Interleukin-1 gene cluster variants in hemodialysis patients with end stage renal disease: An association and meta-analysis

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ABSTRACT

We evaluated whether polymorphisms in interleukin (*IL*-1) gene cluster (*IL*-1 alpha [*IL*-1*A*], *IL*-1 beta [*IL*-1*B*], and *IL*-1 receptor antagonist [*IL*-1*RN*]) are associated with end stage renal disease (ESRD). A total of 258 ESRD patients and 569 ethnicity matched controls were examined for *IL*-1 gene cluster. These were genotyped for five single-nucleotide gene polymorphisms in the *IL*-1*A*, *IL*-1*B* and *IL*-1*RN* genes and a variable number of tandem repeats (VNTR) in the *IL*-1*RN*. The *IL*-1*B* − 3953 and *IL*-1*RN* + *8006* polymorphism frequencies were significantly different between the two groups. At *IL*-1*B*, the T allele of − 3953C/T was increased among ESRD (*P* = 0.0001). A logistic regression model demonstrated that two repeat (240 base pair [bp]) of the *IL*-1*Ra* VNTR polymorphism was associated with ESRD (*P* = 0.0001). The C/C/C/C/C/1 haplotype was more prevalent in ESRD = 0.007). No linkage disequilibrium (LD) was observed between six loci of *IL-1* gene. We further conducted a meta-analysis of existing studies and found that there is a strong association of *IL*-1 *RN* VNTR 86 bp repeat polymorphism with susceptibility to ESRD (odds ratio = 2.04, 95% confidence interval = 1.48-2.82; *P* = 0.000). *IL*-1*B* − 5887, +8006 and the *IL*-1*RN* VNTR polymorphisms have been implicated as potential risk factors for ESRD. The meta-analysis showed a strong association of *IL*-1*RN* 86 bp VNTR polymorphism with susceptibility to ESRD.

Key words: End stage renal disease, haplotype, interleukin-1 gene cluster, meta-analysis, pro-inflammatory, variable number of tandem repeats

Introduction

Cytokines are small, short-acting glycoprotein's that regulate immune response. Inter-individual differences in cytokine production influence immune and inflammatory responses. Patients with end-stage renal disease (ESRD) have an impaired immune response causing an imbalance of Th1/Th2 cytokine network. Interleukin (*IL*-1) is involved in the inflammatory response, cell growth, and tissue repair. It has three well-studied members, two agonists, *IL*-1 alpha (*IL*-1*A*) and *IL*-1 beta (*IL*-1*B*), and the *IL*-1 receptor antagonist (*IL*-1*Ra*).[1] *IL*-1*A* and *B* are proinflammatory cytokines, which bind to the *IL*-1 receptor, while the Ra, a competitive inhibitor at the receptor site of both molecules, which down-regulates the immune response.[2] Expression of *IL*-1*Ra* depends upon *IL*-1*RN* gene, which has a length variation within intron 2 caused by 86 bp variable number of tandem repeats (VNTR).[3] According to the number of 86 bp repeats, there are five alleles corresponding to allele I (410 bp), II (240 bp), III (500 bp), IV (325 bp) and V (595 bp).[3] Allele I and II are involved in the production of *IL*-1*Ra*. *IL*-1 gene polymorphisms have been associated with diseases in which inflammation is suspected to play a role.[4-6] Their localization in regulatory regions suggests that they may modulate *IL*-1 protein production by directly affecting transcription, leading to their association with altered levels of *IL*-1.

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Polymorphisms of IL genes may influence gene transcription and thereby modulate the risk of progression of renal disease. The observed inconsistencies in the data regarding associations between single-nucleotide gene polymorphisms (SNPs) and their presumed phenotypic expression emphasize the need to recognize conceptual and methodological aspects such as haplotypic rather than single SNP variations and the influence of pathway genes with synergistic or antagonistic effects that ultimately determine the phenotype.[7]

The goal of this study was to elucidate whether polymorphisms in *IL*-1 gene cluster (*IL*-1*A*, *IL*-1*B*, and *IL*-1*RN*) are associated with ESRD in north Indians. Both ESRD patients and the controls were genotyped for the *IL*-1*A* -889C/T, *IL-1B* (+3953C/T, -5887C/T, -511C/T), *IL*-1*RN* + 8006C/T and *IL*-1*RN* 86 bpVNTR gene polymorphisms. We have also carried out a meta-analysis of the existing data.

