Association of Pro/Anti-inflammatory Cytokine Gene Polymorphisms with Benign Prostate Hyperplasia Risk

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Abstract Benign prostate hyperplasia (BPH) is a common condition in aged men and result from prolong chronic inflammation in prostate gland. Cytokines are important molecules responsible for inflammation. Single nucleotide polymorphisms (SNPs) in promoter region of cytokine genes have been shown to alter the level of cytokines. Hence we evaluated the association of pro-inflammatory and anti-inflammatory cytokine SNPs in a North Indian cohort of BPH patients. We observed that *IL-1B* -511 CT + TT genotypes conferred protective effect for susceptibility to BPH (OR 0.39, P 0.001). Our results also demonstrated that *TNF-A* -1031 C allele to be associated with risk for BPH (OR 1.89, P < 0.0001). Moreover, we also observed twofold risk for *IL-10* -1082 cytokine gene polymorphism (OR 1.96, P 0.048). No association was observed with risk of BPH for *IFN-G* +874, *IL-1 RN VNTR*, *IL-6* -174, *IL-10* -819 and *TGF-B* +28. Our findings of *IL-1B* -511, *TNF-A* -1031 and *IL-10* -1082 suggested that these variants play important role in susceptibility to BPH. Future studies in large cohort of different ethnicity BPH groups are warranted to establish definite associations with other cytokine gene polymorphisms as well.

Keywords Cytokine · Gene polymorphisms · BPH

Introduction

Benign prostate hyperplasia (BPH) is a very common condition, yet little is known about the etiology and pathogenesis

of the condition, and the only definitive risk factors are an intact androgen system and aging and inflammation [1–3]. Although many epidemiological clinical studies have been conducted worldwide for last 20 years, but incidence of clinical BPH remains difficult to determine. However, its prevalence is age dependent with histological evidence of BPH rarely under 50 years of age, but by age 80 virtually all men have some histological evidence of the process.

Chronic inflammation has been implicated in the development of complex human diseases including BPH. Cytokines are important inflammatory markers secreted in response to tumors and often by tumor cells. Several polymorphisms in cytokines, both functional and non-functional have already been reported to have positive associations with prostatic growth but surprisingly BPH still lacks a definitive and complete picture of subtle genetic polymorphisms that would be useful for early diagnosis.

The role of different cytokines in BPH is not well established. Genetic association studies are likely to shed light on our current understanding of their roles in the risk of BPH. The present study has therefore undertaken to assess the risk of BPH with pro and anti-inflammatory cytokine genes such as *IL-1B*, *TNF-A*, *IFN-G*, *IL-1RN*, *IL-4*, *IL-6*, *IL10*, and *TGF-B* in a case control study in north Indian Population. An attempt was also made to find risk for BPH for various lifestyle related risk factor such as tobacco smoking, tobacco chewing and alcohol consumption in relation to these gene variants.

Materials and Methods

The participants in this study (BPH patients and controls) were unrelated individuals of similar ethnicity from Lucknow and other adjoining cities, visiting department of

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Urology at the tertiary referral centre of North India during May 2005 to December 2007. All of the BPH patients had various degrees of lower urinary tract symptoms and showed an apparent prostatic enlargement by digital rectal examination. The PSA levels were measured in all of the BPH patients, and men with elevated PSA levels (>4.0 ng/ml). Most subjects were enrolled in our ongoing project identifying the gene polymorphisms affecting the prostate cancer (PCa) development and progression. The clinical presentation of patients was lower urinary tract symptoms with a severe American Urological Association symptom score (greater than 20). The patients with a serum prostate-specific antigen (PSA) level greater than 4 ng/ml underwent transrectal ultrasound-guided prostatic True-Cut biopsy to rule out PCa. Cases having previous history of cancer were excluded. Only symptomatic and histologically proven BPH cases ($n = 151$) were enrolled in the study after informed consent. The study was accorded approval by the ethical committee of institute and informed consent was obtained from all subjects. Blood was collected by veni-puncture in sterile, 5 ml ethylenediamine tetra-acetic acid (EDTA) tubes and stored at -20°C .

