Association of IL10, IL10RA, and IL10RB Polymorphisms with Benign Prostate Hyperplasia in Korean Population

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a common disease and occurs in about one third of men in their sixties. In Korea, the overall prevalence of BPH was 40%, and this result suggests an increasing tendency of BPH prevalence (1). BPH is a complex disease from multiple etiologies and pathogenesis point of view. Recently, several reports revealed that BPH is related with immune-mediated inflammatory response (2, 3). Experimental investigations using prostatitis in mice and rats suggested autoimmune responses and genetic background as etiologic factors (4). Chronic inflammation has been documented for years in BPH, and it becomes evident as a major factor in disease initiation and progression (5).

Cytokines such as interleukin 10 (IL10) may play an important role in the process of inflammation. The aim of this study was to analyze the association between IL10, IL10RA and IL10RB single nucleotide polymorphisms (SNPs), and benign prostate hyperplasia (BPH) in Korean population. All patients with BPH were divided into two groups according to international prostatic symptom score (IPSS), prostate specific antigen (PSA) level, Qmax, and prostate volume. We selected two IL10 SNPs (rs1518111 and rs1554286), three IL10RA SNPs (rs2256111, rs4252243, and rs2228054), and two IL10RB SNPs (rs999788 and rs2834167). Genotypes of seven SNPs were determined through direct sequencing. The G/G genotype of IL10RB polymorphism (rs2834167) was associated with a high PSA level compared with the A/G + A/A genotypes (P = 0.009). Of IL10 SNP, the A/A genotype of rs1518111 and T/T genotype of rs1554286 were associated with small prostate volume, respectively (P = 0.011, P = 0.014). Moreover, the T/T genotype of IL10RB polymorphism (rs999788) was associated with high prostatic volume compared with the T/C + C/C genotypes (P = 0.033). The linkage disequilibrium (LD) blocks were formed in IL10 and IL10RA. However, haplotypes in the LD block were not associated with BPH. It is concluded that there is a strong association between the IL10 and IL10RB SNPs, and BPH in Korean population.

MATERIALS AND METHODS

Study population
The present study consisted of 233 patients with BPH and 214 age-matched normal healthy patients. The patients with BPH were enrolled from Kyung Hee University Hospital between January 2002 and December 2008. All healthy control patients underwent screening and had a normal serum prostate-specific antigen (PSA) level (< 4 ng/mL). The patient with BPH complained of moderate or severe lower urinary tract symptoms.
**Sample processing**

Lower urinary tract symptoms were quantified using international prostate symptom score (IPSS). Uroflowmetry was done to measure peak urinary flow rate (Q_{max}) for all patients. Serum PSA levels were checked in all BPH patients. The patients with a serum PSA level more than 4 ng/mL underwent transrectal ultrasound-guided prostate biopsy to rule out prostate cancer. Prostate size was assessed with transrectal ultrasound. Exclusion criteria for this study were prostate cancer, neurogenic bladder, urinary tract infection, uncontrolled diabetes mellitus, and cardiovascular disease. All patients with BPH were divided into two groups according to several multicenter studies; low (0-19) and high (≥ 20) IPSS group, low (< 1.5 ng/mL) and high (≥ 1.5 ng/mL) PSA level, low (< 10 mL/sec) and high (≥ 10 mL/sec) Q_{max}, and small (< 30 mL) and large (≥ 30 mL) prostate volume (8, 9). Genomic DNA was extracted from blood samples collected in EDTA using the Qiagen DNA Extraction kit (Qiagen, Tokyo, Japan).

**SNP selection**

We selected two *IL10* SNPs (rs1518111 and rs1554286), three *IL10RA* SNPs (rs2256111, rs4252243, and rs2228054), and two *IL10RB* SNPs (rs999788 and rs2834167) with greater than 0.3 heterozygosity among SNPs located in the exon and promoter region (http://www.ncbi.nlm.nih.gov/SNP, BUILD 130) for analysis (Fig. 1).

**Direct sequencing**

Genotypes of two *IL10* SNPs (rs1518111 and rs1554286), three *IL10RA* SNPs (rs2256111, rs4252243, and rs2228054), and two *IL10RB* SNPs (rs999788 and rs2834167) were determined through direct sequencing. Genomic DNA was extracted from blood samples collected in EDTA using the Qiagen DNA Extraction kit (Qiagen, Tokyo, Japan).

PCR products were amplified using internal forward and reverse primers and a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice, and then analyzed by the ABI Prism 3730XL DNA analyzer (Applied Biosystems). Sequence data were analyzed using SeqManII software (v2.3; DNAATAR Inc., Madison, WI, USA).