Materials and Methods

A total number of 258 unrelated ESRD patients (226, 87.5% males) were included. Inclusion criteria was an estimated glomerular filtration rate (GFR) <15 ml/min/1.73 m^2. Modification of diet in renal disease formula was used (http://www.radiolopolis.com/index.php/tools/calculators/gfr-calculation-using-mdrd-equation.html) for calculation of GFR. For each patient, the information was collected for various other factors [Supplementary Table 1]. The type of chronic kidney disease was established by history, laboratory investigations, including urine analysis, ultrasound and/or computed tomography scan of the kidneys followed by histopathological evaluation of the renal biopsy specimen if required. Patients were categorized into following subtypes, chronic glomerulonephritis (CGN = 168), chronic interstitial nephritis (CIN = 68), hypertensive nephrosclerosis (HN = 15) and polycystic kidney disease (PKD = 7) group. Patients with diabetic mellitus, receiving corticosteroids, vitamin D or vitamin D derivatives were excluded.

Five hundred and sixty-nine (485 males) age, sex and ethnically matched north Indians from same geographic area were selected as controls. The control subjects were unrelated healthy voluntary blood donors. Subjects with risk factors such as family history of hypertension, diabetes mellitus and hyperlipidemia were excluded. The criterion of defining control sample as normal was based on the absence of any evidence of kidney disease [Supplementary Table 1]. The study was performed in accordance with the ethical standards laid down by the declaration of Helsinki, and all persons involved gave their informed consent prior to the inclusion in the study. The study was approved by the ethical committee of our hospital.

Blood collection, deoxyribonucleic acid extraction and genotyping

Blood samples for measuring the serum biochemical parameters were obtained in the morning after 8 h of fasting. For deoxyribonucleic acid (DNA) extraction, 5.0 ml of venous blood from each study subject was collected in an ethylenediaminetetraacetic acid vial. Genomic DNA was prepared by using QIAmp DNA Blood Mini Kit, Qiagen, Valencia, CA).

Genotyping of the interlukin-1 gene cluster

Genotyping for each marker was conducted in three phase's to rule out the experimental biases. All the samples were coded and double blind. The details of primer and polymerase chain reaction (PCR) condition for studied *IL*-1 gene are shown in Supplementary Table 2. Bi-allelic polymorphisms in the *IL*-1 gene cluster[8,9] were determined by PCR–RFLP analysis.

Statistical analysis

Statistical analyses for the genotypic and allelic frequencies were performed by Chi-Square test using Graphpad Prism (version 3.0, Graphpad Software, Inc. San Diego, CA USA). Fisher's exact test was used to assess deviation of the genotype frequency from that expected under Hardy-Weinberg Equilibrium (HWE) [Supplementary Table 3]. Power of the study was calculated using Quanto version 1.1 (http://hydra.usc.edu/gxe) with input of following variables, case-control study design, significance level <0.05 (two sided), model of inheritance = log additive, minor allele frequency = 0.15, genetic effect (odds ratio) ≤0.6 or ≥1.6. This study achieved 80% of power, which was sufficient to consider OR of ≤0.6 or ≥1.6, with the type 1 error α =0.05. Bonferroni corrections were applied where ever required. To evaluate the synergistic effect of studied *IL*-1 gene polymorphisms and risk of ESRD, we performed multivariate analysis using the SPSS software (version 15.0, Biostatistics Consulting University of Massachusetts School of Public Health, US, URL: https://udrive.oit.umass.edu/statdata/spss.zip). Haplotypes were constructed for *IL*-1 gene through Arlequin software (version 3.1, Computational and Molecular Population Genetics Lab, Institute of Zoology, University of Berne, Baltzerstrasse 6, 3012 Bern, Switzerland, URL: http://cmpg.unibe.ch/software/arlequin3).

The analysis of the linkage disequilibrium (LD) of studied polymorphisms was performed with the use of

Haploview v3.11 program; www.broad.mit.edu/mpg/haploview/index.php. This method was used to provide a D prime (D') value. We performed the χ^2 test for association of haplotypes, as well as 10,000 permutations, to obtain empirical *P* values in order to correct for multiple-testing bias. A D' value of zero indicated no LD between different polymorphisms, and D' value of one indicated complete LD. The genotypes of studied group were in HWE for all the six loci.

Meta-analysis

Search strategy and study selection

Meta-analyses was conducted for loci *IL-1A-889, IL-1B-511* and *IL-1RN* 86 bpVNTR of *IL*-1 gene based on the PubMed database using the terms "ESRD" or "renal failure" and "*IL*-1". Study selection criteria were: (1) ESRD patients with creatinine clearance <15 ml/min/1.73 m^2 and were recommended for renal transplantation; (2) controls were drawn from the same geographic area and ethnic background as patients; (3) authors provided original genotype frequencies; and (4) patient and control groups did not overlap between studies. Six case-control studies including present study met the selection criteria.[10-14]

Meta-analyses was conducted for *IL*-1 gene cluster by fitting random effect models and were checked for small size and publication bias by visually examining the possible asymmetry in funnel plots[15] and Egger's test. Cochran's Q statistic, to test for heterogeneity and the I^2 statistic, to quantify the proportion of the total variation owing to heterogeneity were calculated.[16] Analyses were carried out using MetaAnalyst Version: Beta 2 (Tufts Medical Center, Boston, MA, USA).[17]

Results

Demographic profile and clinical characteristics of patients and controls

The demographic and biochemical profile of both patients and controls is shown in Supplementary Table 1.

