World Journal of Urology (2019) 37:709–718
https://doi.org/10.1007/s00345-018-2422-4

ORIGINAL ARTICLE

Correlation between 5-α reductase type 2 protein expression and methylation of 5-α reductase type 2 promotor gene of benign prostatic hyperplasia

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Received: 11 December 2017 / Accepted: 25 July 2018 / Published online: 1 August 2018

Abstract

Purpose The enzyme 5-α reductase type 2 (5-AR 2) plays a key role in the development and maintenance of the prostate gland. We evaluated the level 5-AR 2 protein expression and the relationship between methylation of the 5-AR 2 gene-promoter and 5-AR 2 protein expression of benign prostatic hyperplasia (BPH).

Materials and methods A total of 37 prostate samples were evaluated. These included 22 samples from men undergoing transurethral prostate resections and 15 non-cancerous transition-zone human prostate tissue samples taken following radical prostatectomy. We quantified 5-AR 2 protein expression and gene-promoter methylation status using common assay procedures. Clinical variables included age, body mass index (BMI), prostate-specific antigen (PSA) levels, lipid profiles, and prostate volumes. Univariate and multivariate statistical analyses were performed followed by stepwise logistic regression modeling.

Results We were able to extract DNA from 36 of the 37 tissue samples and 10 of these (28%) did not express the 5-AR 2 protein. In total, 26 patients (72%) had methylated 5-AR 2 promoter-regions. There was a strong correlation between methylation of the 5-AR 2 promoter-regions and low-absent 5-AR 2 protein expression ($p = 0.0003$). Increasing age significantly predicted methylation status and protein expression level ($p = 0.013$).

Conclusions The level of 5-AR 2 protein expression varies among prostate tissue samples. Methylation of the 5-AR 2 gene-promoter may account for low or absent expression of 5-AR 2 in adult human prostate tissues. Increased age correlates with increased 5-AR 2 gene-promoter methylation and decreased protein expression in men with BPH.

Keywords 5-α Reductase · DNA · Hyperplasia · Methylation · Prostate

Introduction

Benign prostatic hyperplasia (BPH), the nonmalignant enlargement of the prostate gland, is a proliferative condition that typically affects older men. Half of all men have histologically identifiable BPH at 60 years old, and

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the prevalence of cases rises by age, peaking at approximately 80% in men aged above 80 years [1, 2]. BPH is diagnosed histologically and characterized by an increased number of epithelial and stromal cells in the periurethral part of the prostate gland [3]. The precise etiology of prostatic hyperplasia is uncertain; androgens, estrogens, stromal–epithelial interactions, growth factors, inflammatory pathways, cytokines, and genetic factors may all play a role in the hyperplastic process. Androgens must be present for BPH to develop; they are also required for normal prostate development and maintenance during puberty and aging. BPH does not occur in men castrated prior to puberty [4]. In men with BPH and normal prostate glands, androgen stimulation is initiated by the irreversible conversion of testosterone into dihydrotestosterone (DHT) by 5-α reductase (5-AR) within the prostate. Because DHT is the major prostate androgen, 5-AR isozymes play a significant role in prostate development [5, 6].Serum testosterone levels decline in aging men, but the relative levels of DHT increase. The higher affinity of DHT for the androgen receptor makes it a more potent androgen than testosterone, and the hormonal changes that occur as men age are important causes of prostate enlargement.

The two main classes of medication for BPH management are α-adrenergic blockers and 5-α reductase inhibitors (5-ARIs). The α-blockers target alpha-1 receptors, causing relaxation of the prostate and bladder-neck smooth muscle. However, α-blockers do not reduce the size of the prostate or the risk of BPH progression. In contrast, 5-ARIs (e.g., finasteride and dutasteride) inhibit the conversion of testosterone to DHT, which reduces prostatic DHT concentrations and leads to epithelial-cell atrophy, increased rates of apoptosis, and a reduction in prostate gland volume [7, 8]. There are two 5-AR enzymes: 5-α reductase type 1 is found in hair follicles, skin, liver, and the prostate gland, whereas 5-α reductase type 2 (5-AR 2) is found predominantly in prostate and pelvic tissues. 5-ARIs are highly effective and utilized widely in patients with lower urinary tract symptoms that are secondary to BPH. Daily treatment with finasteride decreases prostate size by approximately 20–30% within 4–6 months and improves clinical symptoms [9–11].

