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Interleukin-1β and receptor antagonist (IL-1Ra) gene polymorphisms and the prediction of the risk of end-stage renal disease

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Abstract

Cytokines play an important role in the pathogenesis of kidney disease and its progression to end-stage renal disease (ESRD). Inflammation is regulated by the genes of the interleukin 1 (IL-1) gene cluster. Therefore, it was hypothesized that a polymorphism in this gene cluster may be associated with the risk of ESRD. Polymorphisms in the IL-1 gene cluster were examined in a cohort of 222 ESRD patients and 206 controls of similar ethnicity. These individuals were genotyped for IL-1 β (promoter −511 and exon-5 +3953) genes and a variable number of tandem repeats (VNTR) in the IL-1 receptor antagonist gene (IL-1Ra). There was significant difference in genotype frequencies between ESRD patients and control group for IL-1β (promoter region and exon-5) and IL-1Ra gene polymorphism ($p < 0.001$, 0.006 and < 0.001, respectively). A significant difference was observed in IL-1Ra for 1/1 (410/410) and 1/2 (410/240) genotypes, and the risk for ESRD was higher in those carrying the 1/1 genotype ($p = 0.014$, OR = 1.692, and $p < 0.001$, OR = 0.163). Also identified was a novel, rare allele of a single copy of 86 bp in ESRD patients as compared with the controls. The haplotype 'T-E2-1' frequency distribution between patients and controls revealed greater than threefold risk ($p = 0.001$, OR = 3.572, 95% CI = 1.589–8.032). Genetic linkage between the IL-1β promoter region and exon-5 and between the IL-1β promoter and IL-1Ra of IL-1 gene demonstrated a strong association among the variants in controls ($D' = 0.42$, $p < 0.001$, and $D' = 0.39$, $p = 0.001$). Thus, the three polymorphisms within the IL-1 cluster are associated with ESRD. This finding is perhaps one of the strongest associations between genotype and ESRD reported, and it suggests that the IL-1 gene cluster affects the risk of development of ESRD.

Keywords: *End-stage renal disease (ESRD), interleukin (IL) 1 gene cluster, polymorphism, haplotype.*

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Introduction

End-stage renal disease (ESRD), with its associated glomerulosclerosis and interstitial fibrosis, is a major cause of morbidity and mortality worldwide (Dusel et al. 2005). Although the well-documented increased familial clustering of chronic kidney disease (CKD) may in part depend on environmental factors, the available evidence suggests

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that genetic factors are also contributors (Satko et al. 2005). Substantial evidences suggest that inflammation may be established before the onset of kidney disease and could be a causal factor in the development or progression of CKD. The interleukin (IL)-1 gene products may be particularly important molecular effectors in the process of inflammation (Wetmore et al. 2005).

The IL-1 gene cluster, a 430-kb region of chromosome 2 consists of IL-1α, IL-1β and IL-1 receptor antagonist. The IL-1 system is unique in having a natural inhibitor known as the IL-1 receptor antagonist (IL-1Ra) molecule (Arend et al. 1998). IL-1Ra competitively inhibits at the receptor site of both molecules and down-regulates the immune response and inflammation. Six alleles of the IL-1Ra gene have been reported, corresponding to 410 bp (allele *1 = four repeats), 240 bp (allele *2 = two repeats), 500 bp (allele *3 = five repeats), 325 bp (allele *4 = three repeats, 595 bp (allele *5 = six repeats) and 154 bp (allele *6 = one repeat) (Tarlow et al. 1993, Vamvakopoulos et al. 2002). As there are three potential protein-binding sites located in this 86-bp sequence, the number of repeats influences gene transcription and protein production. Dimers have been shown to be associated with a higher IL-1Ra secretion (Danis et al. 1995). It has been seen that IL-1β (promoter region and exon-5) and in IL-1Ra show a cooperative effect, e.g. IL-1Ra *2 (allele 240) increases IL-1Ra levels in the presence of allele *T of IL-1β (−511) (Labow et al. 1997). Different polymorphisms in the IL-1β gene influence protein production, one of which is located within the promoter region at position −511, the other in exon-5 +3954, representing a C to T base transition in both the polymorphic sites and the allele *T represents an IL-1β 'high secretor' (Di Giovine et al. 1992, Cantagrel et al. 1999).

