

# Promoter Polymorphism (rs12770170, -184C/T) of Microseminoprotein, Beta as a Risk Factor for Benign Prostatic Hyperplasia in Korean Population

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**Purpose:** Benign prostatic hyperplasia (BPH) is the most common prostate disease in aging men. Microseminoprotein-beta (MSMB) is abundant in semen. In this study, we investigated association between single nucleotide polymorphisms (SNPs) at the promoter of the *MSMB* gene and the risk for developing BPH in a Korean population.

**Methods:** We genotyped two promoter polymorphisms (rs12770171, -184C/T and rs10993994, -2C/T) of the *MSMB* gene by direct sequencing. Ninety-five BPH patients and 78 control subjects were recruited for this study. SNPStats and Haploview version 4.2 were used for genetic analyses. Multiple logistic regression models (codominant, dominant, recessive, and log-additive models) were applied to determine the odds ratio (OR), 95% confidence interval (CI), and P-value.

**Results:** Genotype frequency of the rs12770171 SNP showed significant difference between BPH patients and controls (OR, 2.14; 95% CI, 1.07–4.27;  $P=0.032$  in the codominant 1 model; OR, 2.31; 95% CI, 1.19–4.47;  $P=0.011$  in the dominant model; and OR, 2.05; 95% CI, 1.17–3.61;  $P=0.009$  in the log-additive model). Moreover, the SNP also showed association between the two groups (OR, 2.05; 95% CI, 1.19–3.52;  $P=0.009$ ). The rs10993994 SNP was not associated with BPH. In haplotype analysis, CC and TT haplotypes were associated with BPH ( $P<0.05$ ).

**Conclusions:** This result indicates that a promoter polymorphism (rs12770170, -184C/T) in the *MSMB* gene may be associated with BPH development in a Korean population.

**Keywords:** Prostatic hyperplasia; Single nucleotide polymorphism; Beta-microseminoprotein

## INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common prostate disease in aging men [1]. Prevalence of BPH is >50% in 60-year-old men, and it is found in nearly all men more than 80 years of age [2]. BPH results in obstruction in urinary flow and produces lower urinary tract symptoms. Until now, the etiology of BPH is unknown. In order to diagnosis BPH, prostate specific antigen (PSA) marker is used. However, it can be used as an assistant marker to predict BPH development [3].

Some previous studies showed that the family history of BPH

and genetic polymorphisms are risk factors associated with an early age of onset of BPH [4-6]. Recently, several researchers reported a genetic association between BPH development and polymorphisms of specific genes including the vitamin D (1,25-dihydroxyvitamin D<sub>3</sub>) receptor (*VDR*) (FokI, BsmI, ApaI, and TaqI loci), aldehyde dehydrogenase 2 (*ALDH2*), and nitric oxide synthase 2 (*NOS2*) genes [7-9].

Microseminoprotein-beta (MSMB) is the second most abundant protein in semen after PSA [10,11]. MSMB is a cysteine-rich, 16-kDa protein containing 94 amino acids. It is also known as prostate secretory protein of 94 amino acids (PSP94) and

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$\beta$ -inhibin [11-13]. The major biological function of MSMB is to inhibit the activity/secretion of the follicle stimulating hormone [13-15]. MSMB expression is observed in various zones of the prostate [16]. Whitaker et al. [13] reported that MSMB expression is high in normal and benign prostate tissue and is low or absent in prostate cancer tissues. Therefore, they suggested that MSMB might be a useful biomarker to diagnose prostate cancer, and that its serum levels may be useful as a prognostic marker [13].

In this study, we investigated whether two promoter polymorphisms (rs12770171, -184C/T and rs10993994, -2C/T) of the *MSMB* gene were associated with the BPH development in a Korean population.

## MATERIALS AND METHODS

### Study Subjects

We recruited 173 patients for this study. Patients who visited the Kyung Hee University Medical Center between January 2002 and December 2008 for treatment of lower urinary tract symptoms were selected. The clinical symptoms of the patients were evaluated according to the International Prostate Symptom Score, and the prostate volume of all patients was determined using transrectal ultrasonography (TRUS).

The levels of PSA in the sera of the patients was tested. The patients with a serum PSA level  $>4$  ng/mL were further tested by transrectal ultrasound-guided prostate biopsy to rule out prostate cancer. Prostate size was assessed by TRUS. Patients with prostate cancer, neurogenic bladder, urethral stricture, acute/chronic prostatitis, urinary tract infection, uncontrolled diabetes mellitus, hypertension, or those who had undergone previous pelvic surgery were excluded from this study.

We divided the patients into the BPH and control groups according to the diagnosis of BPH (control group, prostate vol-

ume  $<30$  mL; BPH group, prostate volume  $>30$  mL). The demographic characteristics of the BPH and control groups are shown in Table 1. The numbers of patients in the BPH and control groups were 95 and 78, respectively.

All patients provided informed consent for use of their samples and clinical data. The Institutional Review Board at Kyung Hee University Medical Center approved the methods used in this study.

