

**Association of IL-4 and IL-1 Receptor Antagonist (IL-1Ra) Gene
Polymorphisms and the Risk of Benign Prostatic Hyperplasia (BPH)**

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ABSTRACT

OBJECTIVES The genetic and cellular processes involved in aetiopathology of BPH are unknown. Though there is growing evidence of BPH as immune-mediated diseases distinct from prostate cancer, still cytokine gene polymorphisms associated with risk of BPH are less explored. The purpose of this study was to investigate the genetic association of polymorphisms of important cytokine genes (*IL-4* and *IL-1Ra*) with risk of BPH in a case-control study of North Indian population.

METHODS The *IL-4* and *IL-1Ra* gene polymorphism were genotyped with VNTR-PCR in 150 BPH patients and normal healthy controls. Based on their response to combined therapy of α -adrenergic inhibitor + 5- α -reductase inhibitor, patients were grouped as responder and non-responder. Genotype distribution and allelic frequencies between patients and controls were compared and odds ratio (ORs) with 95% confidence intervals (CI) were calculated using SPSS software (version-11.5)

RESULTS The difference of genotype frequency distribution for both *IL-4* and *IL-1Ra* gene polymorphism between BPH and control group were found to be statistically significant ($p < 0.05$). Significant difference ($p < 0.05$) was also observed between responder and non-responder groups in *IL-4* gene variants.

CONCLUSIONS *IL-4* and *IL-1Ra* gene polymorphisms are associated with the risk of BPH. This study for the first time demonstrates association of *IL-4* polymorphism with BPH, particularly influences therapeutic response of the patients.

Recently, BPH has been described as an immune mediated inflammatory disease¹. There is growing evidence of BPH as an immune-mediated disease, distinct from prostate cancer, yet association of cytokine gene variants with risk of BPH are less explored.

IL-4, a member of the Th2 cytokines is a potent anti-inflammatory cytokine, involved in the reduced production of pro-inflammatory cytokines and vicious enzymes by monocytes². Kramer *et al.* (2002) reported that hyperplastic prostate tissues and BPH-T-cell lines express copious levels of IL-4. IL-4 has diverse functions, viz. it increases proliferation of fibroblasts while inhibits smooth muscle cell growth from prostatic stromal clones³.

IL-4 has been located on the long arm of 5th chromosome⁴. Four polymorphic sites, C/T (*BsmFI*) transition at -590 position, C/T transition at -34 position⁵ and two sites of repeat polymorphisms, GT repeat in the intron-3 and 70 bp VNTR in the intron-2 have been reported in *IL-4*⁶. The frequent allelic form of 70bp VNTR in intron-2 consists of three repeats along with a rare allele of double repeats. In addition, there is a rare third allele consisting of four repeats. In a 70bp VNTR association study in Japanese population, it was found that *IL-4* variants exhibit significant difference in the proportion of Th cells producing IL-4 suggesting that *IL-4* genotype could influence the type of immune response⁷. However nothing is known about the association of *IL-4* polymorphism with risk of BPH.

IL-1 α and IL-1 β are pro-inflammatory cytokines of the IL-1 family. IL-1 receptor antagonist (IL-1Ra) competes with IL-1 α and IL-1 β ⁸⁻⁹. IL-1 α is produced by prostatic epithelial cells and induces FGF-7 expression in prostatic stromal cells. FGF-7 in turn, induces epithelial proliferation and further increases in IL-1 α expression, thus creating a

loop, and ultimately leads to increased tissue mass in the prostatic transition zone, which is critical in the pathogenesis of BPH¹⁰. Senescent prostatic epithelial cells are reported as the source of IL-1 α ¹¹. Thus, secreted IL-1 α may be one of the major responsible factors for age-related growth and proliferation of prostatic epithelial cells observed in BPH¹². In a recent expression study, it was found that IL-1 α is expressed in BPH but not in prostate cancer (CaP). IL-1Ra, on the other hand is expressed in both BPH and CaP but its expression is progressively reduced with the tumor grade¹².