Distribution of genotype and allele frequency of *interleukin*-1 alpha, *interleukin*-1 *beta* and *interleukin*-1 receptor antagonist

Genotype, allele and carriage allele frequency distributions for *IL*-1 gene cluster (*IL*-1*A*, *IL*-1*B*, and *IL*-1*RN*) are shown in Table 1.

The frequency of C and T allele of *IL*-1*A*, the rs1800587 (-889 C/T) in the patient group was 83.1% and 16.9%, respectively. In the control group, it was 86.5% and 13.5% respectively, both groups did not differ significantly (*P* = 0.0887).

Interlukin-1 beta rs1143627 (−5887C/T) CC genotype frequency was higher in controls (56.6%) than in patients (47.7%; *P* = 0.0210, OR = 0.69, 95% confidence interval [CI] =0.52–0.94). Allele frequency also differed significantly in both the groups (*P* = 0.0367). The SNP in *IL-1B*, the rs1143634 (3953C/T) located in the promoter region of *IL*-1*B* was significantly different between the patient and control groups. The T allele frequency at rs1143634 was higher in the patients (36.8%) than in the control subjects (23.9%; *P* = 0.0001, OR = 1.85, 95% CI = 1.47–2.31). The distribution of rs1143634 (-3953C/T) TT genotype was found to be higher in ESRD patients than in control subjects (*P* = 0.0027, OR = 2.17, 95% CI = 1.32–3.57).

For *IL*-1*RN*, the CC genotype for rs419598 (+8006T/C) was significantly different between ESRD patients and controls (*P* = 0.0001, OR = 9.35, 95% CI = 2.27-38.58), as well as allele frequencies were significantly different among patients and controls. The two repeats of 86 bp (240 bp) was observed in 32.6% of ESRD and 17.8% in the normal control group. A significant difference was found in the frequency distribution of 2/2 genotypes between both groups (*P* = 0.0098, OR = 2.31, 95% CI = 1.23–4.32) [Table 1]. The frequency of single repeat (410 bp) in control group was 74.4% and in ESRD it was 61.2% and was found to be a protective allele (*P* = 0.0001, OR = 0.55, 95% CI = 0.43–0.68) [Table 1].

None of the genotype or allele frequency was statistically different among patient with CGN and CIN. Further, we compared CGN and CIN group with 569 healthy controls and observed significant association (0.001) at allelic and genotypic level of *IL*-1*RN* + 8006T/C (rs419598) [Table 2]. *IL*-1*B* - 5887 C allele was signifiantly associated for protection (*P* = 0.041, OR = 0.75). *IL*-1*RN* 86 bpVNTR*;* 1/1 genotype was observed with high frequency in control as compared to CGN (54.8% and 36.3%) (*P* = 0.0001, OR = 0.47, 95%CI = 0.33-0.67). However 1/2 genotype was significantly associated with the disease phenotype when compared with control and CGN (*P* = 0.0001, OR = 2.07) and CIN (*P* = 0.0001, OR = 2.76) group. We have not done any comparison with the sub group HN = 15, PKD = 7 with controls due to the small number of sample size and to avoid false significant *P* value.

To demonstrate an independent role of studied *IL*-1 gene in influencing the risk of ESRD, we assessed the association between *IL*-1 genotypes and ESRD risk in a multivariate model. In multivariate logistic regression analysis with ESRD patients as the dependent variable and the polymorphisms as independent variables, the