The mechanism of the IL-1 gene can be well understood (Figure 1). The three polymorphisms, IL-1β (promoter region −511 and exon-5 +3954 and IL-1Ra) directly affect the protein structure and alter the protein function without affecting the sequence of amino acid in polypeptide chains. The protein production at different transcriptional or post-transcriptional levels is only influenced by altering the structure of a gene's regulatory elements with binding sites for transcription factors, transcription rate, mRNA splicing and mRNA stability (Laurincová 2000). Thus, the net effect of the three members of the IL-1 gene family is to control inflammatory and host defence responses.

The pro-inflammatory cytokine IL-1 stimulates kidney mesangial cell proliferation and extra cellular matrix production, which leads to renal damage. Genes within the IL-1 gene cluster are therefore possibly involved in the pathogenesis of diabetic nephropathy and other renal diseases (Hughes et al. 1996). In the present study, the implication of IL-1β (promoter and exon-5) and IL-1Ra gene polymorphism in ESRD in the north Indian population was analysed. The relative frequencies of specific alleles/genotypes of IL-1β (promoter −511 and exon-5 +3953) and IL-1Ra were determined in a cohort of ESRD patients and the relationship was evaluated by employing haplotypes and linkage disequilibrium (LD) within the IL-1 gene cluster.

Materials and methods

Patients and clinical data

A total of 222 north Indian ESRD patients on haemodialysis (HD) (age range 17–64 years; mean age 35.5 ± 9.95 years, males = 183, female = 39) from our tertiary care hospital in Lucknow, India, were studied. Patients were selected with > 4.0 mg dl^{-1}