The landmark Proscar Long-Term Efficacy and Safety (PLESS) study demonstrated that long-term finasteride treatment reduced prostate volume by approximately 18%, improved urinary flow rate and urinary symptom scores, reduced the risk of BPH-related surgery by 55% compared with placebo, and reduced the risk of acute urinary retention by 57%. [10]. However, approximately 30% of patients did not respond to long-term 5-ARI treatment and 22.9% had surgery following the medication [12]. In these cases, the course of the disease was not altered by decreasing the risk of acute urinary retention by 21–43% and the risk of BPH-related surgery by 31–52% [13].

Some reports indicate that older BPH patients with higher baseline symptom scores and larger prostate glands have a greater risk of treatment failure [14]. In addition to these clinical factors, we believe that differences in the levels of 5-AR 2 protein expression and methylation of the 5-AR 2 gene-promotor among prostate tissues could be important. DNA methylation is one of the most common epigenetic mechanisms that can affect or silence gene expression. CpG islands are clusters of CpG dinucleotides found in gene regulatory regions, and they are typically unmethylated. Aberrant hyper-methylation of gene-promoters rich in CpG dinucleotides can alter chromatin structure, recruit methylated DNA-binding proteins and modify histones, leading to gene silencing. This altered regulation of silenced genes can promote the development of disease. Methylation of CpG islands is associated with the regulation of various genes linked to age-related diseases and cancer [15]. It can also inactivate genes as part of the normal aging process [16, 17].

Here we demonstrate that levels of 5-AR 2 protein expression among prostate tissue samples. Additionally, we demonstrate a correlation between DNA methylation of the 5-AR 2 gene-promoter and reduced 5-AR 2 protein expression, providing a potential molecular mechanism for gene silencing. We also evaluated associations between DNA methylation of the 5-AR 2 gene-promoter and age, obesity, lipid profile, and comorbidities to explain why patients are resistant to 5-ARIs.

Materials and methods

Patients and clinical data collection

After obtaining institutional review board approval, prostate specimens were collected from 37 patients between September 2016 and August 2017. A total of 22 samples were taken from men with symptomatic BPH undergoing transurethral prostate resections, and 15 samples were from non-cancerous transition-zone human prostate tissues following radical prostatectomy for prostate cancer. Medical records were reviewed to obtain the clinical and pathological data for each patient, including their age, body mass index (BMI), and comorbidities (hypertension and type II diabetes mellitus). To perform subset analyses, patients were categorized according to World Health Organization Asian BMI criteria, as follows: normal BMI < 23 kg/m^2; overweight 23–24.9 kg/m^2; obese ≥ 25 kg/m^2. Total prostate volumes were measured directly using transrectal ultrasound or calculated from a computerized tomogram within 6 months of surgery using the prostate ellipsoid equation (length × width × height × 0.52). We excluded pelvic floor muscles from prostate contours and we identified prostate apices. This improved prostate volume calculations on CT images. Serum prostate-specific

antigen (PSA) levels, testosterone levels and lipid profiles were collected if preoperative laboratory values obtained < 1 month ago were available.

Tissue processing, immunohistochemistry (IHC), DNA extraction and methylation

Following pathological examination, all prostate tissue samples were frozen and stored at − 80 °C. For the IHC analysis, the samples were incubated with the SRD5A2 primary antibody (Aviva Systems Biology, San Diego, CA, USA) at a concentration of 1:200. After washing, the secondary antibody was applied at a concentration of 1:300. To evaluate immunoreactivity to the 5-AR 2 antibody, 500 cells were assessed manually from three representative areas of each sample at 40× magnification. Levels of immunoreactivity were defined as negative (< 10%), weak (11–30%), moderate (31–60%), or strong (> 60%) staining.

For methylation analyses, DNA was extracted from cell lines and human prostate tissues using the AllPrep®DNA/RNA/Protein Mini kit (QIAGEN, Hilden, Germany). Methylation of CpG islands in the 5-AR 2 was assessed using the Methyl Collector Ultra kit (Active Motif, Inc.). The primers used for polymerase chain reaction (PCR) amplification of the 5-AR 2 gene (*SRD5A2*) were 5′-AAGCGGGAGGTG AATGTAAA-3′ (forward) and 5′-CTTTATGGAGCGCCA GACG-3′ (reverse). The Touch-down PCR amplification conditions were: pre-denaturation at 94 °C for 3 min followed by 16 cycles of amplification (denaturation, 94 °C for 30 s; annealing, 72 °C for 30 s (decreasing by − 0.5 °C/cycle); extension, 72 °C for 20 s). This step was followed by 25 cycles of amplification (denaturation, 94 °C for 30 s; annealing, 64 °C for 30 s; extension, 72 °C for 20 s) and a final extension at 72 °C for 10 min. To determine band intensities, each band was isolated using the ImageJ software selection tool (National Institutes of Health, Bethesda, MD, USA) and its intensity quantified using relative intensity values (ratio of the band's intensity to that if the corresponding loading control). The relative intensities of DNA bands were defined as absent (< 0.10), weak (0.11–0.50), medium (0.51–1.00), and strong (> 1.00). Strong and medium bands were defined as methylation positive.

Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation (SD). Continuous variables are expressed as medians and categorical variables as percentages. Patients were divided into two groups (methylated and unmethylated) and descriptive analyses were performed to compare their clinical information. Statistical analyses were performed using MedCalc (ver. 14.8.1; MedCalc Software, Mariakerke, Belgium).Continuous variables are expressed as mean ± SD and were analyzed using *t* tests. Chi-squared tests or Fisher's exact test were used for dichotomous variables. Univariate logistic regression analysis was performed to identify clinical factors associated with methylation status. Multivariate analysis was performed by stepwise logistic regression modeling using all factors that were significant in the univariate analysis ($p < 0.1$). All tests were two-tailed and a p value < 0.05 was considered statistically significant.

Results

Description of the cohort

We obtained prostate tissue from 22 patients who underwent transurethral resection of the prostate gland for symptomatic BPH, and non-cancerous transition-zone tissue from 15 patients who underwent radical prostatectomy for prostate cancer. We were unable to extract DNA from one of the 37 prostate tissue samples (number 27) and this sample was excluded. Patient characteristics, including age, BMI, prostate volume, PSA and testosterone levels, and lipid profile are shown in Table 1. The mean age of our cohort was 68.4 years. In total, 16% of the men were of normal weight (BMI < 23 kg/m^2), 46% were overweight (BMI 23–24.9 kg/m^2), and 38% were obese (BM I ≥ 25 kg/m^2). The prevalence of diabetes and hypertension in our cohort was 27.7 and 55.5%, respectively. In total, 27 of the 36 patients 75%) had methylated 5-AR2 gene-promoter regions. There were statistically significant differences in age and total cholesterol level between the two groups.

5-AR 2 protein expression in BPH samples

We evaluated for expression of the 5-AR 2 protein on 36 prostate tissue specimens. We found significant variability in 5-AR 2 protein expression among the samples (Fig. 1). 5-AR 2 was expressed mainly in epithelial cells, but expression levels in both the cytoplasm and nuclei varied significantly. There was strong (5%), moderate (17%), and weak (50%) immunoreactivity, and 28% of the prostate tissue samples (10/36) expressed no 5-AR 2 protein (Fig. 1a).

Methylation of the 5-AR 2 gene-promoter in BPH tissue samples

Using methylation-specific PCR methods and primers designed to amplify CpG islands in the 5-AR 2 gene, we found that a significant proportion of human adult prostate transition-zone tissue contained heavily methylated CpG islands. In total, 26 of the 36 (72%) transition-zone tissue samples contained methylated CpG islands in the promoter-region of the 5-AR 2 gene (Fig. 2). These findings

