Association between polymorphisms of estrogen receptor 2 and benign prostatic hyperplasia

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Received October 2, 2014; Accepted August 20, 2015

DOI: 10.3892/etm.2015.2755

Abstract. Estrogens and estrogen receptors (ESRs) have been implicated in the stimulation of aberrant prostate growth and the development of prostate diseases. The aim of the present study was to investigate four single nucleotide polymorphisms (SNPs) of the *ESR2* gene in order to examine whether *ESR2* is a susceptibility gene for benign prostatic hyperplasia (BPH). In order to evaluate whether an association exists between *ESR2* and BPH risk, four polymorphisms [rs4986938 (intron), rs17766755 (intron), rs12435857 (intron) and rs1256049 (Val328Val)] of the *ESR2* gene were genotyped by direct sequencing. A total of 94 patients with BPH and 79 control subjects were examined. SNPStats and Haploview version 4.2 we used for the genetic analysis. Multiple logistic regression models (codominant1, codominant2, dominant, recessive and log-additive) were produced in order to obtain the odds ratio, 95% confidence interval and P-value. Three SNPs (rs4986938, rs17766755 and rs12435857) showed significant associations with BPH (rs4986938, P=0.015 in log-additive model; rs17766755, P=0.033 in codominant1 model, P=0.019 in dominant model and P=0.020 in log-additive model; rs12435857, P=0.023 in dominant model and P=0.011 in log-additive model). The minor alleles of these SNPs increased the risk of BPH, and the AAC haplotype showed significant association with BPH (χ^2=6.34, P=0.0118). These data suggest that the *ESR2* gene may be associated with susceptibility to BPH.

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Key words: benign prostatic hyperplasia, single nucleotide polymorphism, estrogen receptor 2, *ESR2*, estrogen

Introduction

Benign prostatic hyperplasia (BPH), also known as benign prostatic hypertrophy, is the most common prostate disease affecting elderly men. Approximately 50% of men aged 51-60 years and ~90% of men aged 81-90 years have BPH (1,2). Clinically, BPH is associated with lower urinary tract symptoms (LUTS) (3). BPH is commonly viewed as a benign enlargement of the prostate, which contributes to an array of urinary voiding difficulties. The molecular etiology of BPH involves numerous complicated processes, and thus the exact cause remains unknown. Various theories have been suggested, such as embryonic reawakening, aging, androgens, estrogens and inflammation (4).

Estrogen, a female hormone, plays an essential role in the development of female secondary sexual characteristics; however, it is also produced in men. Estrogen is involved in the stimulation of prostate growth and the development of prostatic diseases in men (5,6). Estrogen action is mediated by two estrogen receptors (ESRs), which can be classified into two subtypes: ESR1 (ERα) and ESR2 (ERβ) (5). ESR1 and ESR2, however, are not isoforms but are encoded by separate genes on different chromosomes (5). *ESR1* and *ESR2* are located on chromosome 6q25.1 and chromosome 14q23.2, respectively (7). The expression of *ESR2* in the ventral prostate shows a different pattern from that of *ESR1*. While *ESR1* is usually located in the stromal cells of the prostate, *ESR2* is primarily expressed in the epithelial cells (8). The two ESRs have distinct physical characteristics. Different expression locations and affinities may be associated with diverse biological functions of estrogens within the prostate gland (5).

In the prostate tissue of adults, an ESR2 expression gradient exists, with low proximal levels and increased expression levels distally. This gradient can result in heterogeneity in differentiation and function along the ductal length (9). Although ESR2 is the predominant ESR expressed in the adult prostate gland, its role has not yet been clearly defined. ESR2 may play a role in epithelial differentiation (8). It has also been suggested that ESR2 carries out an anti-proliferative role in the prostate and regulates the androgenic stimulation of prostate growth (8). Previously, several studies investigated the role

of ESR2 in the prostate using knockout animal models. The studies suggested that ESR2 inhibits the proliferation of the prostate and the development of prostatic hyperplasia during the aging process (5,10). The aim of the present study was to investigate whether four single nucleotide polymorphisms (SNPs) (rs4986938, rs17766755, rs12435857 and rs1256049) of the *ESR2* gene were associated with the development of BPH.