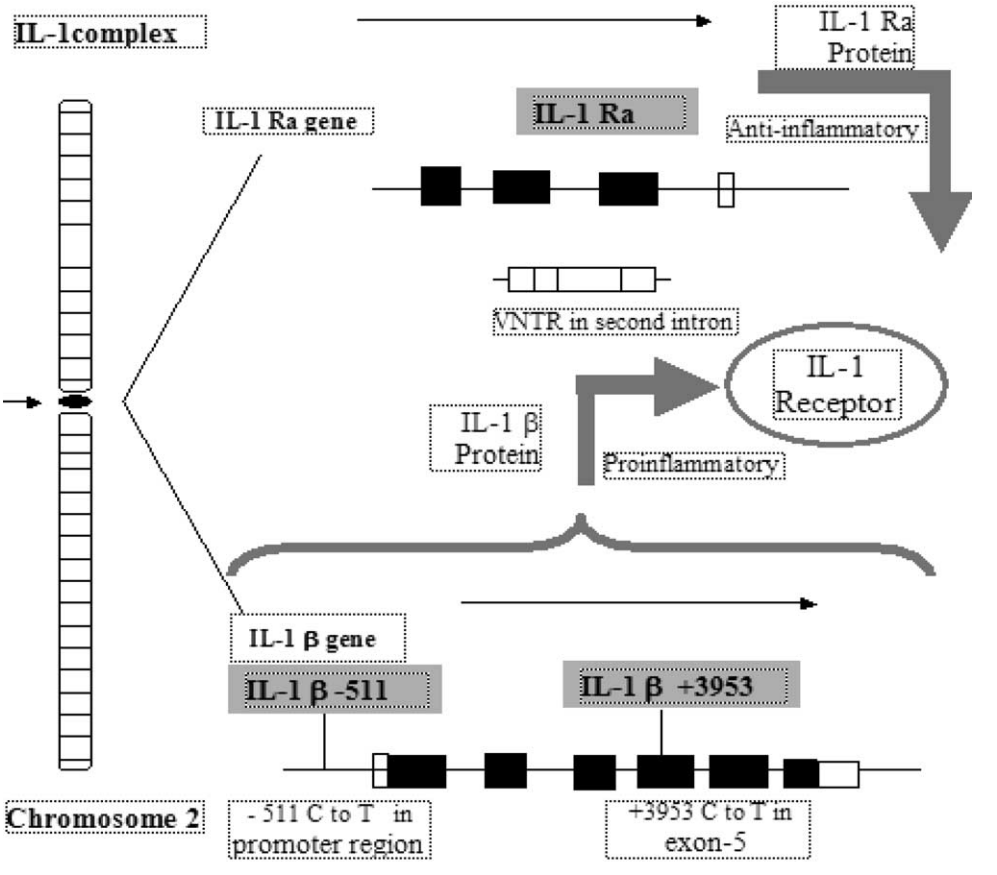

Figure 1. Schematic presentation of the IL-1 gene map demonstrating the different restriction sites for IL-1β promoter region (−511), exon-5 (+3953) restriction enzymes and VNTR in IL-1Ra and their mode of action. The C to T transition in the IL-1β gene in the promoter and exon-5 causes change at transcriptional level and thus cause high production of IL-1 by binding more efficiently to IL-1 receptor. IL-1Ra is a protein that binds to the IL-1 receptor with the same affinity as IL-1 but does not possess agonist activity and therefore, acts as a competitive inhibitor of IL-1.

serum creatinine and had been dialysing for at least 3 months with no evidence of active inflammation. Clinical information and biochemical parameters were retrieved retrospectively from hospital records. The subject characteristics are presented in Table I. Healthy controls ($n = 206$, age range 22–58 years, mean age 38.1 ± 13.6, males = 136, females = 70) for the study were from the same geographical area, with no history of hypertension, diabetes, renal failure, vascular diseases, stroke and/or cardiomyopathy. The Institutional Review Board approved the protocol; informed consent was obtained from patients and controls participating in the study. The hospital ethical committee approved the study.

DNA extraction

Blood (5 ml) was collected in EDTA vials from cases and controls. DNA was extracted from blood lymphocytes using a 'salting out' method (Miller et al. 1988).

Table I. Subject characteristics for patients ($n=222$).

Age (years)	17–64, 35.5±9.5[a]
Gender (male/female)	183/39
Cause of ESRD:	
Glomerulonephritis	172
Focal segmental glomerulosclerosis	6
Diabetic nephropathy	23
Polycystic kidney disease	7
Nephrotic syndrome	10
Alport syndrome	3
Churg Spauss syndrome	1

[a]Mean±SD.

All patients and controls were genotyped for three polymorphisms in IL-1 gene cluster: IL-1 β promoter region −511, IL-1β exon 5 +3953 and IL-1Ra in intron 2.

IL-1β (promoter region). The primer sequences used were: forward, 5′-TGGCATT-GATCTGGTTCATC-3′; and reverse, 5′-GTTTAGGAATCTTCCCACTT-3′, which amplify a 304-bp sequence. Polymerase chain reaction (PCR) products were subjected to digestion by AvaI (MBI Fermentas; Genetix Biotech Ltd., India). The presence of C at position −511, when digested by Ava I, gave two fragments of 190 and 114 bp, while T at the same position remained undigested (Figure 2a). The fragments were analysed by 9% polyacrylamide gel electrophoresis (PAGE) (Cantagrel et al. 1999).

IL-1β (exon 5). The primer sequences used were: forward, 5′-GTTGTCATCCA-GACTTTGACC-3′; and reverse, 5′-TTCAGTTCATATGGACCAGA-3′, which amplify a 249-bp sequence. PCR products were digested by Taq I restriction enzyme (MBI Fermentas). The C (designated as E1) at position exon-5 upon digestion resulted in two fragments of 135 and 114, while T (designated as E2) remained undigested (Figure 2b). The fragments were analysed by 12% PAGE (Cantagrel et al. 1999).

IL-1Ra VNTR. The primer sequences used were: forward, 5′-CTCAGCAA-CACTCCTAT-3′; and reverse, 5′-TCCTGGTCTGCAGGTAA-3′. The region within the second intron of the IL-1Ra gene contained variable numbers of a tandem

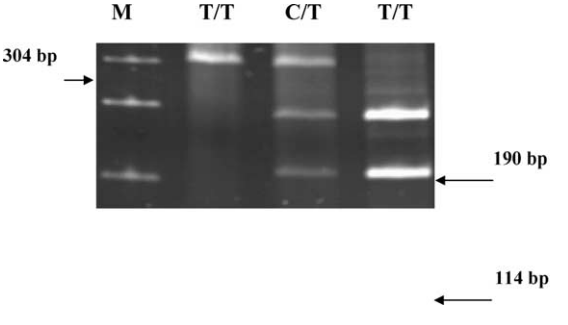

Figure 2a. IL-1β promoter. Lanes M, molecular marker (100 bp); 1, T/T genotype (undigested; 304 bp); 2, C/T genotype (heterozygous; three bands are observed 304: 190 and 114 bp); 3, C/C genotype (homozygous; 190 and 114 bp).