Table 1: Distribution of *IL*-1 gene polymorphism among patients and controls

Genotype		Patients, *n*=258	Control, *n*=569	*P* value	OR (95% CI)
IL-1A–889C/T (rs1800587)					
Genotype frequency					
CC		182 (70.5)	430 (75.6)	0.1493	0.77 (0.55-1.07)
CT		65 (25.3)	124 (21.8)	0.3223	1.21 (0.85-1.71)
TT		11 (4.2)	15 (2.6)	0.3042	1.65 (0.74-3.63)
Allele frequency					
Allele C		429 (83.1)	984 (86.5)	0.0887	1.29 (0.97-1.72)
Allele T		87 (16.9)	154 (13.5)		
IL-1B–5887C/T (rs1143634)					
Genotype frequency					
CC		123 (47.7)	322 (56.6)	0.0210*	0.69 (0.52-0.94)
TC		113 (43.8)	204 (35.8)	0.0357*	1.39 (1.03-1.88)
TT		22 (8.5)	43 (7.6)	0.6760	1.14 (0.66-1.95)
Allele frequency					
Allele C		359 (69.6)	848 (74.5)	0.0367*	1.28 (1.01-1.61)
Allele T		157 (30.4)	290 (25.5)		
IL-1B–3953C/T (rs1143634)					
Genotype frequency					
CC		101 (39.1)	332 (58.3)	0.0001*	0.45 (0.34-0.62)
TC		124 (48.1)	201 (35.3)	0.0007*	1.69 (1.25-2.28)
TT		33 (12.8)	36 (6.3)	0.0027*	2.17 (1.32-3.57)
Allele frequency					
Allele C		326 (63.2)	865 (76.1)	0.0001*	1.85 (1.47-2.31)
Allele T		190 (36.8)	273 (23.9)		
IL-1B–511C/T (rs16944)					
Genotype frequency					
CC		138 (53.5)	327 (57.5)	0.2905	0.85 (0.63-1.14)
TC		100 (38.7)	214 (37.6)	0.7576	1.05 (0.77-1.42)
TT		20 (7.8)	28 (4.9)	0.1108	1.62 (0.89-2.94)
Allele frequency					
Allele C		376 (72.9)	868 (76.3)	0.1406	1.19 (0.94-1.52)
Allele T		140 (27.1)	270 (23.7)		
IL-1RN+8006T/C (rs419598)					
Genotype frequency					
TT		66 (25.6)	491 (86.3)	0.0001*	0.06 (0.04-0.08)
TC		128 (49.6)	76 (13.4)	0.0001*	6.38 (4.53-9.01)
CC		64 (24.8)	02 (0.3)	0.0001*	9.35 (2.27-38.58)
Allele frequency					
Allele T		260 (50.4)	1058 (93.0)	0.0001*	13.02 (9.78-17.32)
Allele C		256 (49.6)	80 (7.0)		
IL-1RN 86 bpVNTR (PubMed ID14563376)					
Genotype frequency					
1/1		90 (34.9)	312 (54.8)	0.0001*	0.44 (0.32-0.59)
1/2		116 (45.0)	151 (26.5)	0.0001*	2.26 (1.66-3.08)
2/2		21 (8.1)	21 (3.7)	0.0098*	2.31 (1.23-4.32)
Others (1/4, 2/3, 2/4, 4/4)		31 (12.0)	85 (15.0)	0.2814	0.77 (0.50-1.21)
Allele frequency					
Number of repeats	Size (bp)				
1	410	316 (61.2)	846 (74.4)	0.0001*	0.55 (0.43-0.68)
2	240	168 (32.6)	203 (17.8)	0.0001*	2.22 (1.75-2.82)
3	500	1 (0.1)	0 (0.0)	-	-
4	325	31 (6.0)	89 (7.8)	0.2196	0.75 (0.49-1.15)

*Significant value ($P<0.05$). OR: Odds ratio, CI: Confidence interval, *IL*-1: Interleukin-1, VNTR: Variable number of tandem repeats, *IL*-1A: Interleukin-1 alpha, *IL*-1B: Interleukin-1 beta, *IL*-1*RN*: Interleukin-1 receptor antagonist

IL-1*B* − 3953C/T polymorphism remained associated with ESRD ($Pc = 0.005$) [Supplementary Table 4]. In order to assess the cumulative effect of different gene polymorphisms with other risk factors we compared all clinical and demographic parameters of the ESRD patients among two genotypic groups for all the markers studied namely "risk genotype" and "nonrisk genotype" like *IL*-1*B* - 5887 TT + CT*;* as risk and CC*;* nonrisk genotype. No comparisons were made for *IL*-1*A* - 889C/T and *IL*-1*B* − 511C/T polymorphism as none of the genotype showed significant association with ESRD [Supplementary Table 4].

Haplotype distribution and linkage disequilibrium

The haplotypes were constructed as shown in Table 3. Our study revealed both protective and susceptible haplotypes. We have avoided any calculations for haplotypes which are less frequent than 5%. The haplotype C/C/C/C/C/1 and C/C/C/C/C/2 was observed to be more frequent in patients with high OR (6.53 and 8.71) and significant *P*c-value ($P = 0.007$), which may cause susceptibility to ESRD. Further the haplotype C/C/C/C/T/1 can be considered as an extended protective haplotype as it was found in 31.9% in the controls when compared to 11.6% in ESRD with highly significant *P* value ($P = 0.0007$) [Table 3].