**Statistical analysis**

We analyzed genetic data in control and BPH subjects. SNPsStats (http://bioinfo.iconcologia.net/index.php), HelixTree (Golden Helix Inc., MT, USA), and SNPAnglyzer (ISTECH Inc., Goyang, Korea) software were used to analyze genetic data. The Hardy-Weinberg equilibrium (HWE) was assessed by SNPsStats in control and BPH cases. Multiple logistic regression models (codominant, dominant and recessive model) were used to calculate odds ratio (OR), 95% confidence interval (CI), and corresponding *P* values with Bonferroni correction, while controlling for age as a covariable (10). A linkage disequilibrium (LD) block of polymorphisms was tested using Haploview version 4.02 (11). *P* < 0.05 was considered significant.

**Ethics statement**

The institutional review board at Kyung Hee University Hospital approved this study (KMC IRB 0913-03). All patients with BPH provided informed consent for the use of their clinical data and samples.

**RESULTS**

The clinical characteristics of the study subject with BPH at baseline were given in Table 2. The median age of the BPH subjects was 66.0 yr. A total of 233 BPH subjects and 214 age-matched control subjects were genotyped to analyze associations between SNPs and BPH.

### Table 1. Primer sequences of seven polymorphisms

| Gene symbol | SNP | Sequence (5'→3') | Size (bp) |
|-------------|-----|------------------|----------|
| *IL10*      | rs1554286 | Forward | CCAATCTCTACACTACCTTTGG GTCCTAAGAGAAATGACAAG | 359 |
|             | rs1518111 | Reverse  | CGACAAAAAGGAGTTAGGAGAT | 385 |
| *IL10RA*    | rs2256111 | Forward  | GCCCTCAAGTCTGCTGGAATTC | 379 |
|             | rs4252243 | Reverse  | GGTTGGTTTACCTCCCTCTCT | 368 |
| *IL10RB*    | rs2228054 | Forward  | CCAACTCTCAATTGAGACGTC | 339 |
|             | rs999788  | Reverse  | GGTTGGTTTACCTCCCTCTCT | 319 |
|             | rs2834167 | Forward  | GGGTCTTTTACGAGCCATGTGG | 404 |
Genotype distributions of SNPs in this study were in Hardy-Weinberg equilibrium in control subjects (IL10 SNPs [rs1518111, \( P = 0.64 \) and rs1554286, \( P = 0.54 \)], IL10RA SNPs [rs2256111, \( P = 0.18 \); rs4252243, \( P = 1.00 \); and rs2228054, \( P = 0.51 \)], and IL10RB SNPs [rs999788, \( P = 0.49 \) and rs2834167, \( P = 0.34 \)]. Genotype distributions of seven SNPs for control and BPH subjects are analyzed. There were no associations between control and BPH subjects, maybe, due to high prevalence in Korean population (IL10 SNPs [rs1518111, \( P = 0.70 \) and rs1554286, \( P = 0.69 \)], IL10RA SNPs [rs2256111, \( P = 0.91 \); rs4252243, \( P = 0.26 \); and rs2228054, \( P = 0.83 \)], and IL10RB SNPs [rs999788, \( P = 0.22 \) and rs2834167, \( P = 0.80 \)]. The genotype distributions of seven SNPs were analyzed according to low (0-19) and high (\( \geq 20 \)) IPSS scores. No significant associations were found between IPSS score (IL10 SNPs [rs1518111, \( P = 0.64 \) and rs1554286, \( P = 0.54 \)], IL10RA SNPs [rs2256111, \( P = 0.18 \); rs4252243, \( P = 1.00 \); and rs2228054, \( P = 0.51 \)], and IL10RB SNPs [rs999788, \( P = 0.49 \) and rs2834167, \( P = 0.34 \)]. In allele analysis, there were no significant differences according to IPSS score. Table 3 shows genotype distributions of seven SNPs for low (<1.5 ng/mL) and high (\( \geq 1.5 \) ng/mL) PSA level. The T/T genotype of IL10RB polymorphism (rs999788) was associated with a high PSA level compared with the T/C + C/C genotypes (95% CI 0.28-0.95, \( P = 0.031 \)). Moreover, the G/G genotype of IL10RB polymorphism (rs2834167) was associated with a high PSA level compared with the A/G + A/A genotypes (95% CI 0.23-0.83, \( P = 0.009 \)). In allele analysis, however, there were no significant differences. The genotype distributions of SNPs were analyzed according to low (<10 mL/sec) and high (\( \geq 10 \) mL/sec) Q\(_{\text{max}}\). There were no significant associations between low and high Q\(_{\text{max}}\). In allele analysis, there were no significant differences according to Q\(_{\text{max}}\).