Table 1 Demographic and clinical characteristics of the cohort

	Overall	Methylated	Unmethylated	*p* value
Patients (%)	36 (100)	26 (72)	10 (28)	–
Age (years)				
Mean ± SD (range)	68.4 ± 6.67 (56–83)	68.3 ± 6.93 (56–83)	62.5 ± 3.64 (57–70)	0.008
< 65, *n*	12	8	7	
≥ 65, *n*	24	19	2	
BMI (kg/m^2)				
Mean ± SD (range)	24.9 ± 2.87 (17.9–32.8)	22.9 ± 3.13 (17.9–32.8)	27.9 ± 2.26 (21.4–28.3)	0.913
WHO Asian BMI cut-offs				
Normal (< 22.9)	6	5	1	
Overweight (23–24.9)	17	12	5	
Pre-obese (25–29.9)	11	8	3	
Obese (≥ 30)	2	2		
Total prostate volume (cc)				
Mean ± SD/median (range)	45.5 ± 21.28 (18.2–103.2)	53.0 ± 20.56 (18.2–103.2)	43.3 ± 23.98 (27.3–89.7)	0.138
≤ 40, *n*	20	16	4	
> 40, *n*	16	11	5	
Transitional zone volume (cc)	23.4 ± 14.8 (7.5–63.4)	28.1 ± 14.1 (7.5–63.4)	22.1 ± 17.4 (9.4–56.9)	0.383
Median PSA, ng/ml (range)	7.68 ± 11.86 (0.46–51.50)	8.77 ± 13.29 (0.46–51.50)	6.64 ± 4.89 (0.32–15.96)	0.654
Median testosterone, ng/dL (range)	271.15 ± 126.35 (128.42–563.87)	244.90 ± 108.23 (128.42–550.27)	385.48 ± 158.59 (158.84–563.87)	0.656
Lipid profile (mg/dL)				
Median total cholesterol (range)	159.9 ± 40.18 (98.8–263.6)	161.8 ± 42.23 (106.0–263.6)	149.3 ± 33.30 (98.8–209.0)	0.002
Median Triglyceride (range)	144.3 ± 100.18 (26.0–485.0)	157.0 ± 111.35 (43.0–485.0)	113.6 ± 51.94 (26.0–175.0)	0.522
Median HDL (range)	38.24 ± 8.43 (25.0–65.0)	37.43 ± 6.03 (27.0–49.0)	37.68 ± 10.46 (25.6–57.8)	0.896
Median LDL (range)	90.72 ± 30.4 (39.0–153.0)	90.15 ± 32.46 (39.0–153.0)	88.74 ± 24.27 (43.9–125.0)	0.822
Number with comorbidity (%)				
Hypertension	18 (50.0)	14	4	0.704
Diabetes	10 (27.7)	8	2	0.671
Pathology data				
Combined chronic prostatitis (%)	21 (59.4)	15	6	0.563
Combined prostate cancer (%)	15 (41.6)	9	6	0.451

SD standard deviation, *BMI* body mass index, *WHO* World Health Organization, *PSA* prostate-specific antigen, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *cc* cubic centimeters

suggest that males with methylated CpG islands in the 5-AR 2 promoter may not express the 5-AR 2 enzyme. To evaluate whether CpG methylation of the 5-AR promoter-region correlated with low-absent levels of 5-AR 2 protein, we repeated our IHC analysis of the transition-zone prostate tissues. Receiver-operating characteristic (ROC) curves were created to evaluate the diagnostic sensitivity and DNA methylation of 5-AR 2, based on the different cut-off values for 5-AR 2 expression. The cut-off values corresponded to the largest Youden index of the ROC curves. The optimal cut-off value was 32% (sensitivity 92%; specificity 60%) (Fig. 3, Table 2). Tissue samples were defined as having low-absent 5-AR 2 protein expression if ≤ 32% of the cells were immunoreactive. If > 32% of the epithelial cells were immunoreactive, the samples were considered positive for 5-AR 2 protein. There was a correlation between methylation of the 5-AR 2 promotor and IHC results showing ≤ 32% 5-AR 2 protein. The six cases where methylation of the 5-AR 2 promotor did not correlate with the IHC results are marked in bold (N) (Table 3). We found a direct correlation between the methylated 5-AR 2 promotor region and decreased 5-AR 2 protein expression using Fisher's exact test. Table 4 shows the results of a two-tailed contingency analysis that demonstrates the strong correlation between these two variables ($p = 0.0003$).