Healthy men ($n = 256$) of similar ethnicity and those attending regular health checkups camp/or participating in prostate cancer screening program were recruited as controls. None of the controls had any history of cancer. Controls were taken in age range 40–80 years to match the age of patients. The total PSA levels were determined in controls and BPH patients using CanAg PSA ELISA kits (Fugirebio, Sweden). Control individuals with PSA levels >4.0 ng/ml or positive DRE were excluded. The study protocol was approved by ethical committee of the Institute. The subjects were personally interviewed and information collected on alcohol consumption and tobacco

intake. Participants were also questioned about their tobacco chewing habits and grouped into non tobacco users and tobacco users. Data on smoking habits were collected by asking whether they had smoked 20 packs of cigarettes or more in their lifetimes and, if yes, whether they currently smoke or had smoked in the past. A subject was considered as former smoker if he had not smoked for past 1 year.

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells using salting out method [4]. PCR method used for genotyping with their respective position and reference is given in Table 1.

Quality Control

To control genotyping errors 10% of the samples and were re-genotyped by other lab personal for another study. We found 99% concordance in re-genotyping results.

Statistical Analysis

The sample size was calculated and found to be adequate using QUANTO software, version 1.0 (<http://hydra.usc.edu/exe>) for the genetic marker. Sample size achieved 80% of the statistical power to consider odds ratio (OR) above 2 and below 0.5. A two tailed P value of less than 0.05 was considered statistically significant. Chi-square (χ^2) analysis was used to assess deviation from Hardy–Weinberg's Equilibrium. Association of cytokine genotypes with risk of BPH was analyzed by unconditional logistic regression test. Patients and controls were used as dependent variable and genotypes, age, cigarette smoking, tobacco chewing

Table 1 Base transition of different polymorphism with their methodology used for genotyping with their reference papers

Cytokine gene	Site of SNP	Base transition	Method used for genotyping	Restriction enzyme	References
Pro-inflammatory cytokines					
<i>IL-1 B</i>	−511	C to T	PCR-RFLP	<i>Ava I</i>	Hartland et al. [5]
<i>TNF-A</i>	−308	G to A	PCR-RFLP	<i>Nco I</i>	Wilson et al. [6]
<i>TNF-A</i>	−857	C to T	ARMS-PCR	–	Grutters et al. [7]
<i>TNF-A</i>	−863	C to A	ARMS-PCR	–	Grutters et al. [7]
<i>TNF-A</i>	−1031	T to C	ARMS-PCR	–	Grutters et al. [7]
<i>IFN-G</i>	+874	T to A	ARMS-PCR	–	
Anti-inflammatory cytokine					
<i>IL-1RA</i>	Intron 2	–	VNTR	–	Hartland et al. [5]
<i>IL-4</i>	Intron 3	–	VNTR	–	Mout et al. [8]
<i>IL-6</i>	−174	G to C	ARMS-PCR	–	Tan et al. [9]
<i>IL-10</i>	−1082	G to A	ARMS-PCR	–	Perrey et al. [10]
<i>IL-10</i>	−819	C to T	ARMS-PCR	–	Perrey et al. [10]
<i>TGF-B</i>	+28 Codon 10	T to C	ARMS-PCR	–	Kruit et al. [11]

and alcohol consumption were used as covariates. Risk was expressed as OR with 95% CI (confidence intervals). Wild type genotype/allele/haplotype was of *TNF-A* promoter polymorphism were taken as reference in risk analysis. ORs were also adjusted for the confounding factors like age, cigarette smoking, tobacco chewing and alcohol consumption by binary logistic regression.

Haplotype Analysis

Haplotypes of each individual consisting of four single nucleotide polymorphisms (SNPs) in *TNF-A* promoter and *IL-10* promoter were constructed and their frequencies were assessed using the maximum-likelihood method, employing an expectation–maximization algorithm through software Arlequin ver. 2.0. Statistical significance was taken if the $P < 0.05$. All statistical analyses were performed with the SPSS ver. 11.5 (SPSS Inc, Chicago, USA) statistical software package. Bonferroni correction was considered when making multiple gene comparison.

Results

Demographic Details

The demographic and clinical details are presented in Table 2. There was no statistical difference between age of controls (60.7 ± 8.4 years) and cases (62.2 ± 8.9 years) PSA concentration in controls exhibited significant difference (2.1 ± 1.0 ng/ml) and cases (9.3 ± 6.5 ng/ml). Higher percentage of smokers in BPH patients (39.7%) was depicted as compared to the controls (21.5%) Our results illustrated 2.4 fold risk for susceptibility to BPH in comparison to those who did not smoke (OR 2.4, $P = 0.001$).