Materials and methods

Study subjects. The present study involved 173 Korean men who had visited Kyung Hee University Medical Center (Seoul, Korea) for LUTS between January 2002 and December 2006. The clinical symptoms of the patients were assessed using the International Prostate Symptom Score (IPSS) and quality of life (QoL) questionnaires (http://www.urospec.com/uro/Forms/ipss.pdf). The prostate volumes of the patients were measured using transrectal ultrasonography, and the serum prostate-specific antigen (PSA) level of each subject was tested. Peak urinary flow rate (Qmax) and average urinary flow rate (Qavg) were measured using an uroflowmetry system. Subjects were excluded from the study if they had suffered from prostate cancer, neurogenic bladder, urethral stricture, acute/chronic prostatitis, urinary tract infection, uncontrolled diabetes mellitus, previous pelvic surgery or hypertension. Based on their symptoms, the subjects were allocated to either the control (prostate volume, <30 ml) or the BPH [prostate volume, ≥30 ml; IPSS, >8; Qmax, <15 ml/sec] group (11,12). The demographic characteristics of the BPH and control groups are shown in Table I. All subjects provided written, informed consent. This study was approved by the institutional review board of Kyung Hee University Medical Center in 2009.

SNP selection and genotyping. The SNP database was searched in order to select SNPs of the *ESR2* gene for study (http://www.ncbi.nlm.nih.gov/SNP, BUILD 141). SNPs with heterozygosity >0.1, minor allele frequency >10% and associations with other diseases were selected (13-16). Four promoter polymorphisms [rs4986938 (intron), rs17766755 (intron), rs12435857 (intron) and rs1256049 (Val328Val)] of the *ESR2* gene were eventually selected. Genotypes were determined by direct sequencing. A polymerase chain reaction (PCR) was conducted prior to sequencing. Genomic DNA was amplified using the following primers (Macrogen, Inc., Seoul, Republic of Korea): rs4986938 sense, 5'-TGTATGACC TGCTGCTGGAG-3' and anti-sense, 5'-AGGCCATTGAGT GTGGAAAC-3'; rs17766755 sense, 5'-GTAGCTCAAGCC ACTTGCTG-3' and anti-sense, 5'-ACATGGAAGCCAGAG TGACC-3'; rs12435857 sense, 5'-CAAGGATTCAGGGTC CTGTG-3' and anti-sense, 5'-GGCAGCAGGATCACTTGA ATC-3'; and rs1256049 sense, 5'-TCAACTCCCTAATGGTTT GTGT-3' and anti-sense, 5'-AGTGAAGGAGCTGATGAT GCTA-3'. During the PCR, 39 cycles were performed at 94˚C for 30 sec, 58˚C for 30 sec and 72˚C for 1 min. Finally, the PCR was conducted at 72˚C for 7 min to terminate the reaction. Each PCR product was identified with 1.8% agarose gel electrophoresis, and the products were sequenced using an ABI Prism® 377 automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were analyzed using SeqManII software, v2.3 (DNASTAR Inc., Madison, WI, USA).

Table I. Clinical data of participants included in the study.

Parameter	Control	BPH
Subjects (n)	79	94
Age (years)[a]	63.2±10.2	67.2±8.1
Total prostate volume (ml)	22.54	53.07
PSA (ng/ml)		
Total	2.16	6.52
Free	0.45	1.46
IPSS	15.41	18.29
QoL	3.32	3.92
Uroflowmetry (ml/sec)		
Qmax	13.69	9.33
Qavg	8.01	5.60

[a]Presented as the mean ± standard error of the mean. BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; IPSS, international prostate symptom score; QoL, quality of life; Qmax, peak urinary flow rate; Qavg, average urinary flow rate.

Statistical analysis. For the analysis of genetic data, SNPStats (http://bioinfo.iconcologia.net/index.php) was used. A Hardy-Weinberg equilibrium test was conducted for each SNP in the control group, and logistic regression models (codominant1, codominant2, dominant, recessive and log-additive) were then applied to obtain odds ratios (ORs), 95% confidence intervals (CIs) and P-values. A linkage disequilibrium (LD) block of *ESR2* gene SNPs was analyzed using Haploview version 4.2 and the algorithm of Gabriel *et al* (17). The level of significance was set at P<0.05.

Results

Clinical characteristics. Prostate volumes were used to categorize the 173 subjects into the BPH group (≥30 ml, n=94) and the control group (<30 ml, n=79); the clinical characteristics of the two groups are shown in Table I. The mean prostate volume in the BPH group was significantly higher than that in the control group (P<0.001). The total PSA, free PSA, IPSS, QoL, Qmax and Qavg in the BPH group were higher than those in the control group (P<0.05).

