Interleukin-1 gene cluster polymorphisms predict risk of ESRD

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Background. Patients with chronic kidney disease manifest an inflammatory state relative to healthy individuals. Inflammation is regulated in part by genes of the interleukin-1 (IL-1) gene cluster. We hypothesized that polymorphisms in this gene cluster may be associated with risk of end-stage renal disease (ESRD).

Methods. Polymorphisms in the IL-1 gene cluster were examined in a cohort of 239 racially diverse hemodialysis (HD) patients and 252 controls. These individuals were genotyped for 3 single nucleotide polymorphisms (SNPs) in the IL-1α and β genes, and a variable-number-of-tandem-repeats polymorphism in the IL-1 receptor antagonist gene (IL-1RN). Polymorphisms were analyzed by logistic regression for their independent associations with ESRD, and the effect of allele dose of IL-1RN on risk for ESRD was examined. The interaction between race and genotype was also investigated.

Results. A logistic regression model demonstrated that homozygosity for allele 2 of the IL-1RN variable-number-of-tandem-repeats (VNTR) polymorphism was associated with ESRD independent of race (P < 0.0005). The IL-1α-889 promoter SNP was associated with ESRD independent of race and of the IL-1RN polymorphism (P = 0.04). The IL-1β-511 promoter SNP is associated with ESRD, but this is accounted for by race (P = 0.04).

Conclusion. Two polymorphisms within the IL-1 gene cluster are associated with ESRD independent of race. This finding is one of the strongest associations between genotype and ESRD reported, and suggests that polymorphisms in the IL-1 gene cluster affect the risk of development of ESRD.

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relationship between IL-1RN allele 2 and more common causes of kidney disease remains unclear. While 2 studies have demonstrated an association of IL-1RN allele 2 with diabetic nephropathy [14, 15], 1 has not [16].

Discordant findings could be the result of several factors. They could reflect differences in study populations because IL-1RN allele frequencies vary widely among different populations [9, 10]. The IL-1RN allele 2 polymorphism may not be the true cause of the observed disease associations; rather, the true disease association may be a second, disease-contributing polymorphism in linkage disequilibrium with the IL-1RN VNTR polymorphism. Additionally, the issue of allelic dosing, or the number of copies of allele 2, has not always been rigorously examined [14, 15], presenting the possibility that disease associations might be driven primarily by individuals who are homozygous for the non–wild-type alleles. Finally, most reports have concentrated principally on a putative association of the IL-1RN polymorphism with renal disease [14, 15]. However, there is evidence that polymorphisms within the IL-1 gene cluster may be coordinately regulated [17], raising the possibility that some combination of alleles within the cluster might have functional implications. In no study has a logistic regression analysis been undertaken to rigorously examine which polymorphisms within the IL-1 gene cluster may be associated with ESRD.

We designed a study to clarify associations between polymorphisms in the IL-1 gene cluster and ESRD. A racially diverse cohort of hemodialysis (HD) patients was assembled, along with race-matched healthy controls. Subjects were genotyped for the IL-1α −889, IL-1β +3954, and IL-1β −511 SNPs were determined by 5′ nuclease activity (Taqman, Applied Biosystems, Foster City, CA, USA). This method utilizes the 5′ nuclease activity of the Taq polymerase along with fluorescence quenching. Primers and probes are as follows: IL-1α −889 forward primer 5′ GGCCACAGGAATTATAAAGCTGAGA, reverse primer 5′ GGAGAAGGAAGGCGATGATTTT, FAM probe CTCTAATGGTGTTGCC, VIC probe CTCTAATGATGTGTC; IL-1β +3954 forward primer 5′ GAGGCTCTCTGCAATTGACAGA, reverse primer 5′ AGGGTGTTGGTCTCTACCTT, FAM probe CTGTTTCTGCTGCTGGGA, VIC probe CTGTTTCTGCTGCTGGGA; IL-1β −511 forward primer 5′ ACCTAACAACATGTCCTACAC, reverse primer 5′ ATCGTGACATAAGCCTGTTA, FAM probe CATGTGTACAAAGGAGA, VIC probe CATGTGTACAAAGGAGA. Reactions were done in 5 μL, with 0.083 μL of the 40× Taqman assay mix (Applied Biosystems), 2.5 μL of 2× Taqman Master Mix, no. UNG (Applied Biosystems), and 10 ng of sample DNA. The reactions were run under the following conditions: 95°C (10 min. hold), then 92°C (15 sec.) and 60°C (1 min.) for 40 cycles.