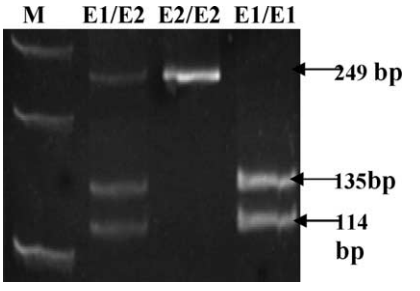

Figure 2b. IL-1β (exon-5). Lanes M, molecular marker (100 bp); 1, E1/E2 genotype (heterozygous; 249, 135 and 114 bp); 2, E2/E2 genotype (undigested; 249 bp); 3, E1/E1 genotype (homozygous; 135 and 114 bp).

repeat (VNTR) of 86 bp. The PCR products of 410 bp (allele 1 = four repeats), 240 bp (allele 2 = two repeats), 500 bp (allele 3 = five repeats), 325 bp (allele 4 = three repeats, 595 bp (allele 5 = six repeats) and 154 bp (allele 6 = one repeat) were analysed by electrophoresis on a 2% agarose gel (Figure 2c) (Bioque et al. 1995).

PCR conditions. The PCR conditions used for the IL-1β promoter and exon-5 were: 95°C for 4 min followed by 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 30 s for 35 cycles and a final extension at 72°C for 10 min; and for IL-1Ra: initial denaturation, 95°C for 5 min followed by 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s for 30 cycles and a final extension at 72°C for 10 min. The size of the PCR product was determined using a 100-bp DNA ladder (Roche, Diagnostics, GmbH Mannheim, Germany). The molecular weight of each band was determined by using software in Alpha Imager 1220 version 5.5 programme.

Statistical analysis

Statistical analysis was performed using the χ^2-test to compare the genotype and allelic frequency distribution in patients and controls with SPSS software (version 11.5). Allele and genotype frequencies were compared using a 2×2 contingency table using Fisher's exact test. $p < 0.05$ was considered to be statistically significant. The odds ratio (OR) at 95% confidence interval (CI) was determined for the disease susceptibility of patients. SNPAnalyzer (a web-based software available at http://www.istech.info/istech/board/login_form.jsp) was used to examine the Hardy–Weinberg equilibrium (HWE), the pairwise LD between each pair of IL-1 loci and haplotype estimation by maximum likelihood method, using the expectation maximization algorithm (Yoo et al. 2005). This algorithm enables the estimation of

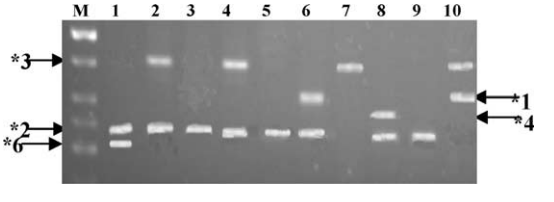

Figure 2c. IL-1Ra. Lanes M, molecular marker (100 bp); 1, 154/240 bp (6/2); 2 and 4, 240/500 bp (2/3); 3, 5 and 9, 240/240 bp (2/2); 6, 240/410 bp (2/1); 7, 500/500 bp (3/3); 8, 240/325 bp (2/4); 10, 410/500 bp (1/3) alleles.

haplotype frequencies reconstruction of an individual haplotype from a diploid genotype. Once the haplotypes were constructed, the comparison in haplotype frequencies between case and control were calculated using a χ^2-test and the OR was determined. p values were derived by empirical simulation and where possible statistical significance was defined at the standard 5% level.

The sample size was calculated using the QUANTO version 1 program (http://hydra.use.edu/gxe). The desired power of the study was set at 80% with a significance level of 0.05 in a two-sided test. As an inheritance model, the log-additive one was chosen, which is the most suitable model in polygenic diseases. By means of this program, the sample size was considered adequate to study these polymorphisms.