Genotype and allele distributions. Genotype and allele distributions of the four SNPs of the *ESR2* gene (rs4986938, rs17766755, rs12435857 and rs1256049) in the BPH patients and control subjects are shown in Table II. Table II also shows the association between each genotype/allele and the risk of BPH by logistic regression analysis. Among the four SNPs, three polymorphisms (rs4986938, rs17766755 and rs12435857) were found to be associated with BPH (Table II), with the minor alleles of these polymorphisms increasing the risk of BPH: i) rs4986938: OR, 1.98; 95% CI, 1.14-3.44; P=0.023 in the log-additive model (C/C genotype vs. C/T genotype vs. T/T genotype); ii) rs17766755: OR, 2.10; 95% CI, 1.06-4.17; P=0.033 in the codominant1 model (G/G genotype vs. G/A genotype); OR, 2.18; 95% CI, 1.13-4.20; P=0.019 in the dominant model

Table II. Genotype and allele frequencies of promoter polymorphisms of the *ESR2* gene in BPH patients and controls.

SNPs	Genotype or allele	Control, n (%)	BPH, n (%)	Models	OR (95% CI)	P-value
rs4986938	C/C	56 (71.8)	53 (56.4)	Codominant1	1.65 (0.84-3.25)	0.150
Intron	C/T	21 (26.9)	34 (36.2)	Codominant2	6.85 (0.80-58.82)	0.080
	T/T	1 (1.3)	7 (7.5)	Dominant	1.89 (0.98-3.64)	0.060
				Recessive	5.82 (0.68-49.39)	0.050
				Log-additive	1.91 (1.08-3.37)	**0.023**
	C	133 (85.3)	140 (74.5)		1	
	T	23 (14.7)	48 (25.5)		1.98 (1.14-3.44)	**0.015**
rs17766755	G/G	58 (73.4)	52 (55.3)	Codominant1	2.10 (1.06-4.17)	**0.033**
Intron	G/A	19 (24.1)	36 (38.3)	Codominant2	2.81 (0.52-15.06)	0.230
	A/A	2 (2.5)	6 (6.4)	Dominant	2.18 (1.13-4.20)	**0.019**
				Recessive	2.21 (0.42-11.66)	0.330
				Log-additive	1.94 (1.09-3.43)	**0.020**
	G	135 (85.4)	140 (74.5)		1	
	A	23 (14.6)	48 (25.5)		2.01 (1.16-3.49)	**0.013**
rs12435857	G/G	43 (54.4)	35 (37.2)	Codominant1	1.86 (0.98-3.54)	0.060
Intron	G/A	33 (41.8)	49 (52.1)	Codominant2	4.15 (1.03-16.66)	0.050
	A/A	3 (3.8)	10 (10.6)	Dominant	2.05 (1.10-3.83)	**0.023**
				Recessive	3.02 (0.78-11.62)	0.090
				Log-additive	1.94 (1.15-3.27)	**0.011**
	G	119 (75.3)	119 (63.3)		1	
	A	39 (24.7)	69 (36.7)		1.77 (1.11-2.82)	**0.017**
rs1256049	C/C	38 (48.1)	53 (56.4)	Codominant1	0.64 (0.34-1.22)	0.180
Val328Val	C/T	38 (48.1)	35 (37.2)	Codominant2	1.30 (0.30-5.64)	0.730
	T/T	3 (3.8)	6 (6.4)	Dominant	0.70 (0.38-1.29)	0.250
				Recessive	1.58 (0.38-6.67)	0.520
				Log-additive	0.82 (0.49-1.38)	0.460
	C	114 (72.2)	141 (75.0)		1	
	T	44 (27.8)	47 (25.0)		0.86 (0.54-1.40)	0.550

P-values were derived from logistic regression analyses with the codominant, dominant, recessive and log-additive models. Bold numbers indicate a significant association. SNP, single nucleotide polymorphism; BPH, benign prostatic hyperplasia; OR, odds ratio; CI, confidence interval.

Table III. Haplotype analysis for the association between *ESR2* gene cluster polymorphisms and BPH.

		Control (n)		BPH (n)			
Haplotype	Frequency	+	-	+	-	χ^2	P-value
GGC	0.425	75	83	72	116	2.955	0.0900
GGT	0.263	44	114	47	141	0.359	0.5500
AAC	0.205	23	135	48	140	6.340	**0.0118**
GAC	0.107	16	142	21	167	0.098	0.7500

Haplotype consists of rs17766755, rs12435857 and rs1256049. Bold numbers indicate a significant association. BPH, benign prostatic hyperplasia; (+), ratio a specific haplotype of case or control; (-), ratio not a specific haplotype of case or control.