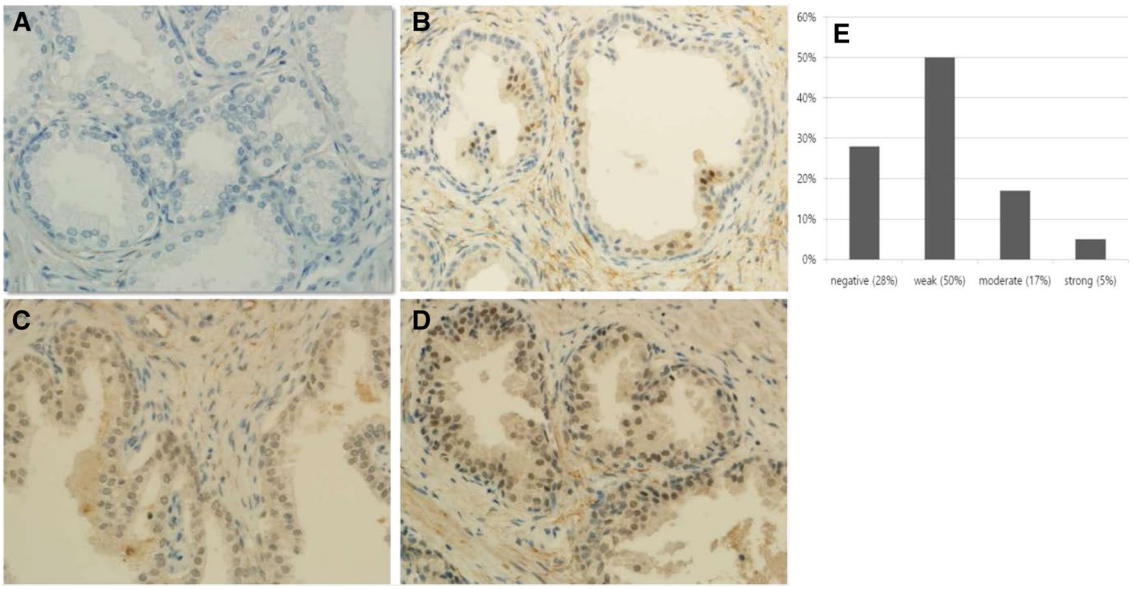

Fig. 1 Heterogeneous expression of 5-α reductase 2 (5-AR 2) in different human prostate tissues 5-AR 2 is expressed in prostate epithelial-cell nuclei: **a** no expression in prostate tissue (0%); **b** weak expression (19%); **c** moderate expression (48%); **d** higher expression in epithelial-cell nuclei (69%); **e** the expression levels of 5-AR 2 protein vary among benign human prostate tissues

Clinical factors associated with methylation status and protein expression

Our univariate analysis demonstrated that 5-AR 2 promotor methylation status was significantly associated with age ($p=0.008$) and total serum cholesterol level ($p=0.002$). BMI, prostate volume, serum PSA level, and comorbidities were not correlated with methylation status. Additionally, there was no association between pathological group (combined chronic prostatitis or prostate cancer) and methylation status in our cohort. Multivariate logistic regression analysis was performed to identify predictive clinical variables and found a significant association between age and 5-AR 2 promotor methylation status ($p=0.013$; Table 5).

Methylation and Expression of 5-AR2 in BPH and Prostate cancer

There are no difference in expression of 5-AR2 nuclear immunostaining between BPH and non-cancerous transition-zone human prostate tissues following radical prostatectomy for prostate cancer ($p=0.557$). Also there has a different outcome in methylation of 5-AR2 gene promotor between the two tissue types ($p=0.362$) (Table 6).

Discussion

Androgens stimulating their receptors play a significant role in prostate gland growth, but testosterone is not the major androgen responsible. DHT is a potent androgen metabolite produced from testosterone by the enzymes 5-AR type 1 (in the liver) and 5-AR 2 (in prostate tissue). DHT is primarily responsible for the development and maintenance of the prostate gland, and also for BPH. Inhibitors of 5-AR can reduce the size of the prostate gland by 20–30% and can also relieve symptoms of the lower urinary tract. The enzyme 5-AR 2 plays a key role in regulating the development and growth of the prostate gland [18].

Reduced 5-AR 2 expression and DHT production during gestation have been linked directly with the development of a smaller prostate gland and with female pseudohermaphroditism, which involves abnormal development of the external genitalia, including hypospadias, preputial adhesions to the glans, a small underdeveloped scrotum, a micro-penis, and a predominant median raphe [19, 20].

However, little is known about the effects of absence of 5-AR 2 expression in adult human males. Niu et al. reported that 29% (6/21) of normal radical prostatectomy tissue specimens did not express 5-AR 2 protein and 39% (7/18) of