Results

Different genotypic and allele frequencies distributions for IL-1Ra and IL-1β gene (promoter and exon-5) are shown in Tables II and III (Figure 2a–c). The genotype distributions for IL-1 cluster in controls were consistent with the HWE. A significant difference was observed in genotype frequencies between ESRD patients and control group for IL-1β (promoter region and exon-5) and IL-1Ra gene polymorphism ($\chi^2 = 37.22$; d.f. $= 2$; $p < 0.001$, $\chi^2 = 10.3$; d.f. $= 2$; $p = 0.006$; and $\chi^2 = 51.5$; d.f. $= 7$; $p < 0.001$, respectively). The TT genotype of IL-1β (promoter region) in ESRD patients showed a greater than twofold higher risk ($p < 0.001$, OR $= 2.89$). The allele frequency for IL-1β-511*T allele (64.1 versus 45.1%) was higher in patients as compared with controls ($p < 0.001$). In IL-1β, the exon-5 heterozygous genotype E1E2 was higher in patients and showed a 1.9 times higher risk ($p = 0.001$, OR $= 1.998$). No difference in allele frequency for IL-1β (exon-5) was observed. A significant difference was observed in IL-1Ra for 1/1 (410/410) and 1/2 (410/240) genotypes, but the risk for ESRD was higher in those carrying the 1/1 genotype ($p = 0.014$, OR $= 1.692$, and $p < 0.001$, OR $= 0.163$). No significant difference was observed in allelic frequencies for IL-1Ra. Interestingly, a rare allele, non-conserved single copy of 86 bp that appears as 154 bp was only observed in ESRD patients (4.0 versus 0%). The patterns of the LD and haplotype distributions were examined by combining the three sites of IL-1 genes using SNPAnalyzer. The eight haplotypes anticipated in normals and patients whose frequencies were $> 1\%$ was projected. There was a significant difference in haplotype 'T-E2-1' frequency distribution between patients and controls with greater than threefold risk ($p = 0.001$, OR $= 3.572$, 95% CI $= 1.589–8.032$). LD was significant between the IL-1β promoter region and exon-5 and between the IL-1β promoter and IL-1Ra of IL-1 gene in controls ($D' = 0.42$, $p < 0.001$, and $D' = 0.39$, $p = 0.001$, respectively). However, in patients the LD was non-significant ($D' = -0.087$, $p = 0.526$).

Discussion

ESRD could be considered a chronic systemic inflammatory state as about 30–50% of dialysis patients exhibit evidence of a markedly activated inflammatory response (Yao et al. 2004). It is possible that factors such as inflammation, oxidative stress and endothelial dysfunction (and genetic variations related to these complications) could be involved in the aetiology and progression of ESRD (Nordfors et al. 2005). IL-1

Table II. Genotype and allele frequencies of IL-1β (promoter region and exon-5) gene in ESRD patients and healthy controls.

Gene	Genotype	Patients ($n=222$) (%)	Controls ($n=206$) (%)	p	*OR at 95% CI
IL-1β promoter region	CC	23 (10.4)	50 (24.3)	< 0.001, d.f. $=2$, $\chi^2=37.22$	0.23 (0.136–0.386), $p<0.001$
	CT	113 (51)	126 (61.2)		1.09 (0.752–1.606), $p=0.63$
	TT	86 (38.7)	30 (14.5)		2.89 (1.85–4.514), $p<0.001$
Allele frequency	−511*C	159 (35.9)	226 (54.9)	< 0.001	0.454 (0.349–0.604)
	−511*T	285 (64.1)	186 (45.1)		
IL-1β exon-5	E1E1	132 (59.5)	149 (72.3)	0.006; d.f. $=2$, $\chi^2=10.3$	0.56 (0.374–0.842), $p=0.006$
	E1E2	81 (36.5)	46 (22.3)		1.998 (1.304–0.386), $p=0.001$
	E2E2	9 (4.0)	11 (5.4)		0.75 (0.304–1.846), $p=0.65$
Allele frequency	+3954*E1	345 (77.7)	344 (83.5)	0.38	0.684 (0.489–0.971)
	+3954*E2	99 (22.3)	68 (16.5)		

regulates complex biological reactions and various polymorphisms of the IL-1 gene cluster are associated with several inflammatory diseases including kidney diseases (Hurme et al. 1998, Syrjanen et al. 2002).

Table III. Genotype and allele frequencies of the IL-Ra gene in ESRD patients and controls.