(G/G genotype vs. G/A genotype + A/A genotype); OR, 1.94; 95% CI, 1.09-3.43; P=0.02 in the log-additive model (G/G genotype vs. G/A genotype vs. A/A genotype); iii) rs12435857: OR, 2.05; 95% CI, 1.10-3.83; P=0.023 in the dominant model (G/G genotype vs. G/A genotype + A/A genotype); OR, 1.94; 95% CI, 1.15-3.27; P=0.011 in the log additive model (G/G

genotype vs. G/A genotype vs. A/A genotype). In addition, allele distributions of the three SNPs (rs4986938, rs17766755 and rs12435857) showed significant association with BPH (rs4986938: OR, 1.98; 95% CI, 1.14-3.44; P=0.015; rs17766755: OR, 2.01; 95% CI, 1.16-3.49; P=0.013; and rs12435857: OR, 1.77; 95% CI, 1.11-2.82; P=0.017) (Table II).

The four SNPs (rs4986938, rs17766755, rs12435857 and rs1256049) underwent LD and haplotype analysis. One LD block was constructed using the method of Gabriel *et al* (17) during pair-wise comparisons among these SNPs (data not shown). The LD block was constructed with rs17766755, rs12435857 and rs1256049. Among the eight possible haplotypes in the LD block, four haplotypes (frequency >0.1) were analyzed (Table III). The AAC haplotype in the *ESR2* gene exhibited a significant association with BPH [χ^2, 6.34; P=0.0118].

Discussion

Although the prostate is commonly considered as a target of androgens, it is also an important target of estrogens. Previous studies have found that estrogens directly and indirectly affect the growth of the prostate and the development of prostate diseases (4-6). In the estrogen pathway, ESRs play an important role in modulating estrogen action. It has been reported that, while ESR1 is an oncogenic factor involved in cell proliferation and survival, ESR2 is a protective factor that is anticarcinogenic and proapoptotic. Numerous previous studies have investigated the association between ESRs and the development of different types of cancer and have focused on the contribution of ESR1 and ESR2 to the onset of such cancers as prostate, ovarian and breast cancer (18-21). The present study, however, focused on investigating the association between ESR2 and the risk of BPH.

Several association studies have investigated the SNPs assessed in the present study (rs4986938, rs17766755 and rs12435857). In the study conducted by Ryan *et al* (13), it was found that an allele of the rs4986938 SNP resulted in a decline in psychomotor speed, and it was thus concluded that this allele was associated with cognitive function. Marini *et al* (14) also showed that rs4986938 SNP gene polymorphism was associated with plasma fibrinogen levels in postmenopausal women, while Yang *et al* (16) reported that the rs17766755 SNP was significantly associated with human height. Kristiansen *et al* (15) showed that rs12435857 was associated with an increased risk of testicular germ cell tumor.

In the present study, an association was found between the rs4986938, rs17766755 and rs12435857 SNPs of the *ESR2* gene and the susceptibility to BPH. The minor alleles of these SNPs were associated with an increased risk of BPH. Previous studies have shown that the serum estrogen level is associated with prostate volume and other characteristics of BPH (22,23). Although the exact role of ESR2 in the mechanism of BPH has not yet been fully determined, it is suggested that ESR2 located in epithelial cells inhibits the proliferation of the prostate and activates apoptosis in BPH (10,24). In the study by Krege *et al* (10), *ESR2*-knockout mice developed prostatic hyperplasia during the aging process. McPherson *et al* (24) demonstrated that ESR2 also activates apoptosis in BPH in an androgen-independent manner. Thus, ESR2 plays an important role in the estrogen-induced proliferation of the prostate (10).

In conclusion, this is the first study, to the best of our knowledge, to investigate whether SNPs of the *ESR2* gene are associated with the susceptibility to BPH. Three polymorphisms (rs4986938, rs17766755 and rs12435857) of the *ESR2* gene were found to be correlated with the development of BPH. Although these three SNPs are intronic and, thus, are unlikely to be directly involved in the development of the disease, they may interfere with the mRNA splicing process and even the gene expression level (25). These results suggest that these polymorphisms of the *ESR2* gene may contribute to the susceptibility to BPH in the Korean population. In the future, additional studies should be conducted with larger study populations to confirm these results.

Acknowledgements

This study was performed with the support of the Cooperative Research Program for Agriculture Science and Technology Development (project no. PJ009558), the Rural Development Administration, Republic of Korea.

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