Table 2: *IL*-1 genotype and allele frequency distribution among primary kidney disease patients and normal controls

Genotype	Controls (*n*=569)	CGN (*n*=168)	CIN (*n*=68)	Chi-Square	*P* value
IL-1A–889C/T (rs1800587)					
CC	430 (75.6)	119 (70.8)	49 (72.1)	0.21	0.9045
CT	124 (21.8)	42 (25.0)	17 (25.0)		
TT	15 (2.6)	07 (4.2)	02 (2.9)		
Allele C	984 (86.5)	280 (83.3)	115 (84.6)	0.11	0.7442
Allele T	154 (13.5)	56 (16.7)	21 (15.4)		
IL-1B–5887C/T (rs1143634)					
CC	322 (56.6)	78 (46.4)*	33 (48.5)	0.19	0.9093
CT	204 (35.8)	75 (44.6)*	30 (44.1)		
TT	43 (7.6)	15 (9.0)	05 (7.4)		
Allele C	848 (74.5)	231 (68.8)*	96 (70.6)	0.15	0.6950
Allele T	290 (25.5)	105 (31.2)	40 (29.4)		
IL-1B–3953C/T (rs1143634)					
CC	332 (58.3)	81 (48.2)*	36 (52.9)	1.24	0.5368
CT	201 (35.3)	67 (39.9)	22 (32.4)		
TT	36 (6.3)	20 (11.9)*	10 (14.7)*		
Allele C	865 (76.1)	229 (68.2)	94 (69.1)	0.04	0.8385
Allele T	273 (23.9)	107 (31.8)**	42 (30.9)		
IL-1B–511C/T (rs16944)					
CC	327 (57.5)	91 (54.2)	35 (51.5)	0.4393	0.8028
CT	214 (37.6)	64 (38.1)	26 (38.2)		
TT	28 (4.9)	13 (7.7)	07 (10.3)		
Allele C	868 (76.3)	246 (73.2)	96 (70.6)	0.33	0.5630
Allele T	270 (23.7)	90 (26.8)	40 (29.4)		
IL-1RN+8006T/C (rs419598)					
TT	491 (86.3)	44 (26.2)***	15 (22.1)***	1.74	0.4187
TC	76 (13.4)	78 (46.4)***	38 (55.8)***		
CC	02 (0.3)	46 (27.4)***	15 (22.1)***		
Allele T	1058 (93.0)	166 (49.4)	68 (50.0)	0.01	0.9067
Allele C	80 (7.0)	170 (50.6)***	68 (50.0)***		
IL-1RN 86 bpVNTR (PubMed ID14563376)					
1/1	312 (54.8)	61 (36.3)***	22 (32.3)**	1.208	0.7512
1/2	151 (26.5)	72 (42.9)***	34 (50.0)***		
2/2	21 (3.7)	12 (7.1)	05 (7.4)		
Others (1/4, 1/5, 2/3, 2/4, 4/4)	85 (15.0)	23 (13.7)	07 (10.3)		

IL-1RN: Interleukin-1 receptor antagonist, *IL-1A*: Interleukin-1 alpha, *IL-1B*: Interleukin-1 beta, PKD: polycystic kidney disease, HN: Hypertensive nephrosclerosis, VNTR: Variable number of tandem repeats, IL-1: Interleukin-1, CGN: Chronic glomerulonephritis, CIN: Chronic interstitial nephritis. ***P<0.0001, **P<0.005, *P<0.05 (patients vs. controls). The Table 2 describes: The difference of frequency of genotype/allele between the subgroups CGN and CIN. *Comparison between subgroup (CGN, and CIN) and control. We have not done any comparison with the sub group HN=15, PKD=7 with controls due to small number of sample size and to avoid false significant *P* value

Table 3: Haplotype distribution of *IL*-1 gene among ESRD patients and control groups (analyzed by Arlequin software v3.1)

Haplotype	Patients (*n*=258)	Controls (*n*=569)	Chi-Square	*P* value	OR (95% CI)	P_c value
C/C/C/C/C/1	27 (10.5)	10 (1.8)	29.45	<0.0001*	6.53 (3.11-13.72)	0.0007*
C/C/C/C/T/1	30 (11.6)	182 (31.9)	37.57	<0.0001*	0.27 (0.18-0.42)	0.0007*
C/C/C/C/T/2	8 (3.1)	41 (7.2)	4.65	0.0310*	0.41 (0.19-0.89)	0.217
C/C/C/T/T/1	6 (2.3)	52 (9.1)	11.61	0.0007*	0.23 (0.10-0.56)	0.0049*
C/C/C/C/C/2	15 (5.8)	4 (0.7)	18.44	<0.0001*	8.71 (2.86-26.54)	0.007*
C/C/C/T/C/1	13 (5.1)	5 (0.9)	12.54	0.0004*	5.98 (2.11-16.97)	0.0028*
C/C/T/C/T/2	15 (5.8)	11 (1.93)	7.55	0.0060*	3.13 (1.42-6.92)	0.042*

IL-1: Interleukin-1, VNTR: Variable number of tandem repeats, *IL-1RN*: Interleukin-1 receptor antagonist, *IL-1A*: Interleukin-1 alpha, *IL-1B*: Interleukin-1 beta, ESRD: End stage renal disease, OR: Odds ratio, CI: Confidence interval. *Significant value (P<0.05), P_c: After Bonferroni's corrections. Haplotypes were constructed for *IL-1A* (–889C/T), *IL-1B* (–5887C/T, –3953C/T, –511C/T), *IL-1RN* (+8006T/C) and *IL-1RN* 86 bpVNTR. We have avoided any calculations for haplotypes which are less frequent than 5%

Linkage disequilibrium was investigated for polymorphisms: *IL*-1*A,* *IL*-1*B,* and *IL*-1*RN* located in the promoter and exonic sequence of the *IL*-1 gene. When we carried out the haplo-view analysis in controls, we identified no LD with D'<1 [Figure 1a]. In patients group there we observed D'<1, which indicates no LD between SNPs [Figure 1b].