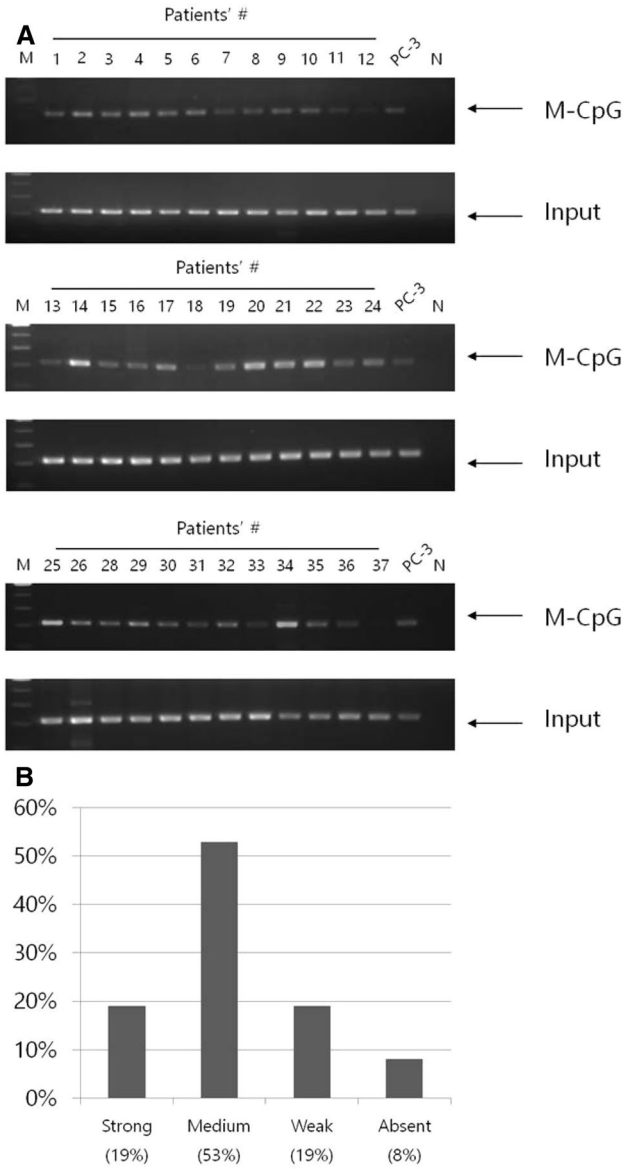

Fig. 2 Methylation of CpG islands in the promoter-region of the 5-AR 2 gene in benign human prostate samples. **a** Examples of methylation of CpG islands in the promoter-regions of the 5-AR 2 gene in human prostate samples. A total of 37 prostate samples are shown; we were unable to extract DNA from one sample (no. 27) and this was excluded. **b** The intensities of the DNA bands of each subgroup were classified using the selection tool in ImageJ software (National Institutes of Health), as follows: strong (19%), medium (53%), weak (19%), and absent (8%)

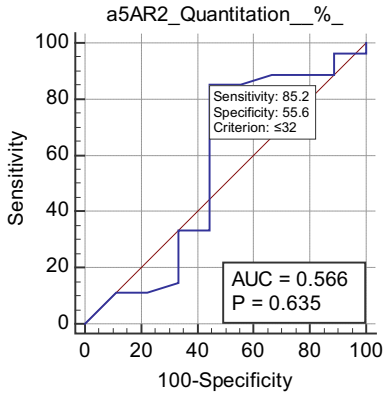

Fig. 3 Receiver-operating characteristic (ROC) curves for 5-AR 2 expression. Area under curve is 0.566

Table 2 Real data showing sensitivity and specificity at various cut-off points of 5-AR 2 expression

Cut-off values	Sensitivity	95% CI	Specificity	95% CI
≤0	11.11	2.4–29.2	88.89	51.8–99.7
≤1	11.11	2.4–29.2	77.78	40.0–97.2
≤3	14.81	4.2–33.7	66.67	29.9–92.5
≤7	33.33	16.5–54.0	66.67	29.9–92.5
≤9	33.33	16.5–54.0	55.56	21.2–86.3
≤32	85.19	66.3–95.8	55.56	21.2–86.3
≤34	85.19	66.3–95.8	44.44	13.7–78.8
≤35	88.89	70.8–97.6	33.33	7.5–70.1
≤40	88.89	70.8–97.6	11.11	0.3–48.2
≤54	96.3	81.0–99.9	11.11	0.3–48.2
≤63	96.3	81.0–99.9	0	0.0–33.6
≤69	100	87.2–100.0	0	0.0–33.6

benign prostate transition-zone specimens were methylated at the 5-AR 2 gene-promoter [21]. Another study showed that 46% (45/96) of patients who underwent transurethral resection of the prostate were symptomatic for BPH with 10% or fewer considered negative for 5-AR 2 expression, while 49% (47/96) had methylation at the 5-AR 2 gene-promotor [22]. These studies suggest that decreased levels of 5-AR 2 protein are associated with resistance to finasteride treatment, which is used to manage BPH, or with different prostate growth rates during adulthood. Here, we demonstrated that 28% (10/36) and 50% (18/36) of histologically benign adult human prostatic samples had absent and low levels of 5-AR 2 protein expression, respectively. We also showed that 72% (26/36) of the patients had methylation at the 5-AR 2 gene-promoter. Additionally, we demonstrated a strong correlation between methylation of the 5-AR 2 promoter-region and absence of 5-AR 2 protein expression. The frequency of 5-AR 2 promotor methylation in our sample differs from that reported by other studies, suggesting that differences in age, race, or other factors may influence promotor methylation.