Table 2 Demographic details of study subjects

	Controls	BPH	<i>P</i> value
Age (years $\pm$ SD)	60.7 ± 8.4	61.5 ± 8.9	NS
Total PSA (mean $\pm$ SD) ng/ml	2.1 ± 1.0	9.3 ± 6.5	<0.0001
	n (%)	n (%)	
Cigarette/bidi smoking ^a			
Non smokers	192 (75.9)	88 (60.7)	0.001
Smokers	61 (24.1)	57 (39.3)	
Non- tobacco users	177 (70.0)	93 (63.3)	NS
Tobacco users	76 (30.0)	54 (36.7)	
Non drinkers	179 (70.8)	102 (70.3)	NS
Drinkers	74 (29.2)	43 (29.7)	

NS non significant

^a Sum is not equal to total due to some missing values

Tobacco chewers and alcohol consumers, however, exhibited no significant difference between case and controls.

Pro-inflammatory Cytokines Gene Variants and Risk for Susceptibility to Benign Prostate Hyperplasia

All the genotypes were in concordance with Hardy–Weinberg equilibrium. Genotypic and allelic frequency distribution is shown in Table 3. *IL-1B* promoter polymorphism illustrated significant difference between cases and controls ($P 0.0004$). Logistic regression analysis shows that CT + TT genotype of *IL-1B* was associated with protective effect for BPH (OR 0.39, 95% CI 0.22–0.68, $P 0.001$). *IL-1B* T allele alone was protective for BPH (OR 0.66, 95% CI 0.49–0.84, $P 0.004$). Genotypic frequency distribution of other pro-inflammatory markers did not show any significant difference. However, frequency of *TNF-A* -1031C allele was significantly higher in patients in comparison to controls ($P < 0.0001$). C allele also demonstrated to be risk factor for BPH (OR 1.89, 95% CI 1.41–2.53, $P < 0.0001$). Other variants of *TNF-A* and *IFN-G* did not demonstrate any risk for BPH. As the four polymorphisms in *TNF-A* region were in same haplotype, risk for each haplotype was also assessed. Haplotype frequency was calculated among BPH cases and controls using genotyping data of four locus of *TNF-A* gene (*TNF-A* -1031, -863, -857, -308) (Fig. 1). We observed risk associated with -1031C, -863C, -857C, -308G (C–C–C–G: OR 1.83, 95% CI 1.25–2.69, $P 0.002$) and C–C–T–G (OR 2.15, 95% CI 1.07–4.31, $P 0.031$) haplotypes associated with increased risk for BPH.

Anti-inflammatory Cytokines Gene Variants and Risk for Susceptibility to Benign Prostate Hyperplasia

All the anti-inflammatory genotypes were in Hardy–Weinberg equilibrium. Table 4 shows the genotype frequency distribution of cases and control for anti-inflammatory cytokine gene polymorphisms. We observed that variant genotype of *IL-4* were lower frequency in cases (35.9%) in comparison to control (26.5%) however, this difference was not significant. On calculating the risk related to the variant genotypes we observed that B1/B2 + B2/B2 genotype were associated with marginally significant reduced risk for BPH (OR 0.61, 95% CI 0.37–0.99, $P 0.047$). B2 allele also demonstrated significantly reduced risk for BPH (OR 0.70, 95% CI 0.46–0.98, $P 0.040$). We also observed risk associated with *IL-10* -1082 GA + AA genotype for BPH (OR 1.96, 95% CI 1.00–3.82, $P 0.048$). None of the other variants like *IL-1 RN VNTR*, *IL-6* -174, *IL-10* -819 and *TGF-B* +28 did not show any risk associated with BPH. *IL-10* 819 and *IL-10*