**METHODS**

**Study subjects**

Patients were enrolled from 4 dialysis units within the San Francisco Bay Area: the San Francisco Veterans Administration Medical Center, San Francisco General Hospital, University of California San Francisco-Mount Zion Dialysis Unit, and Satellite Dialysis Center-Greenbrae. All ESRD patients over age 18 who had been dialyzing for at least 3 months without evidence of active inflammation were eligible to participate. The protocol was approved by the Institutional Review Boards of all study sites; patients provided written informed consent. Control samples were purchased from Coriell Cell Repositories (Camden, NJ, USA). A total of 100 Caucasians, 100 African Americans, 30 Asians, and 10 Hispanics were obtained. As the number of Hispanic samples obtainable from Coriell was limited, we collected blood from an additional 20 individuals of self-declared Central American origin.

**DNA isolation**

For DNA isolation, blood was collected in 15 mL EDTA tubes. Blood was centrifuged within 2 hours of collection and frozen at −70°C. DNA isolation was performed using a commercial kit for whole blood from Gentra-Systems (Minneapolis, MN, USA).

**Genotyping of IL-1α and β SNPS**

The IL-1α −889, IL-1β +3954, and IL-1β −511 SNPs were determined by 5′ nuclease activity (Taqman, Applied Biosystems, Foster City, CA, USA). This method utilizes the 5′ nuclease activity of the Taq polymerase along with fluorescence quenching. Primers and probes are as follows: IL-1α −889 forward primer 5′ GGCCACAGGAATTATAAAGCTGAGA, reverse primer 5′ GGAGAAGGAAGGCGATGATTTT, FAM probe CTCTAATGGTGTTGCC, VIC probe CTCTAATGATGTGTC; IL-1β +3954 forward primer 5′ GAGGCTCTCTGCAATTGACAGA, reverse primer 5′ AGGGTGTTGGTCTCTACCTT, FAM probe CTGTTTCTGCTGCTGGGA, VIC probe CTGTTTCTGCTGCTGGGA; IL-1β −511 forward primer 5′ ACCTAACAACATGTCCTACAC, reverse primer 5′ ATCGTGACATAAGCCTGTTA, FAM probe CATGTGTACAAAGGAGA, VIC probe CATGTGTACAAAGGAGA. Reactions were done in 5 μL, with 0.083 μL of the 40× Taqman assay mix (Applied Biosystems), 2.5 μL of 2× Taqman Master Mix, no. UNG (Applied Biosystems), and 10 ng of sample DNA. The reactions were run under the following conditions: 95°C (10 min. hold), then 92°C (15 sec.) and 60°C (1 min.) for 40 cycles.

**Genotyping of IL-1RN VNTR**

The polymorphic region within intron 2 of the IL-1RN gene was amplified using the polymerase chain reaction (PCR). Genomic DNA (1 ng) served as a template in a 50 μL PCR reaction. This reaction contained the following components: 20 pmol of each forward primer (5′-CTCAGCAACACTCTCAT-3′) and reverse primer (5′-TCCTGGTCTGAGGTA-3′); Taq gold polymerase 2.5U; 0.2 mmol/L of each of the 4 deoxynucleotide-triphosphates; and 1.5 mmol/L of magnesium chloride. Following the initial activation period for the Taq gold
(10 min. at 95°C), 35 cycles of PCR were performed. These consisted of denaturation (95°C × 1 min.), annealing (55°C × 1 min.), and extension (72°C × 1 min.). The size of the amplified products was determined by electrophoresis on a 2% agarose gel.

Statistical analysis

For Hardy-Weinberg equilibrium (HWE) calculations, we estimated the average proportion of each allele in the population. The fourth locus, which is penta-allelic, was collapsed into a biallelic locus (allele 2 vs. nonallele 2). We calculated the expected frequencies of each, and a chi-square test was used to test for departure from HWE. We also calculated the \( P \) value using an enumeration of the trinomial probabilities under the constraint that the number of alleles of each type is constant.