IL-1 α , *IL-1 β* and *IL-1Ra* are located on 2q14–21¹³. Five alleles of the *IL-1Ra* have been reported, corresponding to 2, 3, 4, 5 and 6 copies of an 86-base pair repeats located in intron-2¹⁴, with a recent addition of sixth allele of rare single repeat¹⁵. Out of these only two alleles mainly play roles in the production of IL-1Ra¹⁶. However, role of IL-1Ra genotype in its circulating levels in normal individual is different¹⁷. Till date, only study by Mittal *et al.* (2004) investigated VNTR polymorphism of *IL-Ra* in BPH and reported a significant association of *IL-Ra* variants with the risk of BPH¹⁸.

Role of different cytokines in BPH are not well established. Genetic association studies are likely to shed light in our current understanding of their roles in the risk of BPH. In the present study, we have evaluated the possible genetic association of important cytokines, such as *IL-4* and *IL-1Ra* polymorphisms in the risk of BPH in case-control study in Northern India.

MATERIAL AND METHODS

Study Populations and Sample Processing

The present case-control study comprised of 150 BPH patients (mean age= 63.34 ± 9.04 years) enrolled from the Department of Urology, CSJMMU, Lucknow during the period of July 2005-2007. This study was conducted with prior clearance from the ethical committee of CSJMMU, Lucknow.

The clinical presentation of patients were of lower urinary tract symptoms (LUTS) with severe AUA symptom score ($AUA > 20$). Digital rectal examination (DRE) revealed the enlargement of prostate. The prostate volume of all patients was assessed by Trans-rectal ultrasonography (TRUS). The patients with serum PSA of $> 4 \text{ ng/ml}$ were subjected for TRUS guided prostatic true-cut biopsy to rule out CaP. Patients histologically confirmed for BPH were only included in the study after inform consent.

A total of 150 age matched normal healthy controls (mean age= 62.6 ± 9.4 years) were recruited from patients visiting the hospital for minor medical or surgical problems. All were screened for normal PSA level and absence of symptoms suggestive of BPH and malignancy.

The patients were treated as per standard follow up protocol. The patients who showed improvement in Qmax (maximum flow), reduction in AUA score and post void residue within 2 months of combined therapy (5α reductase inhibitors + β adrenergic blockers) were categorized in Group A (responder) and those who failed or had poor response were categorized as Group B (non-responder). The non-responders were offered trans-urethral

Resection of Prostate (TURP). Patients with recurrent acute urinary retention taken immediately for TURP and lacking data of exact treatment profile were categorized as Group C.

DNA was extracted from peripheral blood using QIAamp DNA mini kit (QIAGEN, Germany). Amplification was performed in a 20µl reaction mixture containing genomic DNA (100-150ng), 2-8 pmol of each primers, 200 nM dNTPs and 0.5U of Taq DNA polymerase (MBI-Fermentas, USA) per tube and using a programmed thermal cycler (Master Cycler gradient, Eppendorf, USA).

Genotyping Assays

***IL-4* INTRON-2 VNTR**

The 70bp VNTR of the *IL-4* were amplified by using PCR primer pairs: Forward, 5'-TAGGCTGAAAGGGGGAAAGC-3' and Reverse, 5'-CTGTTCACCTCAACTGCTCC-3' as per Mout et al, 1991⁶. Alleles of 183 bp (two repeats) and 253 bp (three repeats) were designated as B1 and B2 respectively.

***IL-1Ra* INTRON-2 VNTR**

The 86 bp VNTR of *IL-1Ra* were amplified with the primer pairs: Forward, 5'-CTCAGCAACACTCCTAT-3' and Reverse, 5'-TCCTGGTCTGCAGGTAA-3'. The PCR products of 240 bp (allele A= two repeats), 410 bp (allele B= four repeats), 500 bp (allele C= five repeats), 325 bp (allele D= three repeats), 595 bp (allele E= six repeats) and 86 bp (allele F= single repeat) were analyzed on a 2% agarose gel¹⁹.

Statistical Analysis

Sample size was calculated and found to be adequate using QUANTO software version 1.0 (<http://hydra.usc.edu/gxe>) for each genetic marker, *IL-1Ra* and *IL-4*²⁰. Allele and genotype frequencies and carriage rates of *IL-1Ra* and *IL-4* in all the groups were compared with Fisher's exact test using the programme SPSS software (version-11.5). Allele frequencies were assessed for deviation from the expected Hardy-Weinberg equilibrium using the chi-square test, with $P < 0.05$ considered to indicate statistically significant. Odds ratio (OR) at 95% confidence interval (CI) was determined to describe the strength of association by binary logistic regression model.