Estimation of glomerular filtration rate in healthy individuals on the basis of risk and nonrisk genotypes of *interlukin*-1 gene

An estimation of GFR was computed on the basis of risk and nonrisk genotype of *IL*-1 gene in healthy controls as described in Table 4. We observed that none of the genotype was significantly associated with the risk of lower GFR among healthy controls.

Meta-analysis

As the results of previous studies[10-14] have been inconsistent, we conducted a meta-analysis for three loci *IL*-1*A* - 889C/T, *IL*-1*B* - 511C/T and *IL*-1*RN* 86 bpVNTR polymorphism with susceptibility to ESRD. The *IL*-1*A* - 889C/T polymorphism meta-analysis included seven case-control studies which provided a total of 786 patients and 1079 controls and revealed no association to ESRD (OR = 1.14, 95% CI = 0.78–1.66; P = 0.312) [Figure 2a]. No evidence for heterogeneity was observed between studies (Q = 3.48, P = 0.746 and I^2 = 0%). There was no evidence of publication bias from Egger's regression test (P = 0.253). According to *IL*-1*B* − 511C/T polymorphism meta-analysis which included seven case-control studies (645 cases vs. 996 controls), there was evidence for heterogeneity between studies as P value was significant (Q = 22.70, P = 0.001 and I^2 = 73.6%). We observed no association of this polymorphism with susceptibility to ESRD (OR = 1.38, 95% CI = 0.75–2.54; P = 0.234) [Figure 2b]. The Egger's regression test (P = 0.487) had shown no evidence for publication biasness. The meta-analysis of *IL*-1*RN* 86 bpVNTR polymorphism demonstrated a strong association with susceptibility to ESRD (OR = 2.04, 95% CI = 1.48–2.82; P = 0.000) [Figure 2c]. This analysis included 8 association studies providing total number of 754 ESRD patients and 1230 healthy controls. There was no evidence of publication bias, which has been demonstrated by Egger's regression test (0.253). We observed no heterogeneity between the studies (Q = 4.93, P = 0.667 and I^2 = 0%).

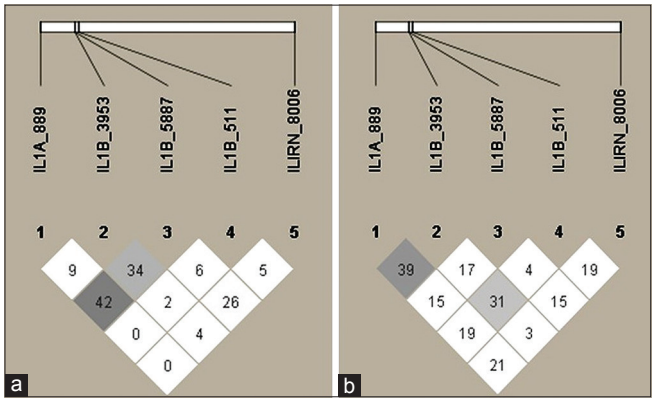

Figure 1: Linkage disequilibrium map of six loci in interleukin-1 (IL-1) alpha, IL-1 beta, and IL-1 receptor antagonist. We estimated the pair wise linkage disequilibrium (LD) by calculating pair wise Dæ and r2. The images were generated with the Haploview software pack. A D' value of θ indicated no LD between different polymorphisms, and D' value of 1 indicated complete LD , (a) Linkage disequilibrium map for six loci of interleukin (IL)-1 alpha, IL-1 beta, and IL-1 receptor antagonist in healthy controls, (b) Linkage disequilibrium map for six loci of interleukin (IL)-1 alpha, IL-1 beta, and IL-1 receptor antagonist in end stage renal disease patients

Discussion

Polymorphisms in cytokine genes may be crucial to understand the mechanisms underlying initiation and progression of ESRD. In recent years, several studies have tried to correlate molecular markers for the *IL*-1 and *IL*-1 receptor genes with severity or outcome of disease.[5,18-23] ESRD could be considered to be a chronic systemic inflammatory state as about 30-50% of dialysis patient's show markedly activated inflammatory response. Inflammation is regulated in part by genes of the *IL*-1 gene cluster. *IL*-1 stimulates hepatocytes to secrete the acute-phase C-reactive protein. This process

Table 4: Estimation of GFR in healthy individuals on the basis of risk and nonrisk genotypes of *IL*-1 gene