### SNP Selection and Genotyping

To select promoter SNPs of the *MSMB* gene, we searched SNPs on the promoter region of the *MSMB* gene in the SNP database (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 141). SNPs with  $>0.1$  heterozygosity and 10% minor allele frequency were selected. Of these, we chose the two promoter polymorphisms rs12770171, -184C/T and rs10993994, -2C/T. The genotypes were determined by direct sequencing. Before sequencing, polymerase chain reaction (PCR) was conducted. Genomic DNA was amplified using the following primers: sense; 5'-CTG GTG GGC TGA CCT GTG GGC T-3' and antisense: 5'-GAT AAG CAG GAC TCC TTA TAG-3'. PCR was performed for 39 cycles at 94°C for 30 seconds, 59°C for 30 seconds, 72°C for 1 minute, and then at 1 cycle at 72°C for 7 minutes to terminate the reaction. The PCR products were identified by electrophoresis on 1.8% agarose gel. The PCR products were sequenced using an ABI Prism 377 automatic sequencer (PE Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using SeqManII ver. 2.3 (DNASTAR Inc., Madison, WI, USA).

### Statistical Analysis

For the analysis of genetic data, SNPStats (<http://bioinfo.iconcolgia.net/index.php>) was used. The chi-square test was used to evaluate Hardy-Weinberg equilibrium of the two promoter SNPs. Next, the effect of SNPs was tested by multiple logistic regression models (codominant 1, codominant 2, dominant, recessive, and log-additive) for odds ratio (OR), 95% confidence interval (CI), and P-values; all the tests were run by adjusting for age as a covariable. A linkage disequilibrium (LD) block of SNPs in the *MSMB* gene was performed using Gabriel's algorithm in Haploview ver. 4.2 [17]. P-value significance was set at 0.05.

## RESULTS

Ninety-five BPH patients and 78 control subjects were genotyped in order to evaluate whether the two promoter polymor-

**Table 1.** Clinical data of participants included in the study

Variable	BPH (n = 95)	Control (n = 78)
Age (yr)	67.19 $\pm$ 8.08	63.19 $\pm$ 10.23
Prostate volume (mL)	52.79 $\pm$ 21.09	22.50 $\pm$ 5.29
IPSS	18.36 $\pm$ 7.40	15.27 $\pm$ 8.10
Serum PSA (ng/mL)	6.48 $\pm$ 6.00	2.16 $\pm$ 2.27

Values are presented as mean  $\pm$  standard deviation.

IPSS, International Prostate Symptom Score; BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen.

phisms (rs12770171, -184C/T and rs10993994, -2C/T) of the *MSMB* gene were associated with BPH. The genotype distributions of the two promoter SNPs in this study were in Hardy-Weinberg equilibrium in the control subjects (rs12770171,  $P=1.00$ ; rs10993994,  $P=0.65$ , data not shown). Table 2 shows the genotype and allele distributions of two promoter SNPs in BPH patients and controls.

In allele distribution analysis, the T-allele frequency of rs12770171 was higher in the BPH group (27.1%) than in the control group (15.4%). This difference indicated that the polymorphism had a significant association with BPH (OR, 2.05; 95% CI, 1.19–3.52;  $P=0.009$ ) (Table 2). The C-allele frequency of rs10993994 was also higher in the BPH group (53.2%) than in the control group (42.9%). However, the rs10993994 allele

did not show a significant association with BPH ( $P=0.06$ ).

Genotype analysis between the BPH and the control group was performed using logistic regression analysis with adjustment for age. There were significant differences between the BPH and control groups (rs12770171: OR, 2.14; 95% CI, 1.07–4.27;  $P=0.032$  in the codominant 1 model [C/C vs. C/T]; OR, 2.31; 95% CI, 1.19–4.47;  $P=0.011$  in the dominant model [C/C vs. C/T + T/T]; and OR, 2.05; 95% CI, 1.17–3.61;  $P=0.009$  in the log-additive model [C/C vs. C/T vs. T/T]) (Table 2).

Two SNPs (rs12770171, -184C/T and rs10993994, -2C/T) of the *MSMB* gene were analyzed for LD and haplotypes using Haploview ver. 4.2. The LD block was composed of rs10993994 and rs12770171 ( $D=1.00$  and  $r^2=0.287$ ). There were three haplotypes in the LD block (haplotype CC, frequency=0.51; haplo-

**Table 2.** Genotype and allele frequencies of promoter polymorphisms *MSMB* gene in BPH patients and controls

SNP	Genotype/allele	BPH, n (%)	Control, n (%)	Models	OR (95% CI)	P-value
rs12770171, -184 C/T promoter	C/C	51 (54.3)	56 (71.8)	Codominant 1	2.14 (1.07–4.27)	0.032
	C/T	35 (37.2)	20 (25.6)	Codominant 2	3.76 (0.75–18.78)	0.110
	T/T	8 (8.5)	2 (2.6)	Dominant	2.31 (1.19–4.47)	0.011
				Recessive	2.96 (0.60–14.57)	0.150
				Log-additive	2.05 (1.17–3.61)	0.009
					1	
rs10993994, -2 C/T promoter	C	137 (72.9)	132 (84.6)		1	
	T	51 (27.1)	24 (15.4)		2.05 (1.19–3.52)	0.009
	C/C	22 (23.4)	24 (31.2)	Codominant 1	1.32 (0.63–2.76)	0.460
	C/T	44 (46.8)	40 (51.9)	Codominant 2	2.29 (0.94–5.58)	0.070
	T/T	28 (29.8)	13 (16.9)	Dominant	1.57 (0.79–3.16)	0.200
				Recessive	1.92 (0.90–4.08)	0.090
			Log-additive	1.50 (0.96–2.33)	0.070	
				1		
				1.52 (0.99–2.33)	0.060	