Table 3 Pro-inflammatory cytokine gene polymorphisms and BPH risk

Genotypes	Controls n (%)	BPH n (%)	OR (95% CI)
IL-1B –511			
CC	32 (12.5)	40 (26.5)	1 ^a
CT + TT	224 (87.5)	111 (73.5)	0.39 ¹ (0.22–0.68)
Alleles			
C	185 (36.1)	140 (46.4)	1
T	327 (63.9)	162 (53.6)	0.66 ² (0.49–0.84)
TNF –1031			
TT	82 (32.0)	36 (23.8)	1 ^a
TC + CC	174 (68)	115 (76.2)	1.41 (0.86–2.32)
Allele			
T	255 (49.8)	104 (34.4)	1
C	257 (50.2)	198 (65.6)	1.89 ³ (1.41–2.53)
TNF –863			
CC	148 (57.8)	95 (62.9)	1 ^a
CA + AA	108 (42.2)	56 (37.1)	0.715 (0.45–1.14)
Allele			
C	380 (74.2)	241 (79.8)	1
A	132 (25.8)	61 (20.2)	0.73 (0.52–1.03)
TNF –857			
CC	196 (76.6)	103 (68.2)	1 ^a
CT + TT	60 (23.5)	48 (31.8)	1.52 (0.92–2.47)
Alleles			
C	448 (87.5)	253 (83.8)	1
T	64 (12.5)	49 (16.2)	1.36 (0.91–2.03)
TNF-308			
GG	215 (84.0)	131 (86.8)	1 ^a
GA + AA	41 (16.1)	20 (13.2)	0.80 (0.43–1.52)
Alleles			
G	467 (91.2)	280 (92.7)	1
A	45 (8.8)	22 (7.3)	0.82 (0.48–1.39)
IFN-G +874			
TT	58 (22.7)	33 (21.8)	1 ^a
TA + AA	198 (77.3)	118 (78.2)	1.31 (0.76–2.26)
Alleles			
T	230 (44.9)	136 (45.0)	1
A	282 (55.1)	166 (55.0)	1.00 (0.75–1.33)

^a Odds ratio adjusted for age, cigarette smoking, tobacco chewing and alcohol consumption

¹ $P = 0.001$, ² $P = 0.004$, ³ $P < 0.0001$

1082 were in linkage disequilibria so haplotype analysis was done and risk for each haplotype was calculated. Figure 2 shows the haplotype frequency of *IL-10* (–819, –1082) gene polymorphism. We observed that haplotype –819C –1082A (C–A) was observed in significantly higher frequency in BPH patients (0.313) than in controls

Fig. 1 *TNF-A* haplotype (–1031, –863, –857, –308) frequency distribution between cases and controls. The figures in the box show OR, 95% CI and P value taking haplotype T–C–C–G as reference. Frequency difference of other haplotypes was not significantly different

(0.248). C–A haplotype demonstrated 60% increased risk for BPH.

Risk of BPH in Tobacco Smoking, Tobacco Chewing and Alcohol Consumer Group in Relation to Cytokine Gene Variants

We also compared the genotypes in different groups based on various lifestyle related risk factors like tobacco smoking, tobacco chewing and alcohol consumption. We observed reduced risk for BPH in *IL-1B* CT + TT genotype in smokers (OR 0.29, 95% CI 0.1–0.87, P 0.027) and alcohol consumers (OR 0.31, 95% CI 0.1–0.95, P 0.040). We also observed two fold risk for BPH associated with *TNF-A* –1031 TC + CC genotype in non cigarette smokers (OR 1.9, 95% CI 1.04–3.58, P 0.039) and non tobacco chewers (OR 2.04, 95% CI 1.1–3.82, P 0.025). Moreover we observed reduced risk in patients with variant genotypes of *IL-4* in cigarette smoker (OR 0.38, 95% CI 0.16–0.93, P 0.033) and tobacco chewer group (OR 0.39, 95% CI 0.17–0.87, P 0.022). None of the other genotypes in other cytokine gene variants demonstrate any association in any of the groups.

Discussion

Inflammation is emerging as an important factor in the etiopathogenesis of BPH [12]. The present case–control study sought to determine the existence of a possible association between the polymorphisms of cytokine genes with BPH.