Genetic variants	GFR		*P* value
	Risk genotype	Nonrisk genotype	
IL-1A–889C/T (rs1800587)	117.8±48.1	120.1±43.5	0.7290
IL-1B–5887C/T (rs1143634)	118.8±48.2	119.8±42.1	0.8655
IL-1B–3953C/T (rs1143634)	118.7±46.5	119.9±43.6	0.8356
IL-1B–511C/T (rs16944)	116.3±41.2	121.3±46.7	0.4042
IL-1RN+8006T/C (rs419598)	123.4±55.1	118.7±42.8	0.5657
IL-1RN 86 bpVNTR (PubMed ID14563376)	142.1±46.5	118.3±44.4	0.0717

VNTR: Variable number of tandem repeats, *IL-1RN*: Interleukin-1 receptor antagonist, *IL-1A*: Interleukin-1 alpha, *IL-1B*: Interleukin-1 beta, GFR: Glomerular filtration rate. *Significant value (P<0.05). *IL-1A*–889C/T: Risk genotype TT+TC versus nonrisk genotype CC, *IL-1B* (–5887C/T, –3953C/T, –511C/T): Risk genotype TT+TC versus nonrisk genotype CC, *IL-1RN* (+8006T/C): Risk genotype CC+TC versus nonrisk genotype TT, *IL-1RN* 86 bpVNTR: Risk genotype 2/2 versus nonrisk genotype others (as the number of female control individuals was less, hence we have not performed the stratification on the basis of gender)

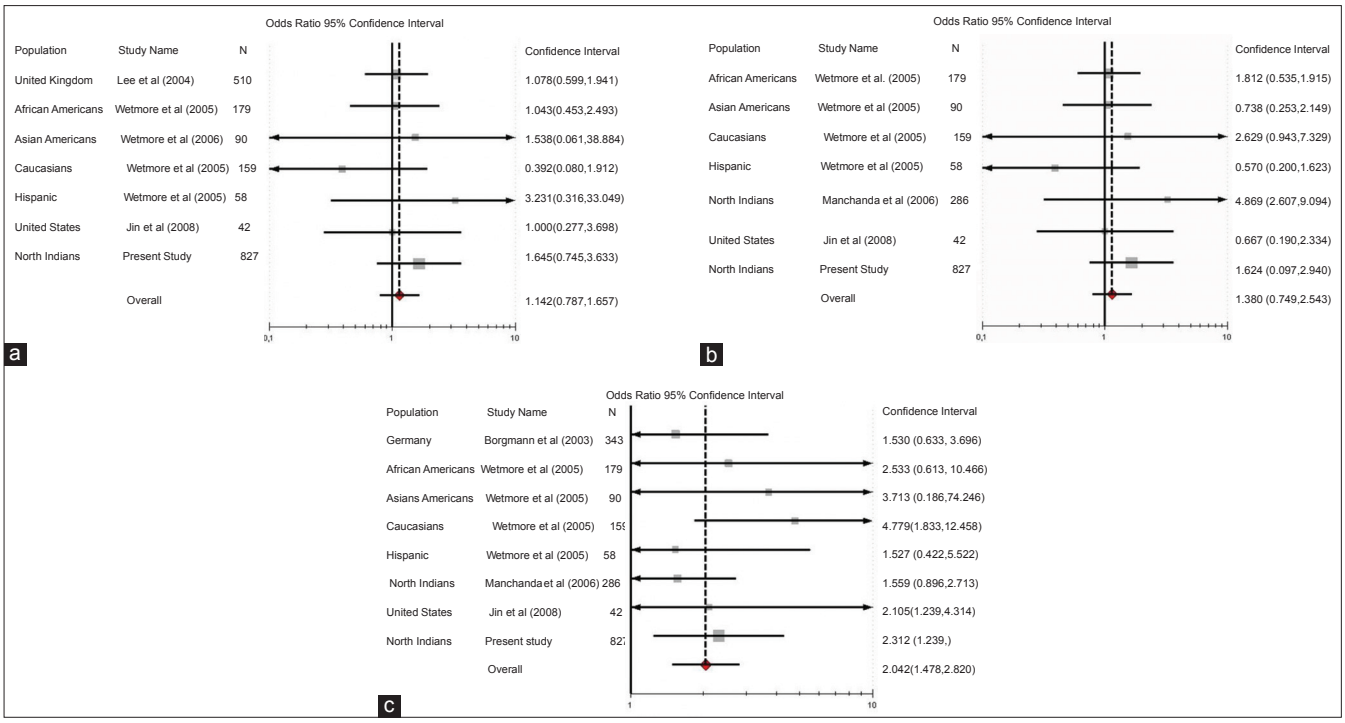

Figure 2: Results of a meta-analysis of interleukin (IL)-1 alpha −889C/T, IL-1 beta −511C/T and IL-1 receptor antagonist 86 base pair variable number of tandem repeat gene polymorphism in end stage renal disease and controls, (a) The forest plot of end stage renal disease risk associated with IL-1 alpha −889C/T polymorphism (TT/CC+CT), (b) The forest plot of end stage renal disease risk associated with IL-1 beta −511C/T polymorphism (TT/CC+CT), (c) The forest plot of end stage renal disease risk associated with IL-1 receptor antagonist 86 base pair variable number of tandem repeat polymorphism (2, 2/1, 1 + 1, 2 + others). The squares and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (CI). The area of the squares reflects the weight (inverse of the variance). The Diamond represents the summary OR and 95% CI

