this study is one reason we think the length of the AC repeats in the *SRD5A3* polymorphism was not associated with AR protein expression or clinical and pathologic parameters such as stage, Gleason score, and surgical margin but was associated with PCa risk. It might be associated with cancer progression, biochemical recurrence, or tumor recurrence if it is regulated by the AR in an androgen-deprived microenvironment in human PCa cells. We need larger samples to study. We also need to evaluate the association between *SRD5A3* polymorphism and progression after long-term follow-up.

CONCLUSIONS

We investigated polymorphisms of the *SRD5A3* gene in PCa risk and evaluated the association between AR protein expression; clinical and pathologic parameters such as stage, Gleason score, and surgical margin; and the length of AC repeats in *SRD5A3* polymorphism. We observed that short AC repeats of the *SRD5A3* polymorphism were associated with an increased risk for PCa. *SRD5A3* polymorphisms may contribute to a genetic predisposition for PCa. However, we did not observe an association between AR protein expression or clinical and pathologic parameters and the number of AC repeats of the *SRD5A3* polymorphism. Comparative analysis of *SRD5A3* genetic predisposition in PCa among different countries may help us to understand the genetic factors of the disease.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

ACKNOWLEDGMENTS

We thank Dr. Riley and Dr. Krieger at the University of Washington for their helpful teaching about STR polymorphism. This project was financially supported by an educational fund (No. 2009-1650) of Chungnam National University, Daejeon, Korea.

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012;62:10-29.
2. Park SK, Sakoda LC, Kang D, Chokkalingam AP, Lee E, Shin HR, et al. Rising prostate cancer rates in South Korea. Prostate 2006;

66:1285-91.
3. Siegel R, Naishadham D, Jemal A. Cancer statistics for Hispanics/Latinos, 2012. CA Cancer J Clin 2012;62:283-98.
4. Muir CS, Nectoux J, Staszewski J. The epidemiology of prostatic cancer: geographical distribution and time-trends. Acta Oncol 1991;30:133-40.
5. Barnholtz-Sloan JS, Raska P, Rebbeck TR, Millikan RC. Replication of GWAS "Hits" by Race for Breast and Prostate Cancers in European Americans and African Americans. Front Genet 2011;2:37.
6. Mohler JL, Armstrong AJ, Bahnson RR, Boston B, Busby JE, D'Amico AV, et al. Prostate cancer, Version 3.2012: featured updates to the NCCN guidelines. J Natl Compr Canc Netw 2012;10: 1081-7.
7. Steers WD. 5alpha-reductase activity in the prostate. Urology 2001;58(6 Suppl 1):17-24.
8. Uemura M, Tamura K, Chung S, Honma S, Okuyama A, Nakamura Y, et al. Novel 5 alpha-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Cancer Sci 2008;99:81-6.
9. Sudduth SL, Koronkowski MJ. Finasteride: the first 5 alpha-reductase inhibitor. Pharmacotherapy 1993;13:309-25.
10. Cindolo L, Fanizza C, Romero M, Pirozzi L, Autorino R, Berardinelli F, et al. The effects of dutasteride and finasteride on BPH-related hospitalization, surgery and prostate cancer diagnosis: a record-linkage analysis. World J Urol 2012 Dec 14 [Epub]. http://dx.doi.org/10.1007/s00345-012-1000-4.
11. Thomas LN, Lazier CB, Gupta R, Norman RW, Troyer DA, O'Brien SP, et al. Differential alterations in 5alpha-reductase type 1 and type 2 levels during development and progression of prostate cancer. Prostate 2005;63:231-9.
12. Luo J, Dunn TA, Ewing CM, Walsh PC, Isaacs WB. Decreased gene expression of steroid 5 alpha-reductase 2 in human prostate cancer: implications for finasteride therapy of prostate carcinoma. Prostate 2003;57:134-9.
13. Andriole G, Bruchovsky N, Chung LW, Matsumoto AM, Rittmaster R, Roehrborn C, et al. Dihydrotestosterone and the prostate: the scientific rationale for 5alpha-reductase inhibitors in the treatment of benign prostatic hyperplasia. J Urol 2004; 172(4 Pt 1):1399-403.
14. Godoy A, Kawinski E, Li Y, Oka D, Alexiev B, Azzouni F, et al. 5a-reductase type 3 expression in human benign and malignant tissues: a comparative analysis during prostate cancer progression. Prostate 2011;71:1033-46.
15. Li J, Ding Z, Wang Z, Lu JF, Maity SN, Navone NM, et al. Androgen regulation of 5a-reductase isoenzymes in prostate cancer: implications for prostate cancer prevention. PLoS One 2011; 6:e28840.
16. Riley DE, Krieger JN. Short tandem repeat (STR) replacements in UTRs and introns suggest an important role for certain STRs in gene expression and disease. Gene 2005;344:203-11.
17. Riley DE, Krieger JN. Short tandem repeats are associated with diverse mRNAs encoding membrane-targeted proteins. Bioessays 2004;26:434-44.
18. Riley DE, Krieger JN. Transcribed short tandem repeats occur in couples with strongly preferred registers. Biochem Biophys Res Commun 2003;305:257-65.
19. Kleiman FE, Ramirez AO, Dodelson de Kremer R, Gravel RA, Argarana CE. A frequent TG deletion near the polyadenylation signal of the human HEXB gene: occurrence of an irregular DNA structure and conserved nucleotide sequence motif in the 3' untranslated region. Hum Mutat 1998;12:320-9.
20. Riley DE, Krieger JN. Diverse eukaryotic transcripts suggest short tandem repeats have cellular functions. Biochem Biophys Res Commun 2002;298:581-6.
21. Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, et al. Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. Cancer Res 2012;72:6142-52.