IL-1B is pro-inflammatory cytokine and it has been earlier investigated that levels of *IL-1B* are up-regulated in BPH tissues [13]. Our results illustrated that *IL-1B* T allele to have a protective effect for BPH. The results also demonstrate that both the T carrying genotypes to be associated to be protective for BPH. When the comparison

Table 4 Anti-inflammatory cytokine gene polymorphisms and BPH risk

Genotypes	Controls n (%)	BPH n (%)	OR (95% CI)
IL-1 RA			
410/410 bp	110 (43.0)	70 (46.4)	1 ^a
All except 410 bp	146 (57.0)	81 (53.6)	0.73 (0.47–1.13)
Alleles			
410 bp	337 (65.8)	205 (67.9)	1
240 bp	165 (32.2)	90 (29.8)	0.89 (0.66–1.22)
500 bp	6 (1.2)	0 (0.0)	NC
325 bp	4 (0.8)	7 (2.3)	NC
IL-4 VNTR			
B1/B1	164 (64.1)	111 (73.5)	1 ^a
B1/B2 + B2/B2	92 (35.9)	40 (26.5)	0.61 ¹ (0.37–0.99)
Alleles			
B1	408 (79.7)	258 (85.4)	1
B2	104 (20.3)	44 (14.6)	0.70 ² (0.46–0.98)
IL-6			
GG	132 (51.6)	84 (55.6)	1 ^a
GC + CC	124 (48.4)	67 (44.4)	0.88 (0.53–1.24)
Alleles			
G	375 (73.2)	218 (72.2)	1
C	137 (26.8)	84 (27.8)	1.06 (0.77–1.45)
IL-10 –819			
CC	64 (25.0)	43 (28.5)	1 ^a
CT + TT	192 (75.0)	108 (71.5)	0.75 (0.46–1.26)
Alleles			
C	252 (49.2)	154 (51.0)	1
T	260 (50.8)	148 (49.0)	0.93 (0.70–1.24)
IL-10 –1082			
GG	44 (17.2)	16 (10.6)	1 ^a
GA + AA	212 (82.8)	135 (89.4)	1.96 ³ (1.00–3.82)
Alleles			
G	191 (37.3)	107 (35.4)	1
A	321 (62.7)	195 (64.6)	1.08 (0.81–1.46)
TGF-β +28			
TT	98 (38.3)	56 (37.1)	1 ^a
TC + CC	158 (61.7)	95 (62.9)	1.11 (0.71–1.73)
Alleles			
T	298 (58.2)	170 (56.3)	1
C	214 (41.8)	132 (43.7)	1.08 (0.81–1.44)

NC not calculated

^a Odds ratio adjusted for age, cigarette smoking, tobacco chewing and alcohol consumption¹ $P = 0.040$, ² $P = 0.047$, ³ $P = 0.048$

was made in different groups based on lifestyle related risk factors, similar results were seen in smokers and tobacco chewers. This clearly indicates that T allele is protective for BPH. A case only study by Mullan et al. have

Fig. 2 *IL-10* haplotype (–819, –1082) distribution between BPH cases and controls. The figures in the box show OR, 95% CI and *P* value taking haplotype C–G as reference. Frequency difference of other haplotypes was not significantly different

demonstrated that promoter polymorphisms in *IL-1 B* promoter region have no significant difference between IPSS scores, peak urinary flow rates, prostate size, and acute urinary retention, when homozygous wild type was compared to heterozygous and homozygous mutant [14].

Previous transactivation studies suggested that *TNF-α* –1031 was a regulatory site for *TNF-α* transcription and *TNF-α* –1031C allele enhanced gene transcription. Increased transcription of *TNF-α* gene resulted in *TNF-α* overproduction, which perpetuated a chronic inflammatory state and therefore higher risk for BPH [15]. Our results complement the studies reported by Higuschi et al. suggesting *TNF-α* –1031C allele to be at risk for BPH. Mak et al. reported *TNF-α* –863A allele to be associated with a decreased basal transcription rate of *TNF-α*. since *TNF-α* 863C/A, is situated at the border of a 10-bp sequence (GGGGACCCCC) showing high similarity to the consensus sequence of nuclear factor- κ B (NF- κ B) binding site [16]. However, our results show BPH with *TNF-α* –863 A allele to be protective. Furthermore, transcription factor OCT1 was reported to bind only *TNF-α* 857T but not *TNF-α* 857C [17]. OCT1 can physically interact with NF- κ B and thus inhibit the transactivating activity of NF- κ B. In the *TNF-α* signaling pathway, NF- κ B is activated by *TNF-α* which subsequently stimulates the production of other cytokines such as IL-1, IL-6, and *TNF-α* itself. Therefore, ablating the activity of NF- κ B can decrease the production of *TNF-α* and other downstream effectors. Many studies have also shown *TNF-α* variant –308 A allele to be associated with over expression of *TNF*. No association, however, of *TNF-α* –857 C > T and *TNF-α* –308 G > A polymorphism was observed with the risk of BPH.