Genotype	Patient ($n=222$)	Control ($n=206$)	p	*OR	95% CI
1/1 (four copies)	142 (64.0)	107 (52.0)	0.014	1.692	1.115–2.418
2/2 (two copies)	51 (23.0)	36 (17.5)	0.186	1.408	0.875–2.268
1/2	11 (5.0)	50 (24.3)	<0.001	0.163	0.082–0.323
3/3 (5 copies)	2 (0.9)	4 (2.0)	0.434	0.459	0.083–2.533
4/4 (3 copies)	2 (0.9)	1 (0.5)	1.000	1.864	0.168–20.708
6/6 (1 copy)	8 (3.6)	0 (0)	–	–	–
1/3	1 (0.5)	6 (3.0)	0.059	0.151	0.018–1.264
1/4	0 (0)	1 (0.5)	–	–	–
2/3	3 (1.4)	0 (0)	–	–	–
6/2	2 (0.9)	0 (0)	–	–	–
2/4	0 (0)	1 (0.5)	–	–	–
$p<0.001$, d.f. $=7$, $\chi^2=51.5$					
Allele frequency:					
Allele 1	296 (66.6)	271 (65.7)	0.828	1.041	0.784–1.382
Allele 2	118 (26.6)	123 (30.0)	0.288	0.850	0.631–1.146
Allele 3	8 (1.8)	14 (3.4)	0.194	0.522	0.217–1.257
Allele 4	4 (1.0)	4 (0.9)	1.0	0.927	0.230–3.732
Allele 6	18 (4.0)	0 (0)	–	–	–

*OR, odds ratio; genotype 1/1: 410/410 bp (low producer of IL-1Ra); 2/2:240/240 (high producer); 1/2:410/240 (intermediate producer); 3/3: 500/500; 4/4: 325/325; 6/6: 154/154 (last three genotypes are also known to be intermediate producers).

Table IV. Association analysis of haplotypes in three LD blocks (IL-1β promoter, exon-5 and IL-1Ra genes) between north Indian ESRD patients and controls.

	Patient		Control				
Haplotype	*n*	Frequency	*n*	Frequency	*p*	*OR	95% CI
T-E1-1	152	0.3428	172	0.4170	0.112	0.726	0.491–1.075
C-E1-1	102	0.2252	84	0.2058	0.558	1.165	0.734–1.847
T-E1-2	64	0.1449	62	0.1454	0.892	0.951	0.557–1.623
C-E2-1	22	0.0506	38	0.0945	0.091	0.513	0.238–1.106
C-E1-2	28	0.0641	22	0.0516	0.687	1.193	0.529–2.692
T-E2-1	56	0.1268	16	0.0414	0.001	3.572	1.589–8.032
T-E2-2	10	0.0228	10	0.0241	1.000	0.926	0.264–3.247
C-E2-2	10	0.0227	10	0.0203	1.000	1.164	0.308–4.394

*OR, odds ratio; C/T, E1/E2 and 1/2 represent the alleles of IL-1β promoter region, exon-5 and IL-1Ra, respectively.

The present study found a highly significant association between the risk of ESRD and IL-1 gene cluster polymorphism. A significant difference in genotype frequencies between ESRD patients and a control group for IL-1β (promoter region and exon-5) and IL-1Ra gene polymorphism ($p < 0.001$, 0.006 and 0.001, respectively) was observed. The TT genotype of the IL-1β promoter was higher in cases than in controls and showed more than twice the higher risk for ESRD ($p < 0.001$, OR = 2.9). The higher frequency for allele 2 (T*) of IL-1β −511*2 (promoter region) in patients is assumed to represent a 'high secretor' phenotype leading to increased pro-inflammatory activity in autoimmune and infectious disease, resulting in over-production of IL-1 that may promote renal dysfunction and injury leading to ESRD (Hurme et al. 1998). The heterozygous genotype 'E1E2' in IL-1β exon-5 +3953 in patients showing a higher risk for renal failure corroborates the findings reported by Loughrey et al. (1998).