P-values were derived from logistic regression analyses with the codominant, dominant, recessive, and log-additive models.

*MSMB*, Microseminoprotein-beta; BPH, benign prostatic hyperplasia; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval. Missing genotype data are omitted.

**Table 3.** Haplotype analysis for association between the *MSMB* gene cluster polymorphisms and BPH

Haplotype	Frequency	BPH		Control		Chi-square	P-value
		+	-	+	-		
CC	0.51	88.0	102.0	89.3	66.7	4.10	0.043
TC	0.27	51.0	139.0	42.7	113.3	0.01	0.910
TT	0.22	51.0	139.0	24.0	132.0	6.62	0.010

Haplotype consists of rs10993994 and rs12770171.

*MSMB*, Microseminoprotein-beta; BPH, benign prostatic hyperplasia; (+), ratio a specific haplotype of case or control; (-), ratio not a specific haplotype of case or control.

type TC, frequency=0.27; haplotype TT, frequency=0.22) (Table 3). Haplotypes CC and TT were found to be associated with BPH (haplotype CC, chi-square=4.10, P=0.043; haplotype TT, chi-square=6.62, P=0.010) (Table 3).

## DISCUSSION

This is the first study to investigate the association between two promoter SNPs (rs12770171, -184C/T and rs10993994, -2C/T) and BPH development. Some previous studies have focused on the correlation between polymorphisms of the *MSMB* gene and prostate cancer [18-20]. Although BPH is not directly linked to prostate cancer, epidemiological studies suggest that patients with BPH have an increased risk of prostate cancer [21]. Therefore, some biomarkers of prostate cancer are thought to be associated with BPH development.

In a genome-wide association study (GWAS), Thomas et al. [22,23] found that the rs10993994 SNP of the *MSMB* gene was significantly associated with development of prostate cancer. The SNP rs10993994 (-59C/T) is located in the proximal promoter region (-59 bp) of the *MSMB* gene. This gene encodes for the prostatic secretory PSP94 protein, which is one of the major proteins secreted by the human prostate gland [10]. Recently, it was reported that the total PSP94 protein level in serum could be used to predict prostate cancer [24].

We analyzed the effect of the SNPs of the *MSMB* gene on BPH development. We also investigated the association of the haplotypes of the SNPs with susceptibility to BPH. We observed a significant association between the promoter polymorphism rs12770171 SNP (-184C/T) of the *MSMB* gene and BPH development, while the rs10993994 SNP did not show any significant association with BPH.

In the SNP database (<http://www.ncbi.nlm.nih.gov/SNP>, BUILD 141), the C- and T-allele frequencies in the rs10993994 SNP have been reported to be 0.659 and 0.341 in Europeans, 0.593 and 0.407 in the Chinese, 0.587 and 0.413 in the Japanese, and 0.288 and 0.712 in Sub-Saharan Africans, respectively. In the control group recruited for this study, the C- and T-allele frequencies of rs10993994 were 0.846 and 0.154, which are higher than those seen in other Asian populations. Moreover, the C- and T-allele frequencies of the rs12770171 SNP did not exist in SNP database. In this study, we first identified the C- and T-allele frequencies of the rs12770171 SNP. The C- and T-allele frequencies of rs12770171 were 0.571 and 0.429, respectively, in the Korean population.

To examine whether the rs12770171 SNP (-184C/T) influences transcription factors, we compared the transcription factor binding sites using the online program AliBaba 2.1 (<http://www.gene-regulation.com/pub/programs/alibaba2>). At the rs12770171 SNP (-184C/T) site, three transcription factors (Sp1) can bind to the C-containing sequence, but not to the T-containing sequence. This difference in transcription factor binding may influence *MSMB* expression.

To our knowledge, this is the first study to examine whether promoter SNPs (rs12770171, -184C/T and rs10993994, -2C/T) of the *MSMB* gene are associated with development to BPH in a Korean population. We found that the promoter polymorphism (rs12770171, -184C/T) of the *MSMB* gene is associated with BPH development. The T-allele frequency of the rs12770171 SNP was higher in the BPH group than the control group. This result indicates that promoter polymorphism (rs12770171, -184C/T) of the *MSMB* gene may contribute to BPH development in the Korean population. In future, additional studies with larger sample sizes or different populations will be needed to confirm our results.

## CONFLICT OF INTEREST

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

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