The haplotypes *TNF-α* –1031C–863C–857C–308G and –1031T–863A–857C–308G demonstrated more than 50% frequency in patients as well as controls which was similar to that observed by a Korean study [18]. *TNF-α* –1031C–863C–857C–308G haplotype revealed increased risk for BPH by 50%. Susceptibility to BPH was,

however, reduced for –1031T–863A–857C–308G haplotypes.

Normal prostate, quantitative RNA analysis reveals that BPH tissues show up to threefold higher IFN- γ levels [19]. BPH derived stromal cells show a considerable increase in proliferation after IFN- γ stimulation. However, our results do not show any risk associated with IFN- γ +874 polymorphism. A recent study from Iran demonstrated IFN- γ +874 to be associated with PCa and BPH [20]. Though, this association may be because of low sample size of the study (41 PCa patients and 100 controls). Our results demonstrate no association of IL-1RA VNTR polymorphism with risk of BPH. However, our earlier studies have demonstrated its association with BPH, however, this may be low sample size (BPH 92 and control 128) [21]. Moreover, a recent study from North India studying the therapeutic aspect of various drugs, like alpha-adrenergic inhibitor plus 5-alpha-reductase inhibitor, which are required for treatment of BPH have shown that IL-1RA VNTR variants do not play a significant role in therapeutic response to these drugs [22]. Similarly a study from North India demonstrated that IL-4 VNTR polymorphism influences the therapeutic response of patients [22]. Our results demonstrate that presence of B2 allele of IL-4 VNTR to be protective for susceptibility of BPH. Surprisingly, comparison in cigarette smokers and tobacco chewers also demonstrated protective effect for BPH susceptibility if B2 allele was present in a genotype. However, this may be due to low sample size in these groups. Our results clearly imply that high producer genotype of IL-4 allele is protective for BPH.

Interleukin-10 (IL-10) is a recently described pleiotropic cytokine secreted mainly by type 2 helper T cells. We observed twofold increased risk associated with GA + AA genotype carriers of IL-10 –1082 gene polymorphism, however, the risk was marginally significant (P 0.048). We did not observe any risk associated with IL-10 –819 gene polymorphism. In haplotype analysis we identified all the four haplotype in significant numbers. Haplotype IL-10 –819C –1082A (C–A) present in significantly higher frequency in BPH patients (31.3%) in comparison to controls (24.8%). C–A haplotype demonstrated moderated increase in risk for BPH (OR 1.59, P 0.027). Our results clearly reveal that IL-10 gene polymorphisms play an important role in etiology of benign prostate hyperplasia.

To the best of our knowledge and extensive literature search, this is perhaps the first study depicting the association of IL-6 –174, IFN- γ +874, TNF- α –1031, TNF- α –863, IL-10 –1082, IL-10 –819 and TGF- β +28, polymorphism with BPH risk. The findings of this study thus indicated that TNF- α variants in the promoter region, susceptible to increased TNF production- may exert significant influence on BPH risk. Our results illustrated that TNF- α –1031 and –863 polymorphisms play an

important role in the pathogenesis of BPH, however, the mechanism still remains to be elucidated. Haplotype analysis revealed that TNF- α –1031C–863C–857C–308G and –1031T–863A–857C–308G may be associated with BPH risk. Moreover our results also convincingly illustrate role of IL-1B –511 and IL-10 –1082 in risk for BPH.