brings forward initial damaging stimuli which are not so effective in nature. However, when the initial stimuli cannot be resolved or when anti-inflammatory systems responsible for regulating inflammation are dysfunctional, inflammation persists. A chronic inflammatory state is harmful, rather than protective, as it may result in end organ and vascular damage.[24]

A silent mutation in the exon 5 of the *IL*-1*B* (-5887C/T) gene, which is represented as the minor T allele is associated with the *IL*-1*B* high-secretor phenotype,[25] which can counter act *IL*-1*Ra* proinflammatory property. Intron 2 of the *IL*-1*Ra* gene includes a region of a variable number of tandem repeats,[19] carrying 240 bp; two repeats polymorphism, which is related to enhanced production of *IL*-1*Ra*. In this study, significant difference was observed between patients and controls. Our results demonstrate that the frequency of allele two (240 bp; 2 repeats) of *IL*-1*Ra* in ESRD was ~ 2 times higher than in controls, similar results have been demonstrated by previous studies.[26] Some studies have suggested that the allelic polymorphism located within intron 2 of *IL*-1*RN* differentially modulate *IL*-1 activity. The *IL*-1*Ra* allele two is associated with increased *IL*-1Ra protein production *in vitro*.[27] The C allele of + 8006T/C in exon 2 of *IL*-1*RN* is associated with the VNTR allele two (240 bp; two repeats) and is associated with lower expression of *IL*-1*Ra*.[28,29] In this study, two additional SNP at - 5887C/T *BsoFI* digested and - 3953C/T *TaqI* digested have been included as they are present in the promoter region. Interestingly, we observed a positive association of these SNPs with ESRD.

An *in vivo* study showed that the *IL*-1*A* - 889 T allele was associated with increased *IL*-1*A* and IL-1*B* protein levels.[30] Furthermore, *ex vivo* analysis of lipopolysaccharide-stimulated peripheral blood mononuclear cells indicated that production of IL-1*A* from *IL*-1*A* with the *IL*-1*A* - 889 T allele increased.[30] *IL*-1*A* (-889C/T) allele was not associated with ESRD in our study. Lee *et al*. has also reported no association of *IL*-1*A* - 889 SNP with renal disease.[12] At the *IL*-1*B* - 511C/T position, there was no association with ESRD.

Earlier studies suggested the importance of *IL*-1 haplotype reflecting differential regulation of *IL*-1*Ra* expression by *IL*-1*B* and coordinated effects of polymorphisms that regulate *IL*-1 bioactivity *in vivo*.[31] It has been postulated that *IL*-1*Ra* genotype and the haplotype of the *IL*-1*Ra* and *IL-B* is important in modulating the susceptibility

of certain diseases.[32] The haplotype C/C/C/C/T/1 of studied *IL*-1 gene was observed to be associated with protection, so the polymorphisms and haplotypes had an influence on the dynamics of renal disease in patients group. This protective haplotype contains the *IL*1*RN* + 8006 'T' allele, which is associated with elevated *IL*-1*RN* expression.[29] An effort to treat rheumatoid arthritis using *IL*-1*Ra* was proved to provide promising results in animal models and humans.[33] Therefore, these studies suggest that altered or imbalanced *IL*-1 production may affect the risk of developing ESRD.

One major limitation of association studies for multifactorial diseases is low sample size due to inclusion of patients meeting all stringent criterions. Meta-analysis helps to overcome this problem. However, we could take only three loci in *IL*-1 gene cluster polymorphism for this purpose as only these studies met the criterion as per our study design. Meta-analysis suggests a strong association of the *IL*-1*RN* 86 bpVNTR polymorphism with susceptibility to develop ESRD.

Conclusion

Interlukin-*1B* -5887, *IL*-1*B* -3953, *IL*-1*RN* + 8006 and the *IL*-1*RN* 86 bpVNTR polymorphisms have been implicated as potential risk factors for ESRD among unrelated ESRD patients. A meta-analysis showed an association of *IL*-1*RN* 86 bpVNTR polymorphism with susceptibility to ESRD. Our results implicate the *IL*-1 gene cluster as an important target of investigation in the development of strategies to slow progression to ESRD and could conceivably provide the basis for defined anti-inflammatory strategies to limit renal disease progression.

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