Many of the changes that occur during prostate cancer are epigenetic, but few independent studies have been performed on BPH patients. Hyper-methylation of *GSTpi*is one of the most frequent epigenetic modifications observed in high-grade prostatic intraepithelial neoplasia, which

Table 3 Summary of the 5-AR 2 protein immunoreactivity and 5-AR 2 promotor methylation results

Prostate sample number	Quantity of 5-AR 2 protein based on IHC analysis (%)	5-AR 2 protein low-absent (cut-off ≤ 32%)	5-AR 2 CpG methylation	Relative intensity of DNA band (target/input)	CpG methylation status correlates with IHC expression
37	69	N	N	0.01	Y
12	61	N	N	0.19	Y
36	38	N	N	0.03	Y
25	37	N	Y	1.44	**N**
15	35	N	N	0.29	Y
18	35	N	N	0.04	Y
20	34	N	Y	1.18	**N**
11	34	N	N	0.19	Y
22	31	Y	Y	1.12	Y
14	31	Y	Y	1.46	Y
24	30	Y	Y	0.77	Y
3	27	Y	Y	0.58	Y
17	27	Y	Y	0.65	Y
13	23	Y	N	0.17	**N**
16	21	Y	Y	0.74	Y
7	20	Y	Y	0.59	Y
33	19	Y	N	0.14	**N**
21	19	Y	Y	0.85	Y
29	18	Y	Y	1.07	Y
23	18	Y	N	0.36	**N**
26	16	Y	Y	0.52	Y
19	15	Y	Y	0.54	Y
35	15	Y	Y	0.82	Y
28	14	Y	Y	0.69	Y
4	12	Y	Y	0.86	Y
32	11	Y	Y	0.58	Y
31	9	Y	N	0.27	**N**
5	7	Y	Y	0.77	Y
6	6	Y	Y	0.72	Y
30	6	Y	Y	0.64	Y
34	6	Y	Y	2.86	Y
2	5	Y	Y	1.10	Y
9	3	Y	Y	0.57	Y
1	0	Y	Y	0.58	Y
8	0	Y	Y	0.60	Y
10	0	Y	Y	0.58	Y

IHC immunohistochemistry, *Y* yes, *N* no

Table 4 Methylation status of the 5-AR 2 promotor correlates with 5-AR 2 protein expression in benign prostatic tissue samples

5-AR 2 protein expression	Methylated	Unmethylated	Total	*p* value
Low–absent	24	4	28	0.0003
High	2	6	8	

Table 5 Results of multiple logistic regression analysis to determine factors associated with 5-AR 2 methylation status and protein expression

5-AR 2 methylation status	OR	95% CI	*p* value
Age	1.26	1.04–1.52	0.013
Total cholesterol	1.02	0.99–1.05	0.164

OR odds ratio, *95% CI* 95% confidence interval

Table 6 Comparison of methylation and expression of 5-AR2 in BPH and prostate cancer

	BPH ($n=22$)	PCa ($n=15$)	p value
5-AR 2 methylation status			0.3624
Methylated	17	9	
Unmethylated	4	6	
5-AR 2 protein expression			
Low-absent	16	12	0.5572
High	5	3	

precedes the development of prostate cancer. BPH tissue samples have significantly lower global 5-methylcytosine levels than normal prostate tissue samples [23]. Additionally, several genes, including *MDR1*and *RASSF1α*, are reportedly hyper-methylated in BPH tissue samples, and this may be linked to prostate growth dysfunction [24].

Gene expression may be regulated at the level of transcription, translation, or post-translation. In addition, epigenetic modifications, including methylation in promoter-regions and histone modifications can lead to gene silencing [25]. During DNA methylation, methyl groups (CH_3^-) are added to the carbon-5 position of cytosine nucleotides. Methylation can change the activity of a DNA segment. Epigenetic control by DNA methylation of promoter-regions within CpG dinucleotide islands can alter chromatin structure, recruit DNA-binding proteins and inactivate transcription, leading to gene silencing [21, 25]. DNA methylation plays a prominent role in regulating gene expression and influencing the downstream functions that can lead to disease phenotypes.

Many pathological processes, including cancer and age-related diseases, are associated with CpG island methylation in gene-promoters. Cancer cells generally show a high degree of aberrant DNA methylation, and older cells have increased levels of methylation in gene-promoter CpG islands [26]. Because the prostate gland grows as it ages, it may be influenced by epigenetic mechanisms similar to those that occur during tumor growth.

There is difficulty to explain the link between increased cholesterol and 5-AR 2 promotor methylation status.

In some population studies have reported on the association between inter-individual variation in blood lipid levels and genome-wide DNA methylation, a key component of the epigenome, in circulating immune cells [27–29].