There were significant associations between IL10 and IL10RB SNPs, and prostatic volume in Table 4. Of IL10 SNP, rs1518111 (95% CI 1.01-2.31, \( P = 0.044 \) in codominant model, 95% CI 1.17-3.34, \( P = 0.011 \) in dominant model) and rs1554286 (95% CI 1.00-2.30, \( P = 0.047 \) in codominant model, 95% CI 1.16-3.32, \( P = 0.014 \) in dominant model) were associated with prostatic volume. The G allele of rs1518111 and the C allele of rs1554286 were found to be significantly associated with large prostatic volume and a

**Table 2. The clinical characteristics of 233 BPH patients**

| Parameters | Mean ± SE |
|------------|-----------|
| Age (yr)   | 65.85 ± 0.60 |
| IPSS       | 17.71 ± 0.54 |
| QoL        | 3.57 ± 0.09 |
| PSA (ng/mL)| 4.43 ± 0.35 |
| Free PSA (ng/mL) | 0.98 ± 0.08 |
| Qmax (mL/sec) | 11.34 ± 0.38 |
| Qave (mL/sec) | 6.49 ± 0.25 |
| VV (mL)   | 209.10 ± 12.11 |
| PVR (mL)   | 60.09 ± 6.81 |
| Total prostate volume (mL) | 38.97 ± 1.39 |
| Inner prostate volume (mL) | 18.13 ± 1.23 |

BPH, benign prostatic hyperplasia; IPSS, international prostate symptom score; QoL, quality of life; PSA, prostate-specific antigen; Qmax, peak urinary flow rate; VV, voided volume; PVR, postvoid residual urine.

**Table 3. Analysis of genotype frequencies in IL10, IL10RA, and IL10RB gene polymorphisms, based on low (<1.5 ng/mL) and high (\( \geq 1.5 \) ng/mL) PSA level, in subjects with BPH**

| SNP (Locus) | Genotype | Low PSA n = 86 (%) | High PSA n = 146 (%) | Model | OR (95% CI) | P   |
|------------|----------|-------------------|---------------------|-------|-------------|-----|
| IL10       |          |                   |                     |       |             |     |
| rs1518111  | A/A      | 45 (52.3)         | 61 (41.8)           | Codominant | 1.17 (0.77-1.78) | 0.46 |
|            | A/G      | 31 (36.0)         | 74 (50.7)           | Dominant   | 1.53 (0.89-2.61) | 0.12 |
| rs1554286  | G/G      | 10 (11.6)         | 11 (7.5)            | Recessive  | 0.62 (0.25-1.53) | 0.30 |
|            | T/T      | 43 (60.0)         | 61 (41.8)           | Codominant | 1.11 (0.73-1.68) | 0.64 |
|            | C/C      | 33 (48.4)         | 74 (50.7)           | Dominant   | 1.39 (0.82-2.38) | 0.22 |
| IL10RA     | rs2256111| G/G               | 36 (41.9)           | 59 (40.4) | Codominant | 1.03 (0.69-1.55) | 0.87 |
|            | A/G      | 40 (46.5)         | 70 (48.0)           | Dominant   | 1.06 (0.62-1.82) | 0.83 |
| rs2228054  | G/G      | 43 (60.0)         | 69 (47.3)           | Codominant | 1.05 (0.68-1.63) | 0.82 |
| Pro175Pro  | A/G      | 37 (43.0)         | 68 (46.6)           | Dominant   | 1.12 (0.65-1.90) | 0.69 |
|            | A/A      | 6 (7.0)           | 9 (6.2)             | Recessive  | 0.88 (0.30-2.55) | 0.81 |
| IL10RB     | rs999788  | T/T               | 19 (22.1)           | 51 (35.4) | Codominant | 0.72 (0.49-1.06) | 0.097 |
| -641T>C    | G/G      | 48 (55.0)         | 65 (45.1)           | Dominant   | 0.52 (0.28-0.95) | 0.031 |
| rs2834167  | G/G      | 16 (18.0)         | 50 (34.2)           | Codominant | 0.70 (0.48-1.03) | 0.07 |
| Lys47Glu   | A/G      | 51 (59.3)         | 66 (45.2)           | Dominant   | 0.44 (0.23-0.83) | 0.009 |
|            | A/A      | 19 (22.1)         | 30 (20.6)           | Recessive  | 0.91 (0.48-1.74) | 0.78 |

BPH, benign prostatic hyperplasia; CI, confidence interval; IL10, interleukin-10; IL10RA, interleukin-10 receptor; alpha; IL10RB, interleukin-10 receptor; beta; OR, odds ratio; PSA, prostate-specific antigen; SNP, single nucleotide polymorphism.
risk factor for increased prostatic volume in present study. Moreover, the T/T genotype of IL10RB polymorphism (rs999788) was associated with high prostatic volume compared with the T/C + C/C genotypes (95% CI 0.30-0.96, P = 0.033). In allele analysis, however, there were no significant differences according to prostatic volume.