RESULTS

Clinical details of BPH patients showed in Table 1. The mean age comparison of BPH patients and normal controls is statistically insignificant ($p > 0.05$). The mean values of the prostate volume and PSA were $48 \text{ cm} \pm 10$ and $1.8 \pm 0.5 \text{ ng/ml}$ respectively.

Genotype distribution of *IL-4* and *IL-1Ra* polymorphism, allele frequencies and carriage rate between BPH patients and control group are shown in Table 2. Significant genotype difference of *IL-4* and *IL-1Ra* polymorphisms in both the groups ($p = 0.005$ & 0.031) was observed. No significant association was observed when comparing the allelic frequency and carriage rate of *IL-1Ra* and *IL-4* between the groups (Table 2). Moreover, when the genotypes of *IL-1Ra* and *IL-4* between Group-A and Group-B patients were compared, a significant difference ($p = 0.001$) was found in *IL-4* genotypes but not in case of *IL-1Ra* (p

= 0.789). We also analyzed the combined effect of *IL-4* and *IL-1Ra* polymorphism with BPH. Genotypes were grouped into high or low producer phenotypes for *IL-4*- B1B1 or B1B2=high producer (HP), B2B2=low producer (LP); and for *IL-1Ra* - 240/240=HP, all others=LP. The high and low risk alleles in both patients and controls comprised the combinations of *IL-4* and *IL-1Ra* genotypes. Taking *IL-4* and *IL-1Ra* high producing genotypes as reference, the combined *IL-4* and *IL-1Ra* genotypes did not exhibit significant difference in the frequency distribution between both the groups (Table 2). We also observed 1.6 folds higher risk in patients having $IL-4^{low}-IL-1Ra^{low}$ and $IL-4^{low}-IL-1Ra^{high}$ genotypes, while p-value was not significant.

Comparison of B1B1 and B1B1/B2B2 genotypes of *IL-4* and AA, and others genotypes of *IL-1Ra* with different parameters (e.g. PSA value, prostate volume and Pdet at Qmax) of BPH were also studied, but none found to be significant (data not shown).

COMMENT

Inflammation is emerging as an important factor in the aetiopathogenesis of BPH¹. The present case-control study is sought to determine existence of a possible association between polymorphisms of cytokine genes viz., *IL-4* and *IL-1Ra* with BPH.

IL-4 serves as growth co-stimulator for a variety of cells, at the same time inhibiting the release of inflammatory mediators such as $TNF\alpha$, *IL-6* and *IL-1\alpha* from activated monocytes². *IL-4* has been reported to regulate growth and proliferation of different prostatic cells³. Associations between the 70 bp VNTR allele of *IL-4* and severity of

several diseases have been reported⁷. Currently there is not a single report on the association of *IL-4* polymorphism with BPH. Interestingly, in our study the *IL-4* polymorphism showed significant association with BPH, indicating that this gene is likely to be associated with this disease. In addition, we found a significant association of *IL-4* variants with patient responsiveness to combined therapy of α -adrenergic blocker + 5- α reductase inhibitors. This indicates that *IL-4* polymorphism is not only associated in the susceptibility of BPH, but also influences drug responsiveness of BPH patients, with some yet unknown mechanism.

We also observed a significant association between genotype distribution of *IL-1Ra* and the risk of BPH. Non-significant association was observed in allelic frequency and carriage rate of *IL-Ra* polymorphism and AA/BB (240/410 bp) genotype showed ten fold risk using logistic regression analysis (**Table 2**). We further observed 2.7 folds higher risk in patients having both low *IL-4* and *IL-1Ra* genotypes (OR=2.778 95%CI=0.59-13.03). *IL-1Ra* is a G protein-coupled receptor where a single change in the intracellular domain may occasionally be critical. It is conceivable that allelic variants of the *IL-1Ra* may impact variation of intracellular signaling pathways resulting in the diseases²¹. The significant association of *IL-1Ra* with BPH suggests that its polymorphism may contribute in the risk of BPH. However, *IL-Ra* genotypes fail to reveal any significant association with drug responsiveness in the BPH patients.