Although our patient numbers were small, we could not find a link between an increased total serum cholesterol level and obesity; the general consensus is that the total cholesterol level rises as the BMI increases [30–32].

A significant positive association between hypercholesterolaemia and BMI has been reported [33].

Increasing BMI is associated with increased global methylation of genes associated with obesity in both subcutaneous and omental adipose tissue [34, 35].

Interestingly, after gastric bypass followed by significant weight loss, patients experienced a global decrease in gene methylation accompanied by hypomethylation of skeletal muscle genes involved in metabolic processes and mitochondrial function, highlighting the dynamic nature of epigenetic modifications [36].

These studies suggest that the internal environment is influenced by changes in total body weight and the associated epigenetic signature. However, these observations are not adequate to explain why cholesterol levels rise as 5-AR2 promotor methylation increases, or how this may be affected by lifestyle.

The causes of alterations in DNA methylation status remain unknown, but they may be strongly associated with aging, chronic inflammation, and epidemiological factors, notably diet and exposure to environmental chemicals.

Recent work has demonstrated that methylation drift can occur in the genes of many tissues during aging due to epigenetic changes that are driven by chronic inflammation. This includes the effects of *Helicobacter pylori* infection on the stomach, inflammatory bowel disease on the colon, and smoking on the lungs [37–39].

These studies suggest that the internal environment of an individual is influenced by changes in total body weight and its associated epigenetic signature. However, these observations are not enough to explained the mechanism of rising cholesterol levels with 5-AR 2 promotor methylation status and how it may be influenced by lifestyle.

Additionally, significant increases in methylation of the estrogen receptor-α gene and estrogen receptor-β promoter-area were reported in aging cardiovascular tissues and atherosclerotic plaques [40].

There is also significant interest in the possible effects of dietary and environmental chemical exposure on epigenetic changes, and in particular the effects of folate and vitamin B12. However, the effects of these factors on DNA methylation in adults are uncertain. Our findings suggest that hyper-methylation associated with aging may function as an epigenetic marker for a distinct BPH pathology. Although the roles and interactions of abnormal DNA methylation are uncertain, the evidence suggests that aging may cause aberrant DNA methylation [41].

Thomas et al. reported that the expression level of 5-AR2 was lower in PIN and PCa than BPH [42]. In contrast, Titus et al. observed high-level expression of 5-AR1 and 5-AR2 in PCa; and higher-level expression in high-grade PCa than low-grade PCa [43, 44].

Thus, each study reported a different outcome. We harvested non-cancerous, transition-zone, human prostate tissues from prostate cancer specimens, to reduce bias.

We found no difference between BPH and non-cancerous, transition-zone, human prostate tissues following radical prostatectomy to treat prostate cancer.

This study had some limitations. Our sample size was relatively small, and we did not use a longitudinal design. Therefore, our observations are restricted to one time point. As a result, we are currently evaluating whether slower prostate growth rates in adult men and/or resistance to treatment with 5-ARIs are associated with reduced levels of 5-AR 2 protein.

Our results provide a basis for future studies to assess how changes in prostate gland volume and symptoms may be linked to 5-ARI medication, 5-AR 2 protein expression, and gene-promoter methylation. Understanding silencing in the 5-AR 2 gene may enable clinicians to predict the prognosis for BPH therapy in individual cases.

The level of 5-AR 2 protein expression varied significantly among tissue samples from patients with BPH. Methylation of the 5-AR 2 gene-promoter correlated strongly with reduced or absent protein expression. There was also a correlation between patient ages and methylation of the 5-AR 2 gene-promoter in BPH tissue samples. These results suggest an epigenetic signature, which may be one reason for differences in efficacy among 5-ARIs used to treat BPH patients.

Acknowledgements This study was supported in part by a grant from Kosin University College of Medicine (2016) (to P.M.K), and also by Korea National Research Foundation (KNRF) grants 2015M3A9B6073646, 2015R1D1A1A01058387 and 2017M3A9G7072564 (to J.Y.J).

Author contributions PMK: first author, conception and design, drafting the manuscript, statistical analysis, YJK: co-first author (PMK and YJK contributed equally to the work) performed the experiments (Tissue processing, DNA extraction and methylation analysis), WTS: protocol/project development, data analysis, SHK: critical revision of the manuscript for scientific and factual content, TSK: critical revision of the manuscript for scientific and factual content, BKC: pathological examination, immunohistochemistry analysis, WIS: critical revision of the manuscript for scientific and factual content, JYJ: co-corresponding author (JYJ and JIC contributed equally to the work) contributed reagents/materials/analysis tools, JIC: conception and design, Supervision.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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