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References

1. Bouraoui Y, Ricote M, García-Tuñón I, Rodríguez-Berriguete G, Touffehi M, Rais NB, et al. Pro-inflammatory cytokines and prostate-specific antigen in hyperplasia and human prostate cancer. *Cancer Detect Prev*. 2008;32:23–32.
2. Imperato-McGinley J, Peterson RE, Gautier T, Sturla E. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5alpha-reductase deficiency. *N Engl J Med*. 1979;300:1233–7.
3. Masumori N, Tsukamoto T, Kumamoto Y, Miyake H, Rhodes T, Girman CJ, et al. Japanese men have smaller prostate volumes but comparable urinary flow rates relative to American men: Results of community based studies in 2 countries. *J Urol*. 1996;155:1324–7.
4. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16:1215.
5. Hartland S, Newton JL, Griffin SM, Donaldson PT. A functional polymorphism in the interleukin-1 receptor-1 gene is associated with increased risk of *Helicobacter pylori* infection but not with gastric cancer. *Dig Dis Sci*. 2004;49:1545–50.
6. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA*. 1997;94:3195–9.
7. Grutters JC, Sato H, Pantelidis P, Lagan AL, McGrath DS, Lammers JW, et al. Increased frequency of the uncommon tumor necrosis factor –857T allele in British and Dutch patients with sarcoidosis. *Am J Respir Crit Care Med*. 2002;165:1119–24.
8. Mout R, Willemze R, Landegent LE. Repeat polymorphisms in the IL-4 gene. *Nucl Acid Res*. 1991;19:3763.
9. Tan D, Wu X, Hou M, Lee SO, Lou W, Wang J, Janarthan B, Nallapareddy S, Trump DL, Gao AC. Interleukin-6 polymorphism is associated with more aggressive prostate cancer. *J Urol*. 2005;174(2):753–6.
10. Perrey A, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. *Transpl Immunol*. 1999;7:127–8.
11. Kruit A, Grutters JC, Ruven HJ, van Moorsel CH, Weiskirchen R, Mengsteab S, et al. Transforming growth factor-beta gene polymorphisms in sarcoidosis patients with and without fibrosis. *Chest*. 2006;129:1584–91.
12. Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? *Eur Urol*. 2007;51:1202–16.
13. Penna G, Mondaini N, Amuchastegui S, Degli Innocenti S, Carini M, Giubilei G, et al. Seminal plasma cytokines and chemokines

- in prostate inflammation: interleukin 8 as a predictive biomarker in chronic prostatitis/chronic pelvic pain syndrome and benign prostatic hyperplasia. *Eur Urol*. 2007;51:524–33.
14. Mullan RJ, Bergstralh EJ, Farmer SA, Jacobson DJ, Hebbing SJ, Cunningham JM, et al. Growth factor, cytokine, and vitamin D receptor polymorphisms and risk of benign prostatic hyperplasia in a community-based cohort of men. *Urology*. 2006;67:300–5.
 15. Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, et al. Polymorphism of the 5'-flanking region of the human tumor necrosis factor (TNF)-alpha gene in Japanese. *Tissue Antigens*. 1998;51:605–12.
 16. Mak YT, Chiu H, Woo J, Kay R, Chan YS, Hui E, et al. Apolipoprotein E genotype and Alzheimer's disease in Hong Kong elderly Chinese. *Neurology*. 1996;46:146–9.
 17. van Heel DA, Udalova IA, De Silva AP, McGovern DP, Kinouchi Y, Hull J, et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF-kappa B transcription factors. *Hum Mol Genet*. 2002;11:1281–9.
 18. Park K, Kim N, Nam J, Bang D, Lee ES. Association of TNFA promoter region haplotype in Behçet's Disease. *J Korean Med Sci*. 2006;21:596–601.
 19. Kramer G, Steiner GE, Handisurya A, Stix U, Haitel A, Knerer B, et al. Increased expression of lymphocyte-derived cytokines in benign hyperplastic prostate tissue, identification of the producing cell types, and effect of differentially expressed cytokines on stromal cell proliferation. *Prostate*. 2002;52:43–58.
 20. Omrani MD, Bazargani S, Bageri M. Interleukin-10, interferon-gamma and tumor necrosis factor-alpha genes variation in prostate cancer and benign prostatic hyperplasia. *Curr Urol*. 2008;2:175–80.
 21. Mittal RD, Mishra DK, Bid HK, Mandhani A. Interleukin-1 receptor antagonist polymorphism in patients with prostate cancer and benign prostatic hyperplasia: a case control study from north India. *Urooncology*. 2004;4:131–4.
 22. Konwar R, Gara R, Singh M, Singh V, Chattopadhyay N, Bid HK. Association of interleukin-4 and interleukin-1 receptor antagonist gene polymorphisms and risk of benign prostatic hyperplasia. *Urology*. 2008;71:868–72.