In IL-1Ra a significant difference was observed in 1/1 (410/410) and 1/2 (410/240) genotypes, but the highest risk for ESRD was observed in those carrying the 1/1 genotype. The results are contrary to previous studies where association of allele*2 has been reported with ESRD (Freedman et al. 1997, Wetmore et al. 2005), while Loughrey et al. (1998) and Bensen et al. (2003) reported non-association of allele 2* of IL-1Ra with diabetic and non-diabetic ESRD. The possible explanation for the discordance of these findings might be that either disease classification may impact on the nature and strength of association between IL-1Ra alleles and the outcome examined, or it could be related to ethnicity and/or geographical variation as compared with other populations including ours in studies reported. Laurincova (2000) postulated that production of IL-1Ra is regulated by IL-1β by counteracting its pro-inflammatory property. Further, IL-1Ra acts as a key factor in early down-regulation of the allogeneic immune response. Genotype 410/410 of IL-1Ra is a 'low producer' and cannot counteract the pro-inflammatory response of IL-1. This results in kidney disease as individuals with lower levels of IL-1Ra may therefore be less protected against the pro-inflammatory effects of IL-1 (Hurme et al. 1998).

The salient feature of the present study was an identification of a novel, sixth variant of IL-1Ra polymorphism containing a single, non-conserved copy of a 86-bp sequence present in 4% of the patient group similar to the observations made by Vamvakopoulos et al. (2002) in renal transplant recipients.

Single nucleotide polymorphism (SNP) at a given locus in any individual may be a part of a haplotype that has an effect on protein expression or function, whereas exactly the same SNP in other individual may not form part of the functional haplotype. Before embarking on large-scale haplotype-based studies on samples from genetically distinct populations, it is important to consider the variation both in LD and in haplotype frequencies within and across populations, as both vary considerably. Such diversity will ultimately impact on the choice and number of 'tagging' SNPs to be typed for studies of candidate genes or for whole genome association studies. Therefore, a subset of SNPs is functionally important in complex disease traits, such as renal disease (Uitterlinden et al. 2004). It is well established that the three polymorphic sites studied in the IL-1 gene cluster show strong LD across IL-1β + 3954/IL-1β −511/IL-1Ra. The haplotypes were therefore constructed based on the existence of LD. As a recent report by Bensen et al. (2003) suggests several nucleotide variations are associated with haplotypes in the IL-1 gene cluster with ESRD, we, too, found a significant difference in haplotype 'T-E2-1' (greater than a threefold risk) frequency distribution between patients and controls ($p = 0.001$, OR = 3.572). The alleles *T and *E2 represent the 'high secretor' of IL-1β, while the allele *1 of IL-l-1Ra is a 'low producer'. Therefore, it is presumed that individuals possessing this haplotype are at higher risk for developing the disease as the pro-inflammatory effects of IL-1 could not be counteracted by IL-1Ra thus cause the tissue damage leading to ESRD. LD was significant between the IL-1β promoter region and exon-5 and between the IL-1β promoter and IL-1Ra of the IL-1 gene in the controls indicating a strong association among the variants.

The results implicated the IL-1 gene cluster as an important target of investigation in the development of strategies in ESRD and could conceivably provide the basis for defined anti-inflammatory approaches to limit renal disease progression. By determining the genotypes of relevant genes in this subgroup of patients with chronic renal disease, one may be able to identify individuals at a higher risk for progressive renal insufficiency.

Hopefully in the future finding the markers of ESRD and recognizing genetic changes affecting the initiation and progression of renal failure will recompense the nephrologists with a more precise approach for the identification of 'high-risk' ESRD patients and the development of accurate individual treatment strategies targeting pro-inflammatory components, thus meeting a crucial medical need as well as planning therapeutic interventions.

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References

Arend WP, Malyak M, Guthridge CJ, Gabay C. 1998. Interleukin-1 receptor antagonist: role in biology. Annual Review Immunology 16:27–55.

Bensen JT, Langefeld CD, Hawkins GA, Green LE, Mychaleckyj JC, Brewer CS, Kiger DS, Binford SM, Colicigno CJ, Allred DC, et al. 2003. Nucleotide variation, haplotype structure, and association with end-stage renal disease of the human interleukin-1 gene cluster. Genomics 82:194–217.

Bioque G, Crusius JBA, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SGM, Pena AS. 1995. Allelic polymorphism in IL-1β and IL-1 receptor antagonist (IL- 1Ra) genes in inflammatory bowel disease. Clinical and Experimental Immunology 102:379–383.

Cantagrel A, Navaux F, Lescoulie LP, Nourhashemi F, Enault G, Abbal M, Constantin A, Laroche M, Mazieres B. 1999. Interleukin-1β, interleukin-1 receptor antagonist, interleukin-4 and interleukin-10 gene polymorphisms. Arthritis and Rheumatism 42:1093–1100.