Epidemiological data support genetic susceptibility of BPH^{22,23}, but association of genetic polymorphisms with risk of BPH is still incomprehensible. Our data revealed significant

association of *IL-4* and *IL-1Ra* polymorphism with BPH. This may further indicate that immunomodulatory processes could significantly influence risk of BPH. However, the exact mechanism of *IL-4* and *IL-1Ra* polymorphism modulating pathophysiology of BPH is not known. The present study is the first to investigate genetic polymorphism of *IL-4* and *IL-1Ra* in BPH as an independent disease. Significant association of *IL-1Ra* has been reported in CaP¹³ and BPH¹⁷. However, these study were done at a time when BPH was considered as early stage of CaP whereas the current understanding suggests that CaP and BPH are independent pathogenetic events. Moreover, association of *IL-4* polymorphism with CaP is also not reported. In addition, measurement of the *IL-4* and *IL-1Ra* levels in serum as well as their expression profiles in BPH tissue samples would have help in getting insights into their functional implications.

CONCLUSIONS

Our data suggests *IL-4* and *IL-1Ra* polymorphisms are associated with the risk of BPH. Though this report confirms association of cytokine polymorphisms with BPH, warrants further investigation in large cohort for understanding BPH susceptibility as well as functions allelic variants.

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Table 1. Demographic Details of Study Subjects

Characteristic	Median
Age (yrs)	63.34 \pm 9.04
AUA Score	
1. Moderate (8-19)	110
2. Severe (20-35)	30
PSA value (ng/ml)	1.8 \pm 0.5
Prostate volume (cm ³)	48 cm \pm 10
Uroflow Q max (ml/sec)	9 (6-12)
Treatment	
1. Surgery	40
2. Medication	110

Table 2. Distribution of genotypes, allele frequency and carriage rate of IL-4 and IL-1Ra gene polymorphism and the risk of individual genotypes between responder and nonresponder group of patients along with combined genotypes of IL-4 and IL-1Ra in BPH patients and controls

	IL-4 Gene Polymorphism				IL-1Ra Gene Polymorphism				
Genotypes	B1B1	B1B2	B2B2	P value	AA (240)	BB (410)	A/B (240/410)	Others	P value
BPH (n=150)	13 (8.67)	48 (28)	89 (56.67)	0.005	31 (20.7)	87 (58.0)	28 (18.7)	4 (2.7)	0.031
Control (n=150)	9 (6.0)	76 (50.67)	65 (43.33)		23 (15.3)	73 (48.7)	43 (28.7)	11 (7.3)	
Allele Frequency	B1	B2		0.069 1.394 (0.974 - 1.994)	A (240)	B (410)		Others	0.076 0.95 (0.66-1.35)
BPH (n=150)	74 (24.67)	226 (75.33)			90 (30.0)	202 (67.33)		8 (2.67)	
Control (n=150)	94 (31.33)	206 (68.67)			89 (29.67)	189 (63.0)		22 (7.33)	
Carriage Rate	B1	B2		1.00 1.00 (0.683-1.463)	A	B		Others	0.642 0.90 (0.58-1.39)
BPH (n=150)	61 (40.67)	137 (91.33)			59 (39.33)	115 (76.67)		8 (2.67)	
Control (n=150)	85 (56.67)	141 (94)			66 (44.0)	116 (77.33)		11 (7.33)	
Genotypes of IL-4 and IL-Ra between responder and non-responder groups									
	IL-4 Genotypes				IL-1Ra Genotypes				
Genotypes	B1B1	B1B2	B2B2	P value	AA (240)	BB (410)	A/B (240/410)	Others	P value
Group A (n=110)	4 (3.64)	38 (34.54)	68 (61.82)	0.001	23 (29.91)	62 (56.36)	22 (20)	3 (2.73)	0.789
Group B (n=30)	7 (23.33)	7 (23.33)	16 (53.34)		7 (23.33)	18 (60.0)	5 (4.55)	0 (0.0)	
Combined genotype									
Combined genotype	IL-4 ^{low} -IL-1Ra ^{low}			IL-4 ^{high} -IL-1Ra ^{low}		IL-4 ^{low} -IL-1Ra ^{high}		IL-4 ^{high} -IL-1Ra ^{high}	
Control (n=150); (%)	72 (48.0)			48 (32.0)		10 (6.67)		20 (13.33)	
BPH (n=150); (%)	87 (58.0)			36 (24.0)		12 (8.0)		15 (10.0)	
P, OR at 95% CI	0.206, 1.61 (0.77-3.372)			1.0, 1.0 (0.451-2.218)		0.391, 1.60 (0.547-4.681)		1.0 (Ref)	