Seven SNPs were analyzed for LD and haplotypes using Haploview (version 4.2) according to IPSS score, serum PSA level, Qmax, and prostatic volume. The LD blocks were formed in IL10 and IL10RA (Fig. 2). The LD block of IL10 gene consisted of rs1554286 and rs1518111. The LD block of IL10RA gene consisted of rs2256111 and rs2228054. However, haplotypes in the LD block of IL10 and IL10RA were not associated within BPH subjects based on prostatic volume (Table 5).

**DISCUSSION**

Cytokines play multiple roles in both immune disorders and...
many complex diseases. Inflammatory processes are strongly influenced by the balance between the effects of pro-inflammatory and anti-inflammatory cytokines. Several cytokines have been suggested for their role in BPH (7). Cytokines are crucial regulators of cellular function and polymorphisms in cytokines related with BPH could be very important, not only for assessing the risk of BPH but also in helping to explain the pathogenesis and progression of disease.

IL10 is a multifunctional cytokine with anti-inflammatory and anti-angiogenic properties (12-14). IL10 has been reported to reduce tumor growth and angiogenesis (15-17). IL10 mediates tumor promoting and tumor suppressive activities such as apoptosis (18). A large number of polymorphisms have been identified in the IL10 gene promoter (19). Low IL10 producing genotypes were associated with increased susceptibility and advanced stage of several diseases such as malignant melanoma and renal cell carcinoma. However, high IL10 producing genotypes were associated with poor outcome of cervical cancer, gastric cancer, and hepatocellular carcinoma (19).

Several studies were reported in IL10 polymorphism related with prostate cancer and BPH (18, 20-24). Two of these studies found significant results. McCarron et al. (20) reported a significant association of the homozygous variant at -1082 with prostate cancer risk. Both the A/G and A/A genotype at -1082 have been associated with low IL10 production. The T allele at the -819 has been associated with low IL10 production. They concluded that SNP associated with differential production of IL10 are risk factor for prostate cancer acting via their influence on angiogenesis. Faupel-Badger et al. (24) examined the hypothesis that genotypes correlated with low IL10 production may be associated with increased prostate cancer risk among Finnish male participants from the Alpha-tocopherol Beta-carotene Cancer Prevention Study. The -819 T/T and -592 A/A low expression genotypes were highly correlated. These two genotypes also were associated with increased prostate cancer susceptibility and high grade tumors. These data revealed that genotypes correlated with low IL10 production are associated with increased risk of prostate cancer and with high-grade disease in Finnish male (24). Mullan et al. (18) reported the associations between BPH and polymorphism in genes that encode growth factor, cytokines, and vitamin D and receptor. The C/C genotype of the transforming growth factor-beta 1 gene was inversely associated with treatment for BPH. The presence of at least one allele with 17 or more CA repeats of the epidermal growth factor receptor gene was positively associated with high IPSS score. However, IL10 SNP (rs1800896) was not significantly associated with BPH.

In our study, G/G genotype of rs2834167 had a significant association with high PSA level. Moreover, the G allele of rs1518111 and the C allele of rs1554286 were found to be significantly associated with large prostate volume. We suggest that IL10 may be linked to reduce tumor growth and angiogenesis. He et al. (6) reported that the IL10 levels of expressed prostate secretion were higher in type II and type IIIa chronic prostatitis patients than in controls. They concluded that IL10 presumably play an important role in the process of prostate inflammation.

The limitation of our study was small sample number used for comparison within BPH group. But we have performed the first genetic examination of the relationships between IL10, IL10RA and IL10RB SNPs, and BPH, using standard diagnostic tools. Our results revealed a strong association between rs1518111, rs1554286, rs999788, and rs2834167 SNPs and BPH.

In conclusion, there is a strong association between the IL10 SNPs (rs1518111, rs1554286) and IL10RB SNPs (rs999788, rs2834167), and BPH in Korean population. Although our results have confirmed an association of IL10 and IL10RB SNP and BPH, additional investigation is needed for understanding protein expression in BPH.

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Inflammation has been documented in benign prostatic hyperplasia (BPH). Cytokines such as interleukin 10 (IL10) may play an important role in the process of inflammation. Therefore we analyzed the association between the BPH and single nucleotide polymorphisms (SNPs) of IL10, IL10RA and IL10RB in Korean population (233 BPH subjects and 214 age-matched healthy subjects). All patients with BPH were divided into two groups according to IPSS, PSA level, Qmax, and prostate volume. Genotypes of seven SNPs were determined through direct sequencing. The T/T genotype of rs999788 and the G/G genotype of rs2834167 were associated with a high PSA level. Of IL10 SNP, the A/A genotype of rs1518111 and the T/T genotype of rs1554286 were associated with small prostatic volume. Moreover, the T/T genotype of rs999788 was associated with high prostatic volume. We conclude that there is a strong association between the IL10 and IL10RB SNPs, and BPH in Korean population.