Danis VA, Millington M, Hyland VJ, Grennan D. 1995. Cytokine production by normal human monocytes: inter-subject variation and relationship to an IL-1 receptor antagonist (IL-1Ra) gene polymorphism. Clinical and Experimental Immunology 99:303–310.

Di Giovine FS, Takhsh E, Blakemore AIF, Duff GW. 1992. Single base polymorphism at –511 in the human interleukin1β gene. Human and Molecular Genetics 1:450.

Dusel JA, Burdon KP, Hicks PJ, Hawkins GA, Bowden DW, Freedman BI. 2005. Identification of podocin (NPHS2) gene mutations in African Americans with nondiabetic end-stage renal disease. Kidney International 68:256–262.

Freedman BI, Yu H, Spray BJ, Rich SS, Rothschild CB, Bowden DW. 1997. Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. Kidney International 51:819–825.

Hughes ME, Wilson RM, Ward JD, Duff GW. 1996. Interleukin-1 receptor antagonist allele (IL1RN*2) associated with nephropathy in diabetes mellitus. Human Genetics 97:369–374.

Hurme M, Santtila S. 1998. IL-1 receptor antagonist (IL-1RA) plasma levels are co-ordinately regulated by both IL-1R and IL-1beta genes. European Journal of Immunology 28:2598–2602.

Hurme M, Lahdenpohja N, Santtila S. 1998. Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. Annals of Medicine 30:469–473.

Labow M, Schuster D, Zetterstrom M, Nunes P, Terry R, Cullinan EB, Bartfai T, Solorzano C, Moldawer LL, Chizzonite R, McIntyre KW. 1997. Absence of IL-1 signaling and reduced inflammatory response in IL-1-type I receptor deficient mice. Journal of Immunology 159:2452–2461.

Laurincová B. 2000. Interleukin-1 family: from genes to human disease. Acta Univ Palacki Olomuc Fac Med 143:19–29.

Loughrey BW, Maxwell AP, Fogarty DG, Middleton D, Harron JC, Patterson CC, Darke C, Savage DA. 1998. An interleukin 1B allele, which correlates with a high secretion phenotype, is associated with diabetic nephropathy. Cytokine 10:984–988.

Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 16:1215.

Nordfors L, Lindholm B, Stenvinkel P. 2005. End-stage renal disease – not an equal opportunity disease: the role of genetic polymorphisms. Journal of Internal Medicine 258:1–12.

Satko SG, Freedman BI, Moossavi S. 2005. Genetic factors in end-stage renal disease. Kidney International 67(Suppl. 94):S46–S49.

Syrjanen J, Hurme M, Lehtimaki T, Mustonen J, Pasternack A. 2002. Polymorphism of the cytokine genes and IgA nephropathy. Kidney International 61:1079–1085.

Tarlow JK, Blakemore AIF, Lennard A, Solari R, Hughes HN, Steinkasserer A, Duff GW. 1993. Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86 base pair tandem repeat. Human Genetics 91:403–404.

Uitterlinden AG, Fang Y, Van-Meurs JB, Pols HA, Van Leeuwen JP. 2004. Genetics and biology of vitamin D receptor polymorphisms. Gene 338:143–156.

Vamvakopoulos JE, Taylor CJ, Morris-Stiff GJ, Green C, Metcalfe S. 2002. The interleukin-1 receptor antagonist gene: a single-copy variant of the intron 2 variable number tandem repeat (VNTR) polymorphism. European Journal of Immunogenetics 29:337–340.

Wetmore JB, Hung AM, Lovett DH, Sen S, Quershy O, Johansen KL. 2005. Interleukin-1 gene cluster polymorphisms predict risk of ESRD. Kidney International 68:278–284.

Yao Q, Lindholm B, Stenvinkel P. 2004. Inflammation as a cause of malnutrition, atherosclerotic cardiovascular disease, and poor outcome in hemodialysis patients. Hemodialysis International 8:118–129.

Yoo J, Seo B, Kim Y. 2005. SNPAnalyzer: a web-based integrated workbench for single-nucleotide polymorphism analysis. Nucleic Acids Research 33(Web Server issue): W483–W488.