The IL-1\( \alpha \) -889 and \( \beta +3954 \) SNPs are known to be in linkage disequilibrium (LD), but the last 3 loci appear not to be, according to previous studies [18]. We tested for LD, within each race separately, using a likelihood ratio test as utilized in the haplo.score package [19]. To test the overall LD over the whole dataset, we combined the race-specific likelihood ratio tests into a single test by adding the log-likelihood ratios.

The combined dataset was examined for the association between the 4 loci (3 SNPs and 1 VNTR), race, and outcome (ESRD status). The SNPs were treated as an exposure with 3 categories (homozygous dominant, heterozygous, homozygous recessive). To investigate the issue of allelic dosing, the VNTR was examined as both a 3-category exposure and as a 2-category exposure (homozygous recessive vs. others). For the allelic-dose calculations, confidence intervals for the odds ratios were calculated using standard large-sample approximations appropriate for generalized linear models [20].

Since allele 2 status has been previously associated with the presence of diabetic kidney disease [14, 15], we conducted separate analyses on the diabetic and nondiabetic ESRD cases. A Fisher exact test was used to compare allele frequencies in the 2 subgroups.

Logistic regression analysis was then employed to study the association between ESRD status and genotype. We arrived at our final model using model selection using the Akaike Information Criterion (AIC) [21]. The criterion counterbalances 2 components of model selection: the explanatory power of the model, as measured by the deviance (which is an analogue for residual variance for logistic regression models), and model complexity, as measured by the number of parameters in the model. We always adjusted for race because differential proportions of cases and controls between races merely reflected our sampling strategy.

We then used forward selection: each polymorphism was tested individually for its association with ESRD, then tested individually for independent and interactive effects with other polymorphisms, as well as with race.

RESULTS

A total of 239 HD patients and 252 healthy controls were studied. Characteristics of the ESRD study subjects are shown in Table 1. The ESRD patients (cases) were a racially diverse group, typical of the population of Northern California. Table 2 shows the genotype frequencies at each locus by race. Caucasian controls were in HWE at all 4 polymorphic sites (data not shown). Caucasian cases were in disequilibrium at the VNTR locus, as shown in Table 3, with a larger-than-expected frequency of allele 2 homozygotes (\( P < 0.0001 \)). African American controls were in HWE; cases were also in disequilibrium at the VNTR (\( P < 0.001 \)), although there were small numbers of allele 2 homozygotes in the African American cases and controls. Hispanics and Asian Americans were not tested because the numbers were smaller than in the other 2 groups.

We then examined the linkage disequilibrium (LD) between each pair of consecutive loci. Significant LD existed only for the first 2 polymorphisms, IL-1\( \alpha \) -889/IL-1\( \beta +3954 \) (\( P < 0.0001 \)) and not between the other 2 consecutive pairs. As LD did not exist across the final 3 positions, IL-1\( \beta +3954/IL-1 \) -511/IL-1RN VNTR (\( P = 0.22 \)), haplotypes were not constructed. Given this pattern of linkage disequilibrium, we elected to perform polymorphic marker-based association tests rather than constructing haplotypes [22].

We assessed for existence of an allelic-dose effect of the IL-1 VNTR. Subjects were analyzed by the number of copies of allele 2 (Table 4). There were significantly more allele 2 homozygotes in the cases than in the controls (\( P < 0.01 \)). As there was no difference between heterozygotes and non-2 homozygotes (that is, between heterozygotes and individuals homozygous for alleles 1, 3, 4, or 5),
Table 2. Genotype in cases and controls at the four loci by race

<table>
<thead>
<tr>
<th>Race (N)</th>
<th>IL-1α -889</th>
<th>IL-1β +3954</th>
<th>IL-1β -511</th>
<th>IL-1RN VNTR&lt;br&gt;a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td>CC</td>
</tr>
<tr>
<td>Caucasian Cases (60)</td>
<td>43</td>
<td>54</td>
<td>3</td>
<td>52</td>
</tr>
<tr>
<td>Controls (99)</td>
<td>62</td>
<td>30</td>
<td>8</td>
<td>65</td>
</tr>
<tr>
<td>African American Cases (81)</td>
<td>32</td>
<td>53</td>
<td>15</td>
<td>78</td>
</tr>
<tr>
<td>Controls (98)</td>
<td>39</td>
<td>47</td>
<td>14</td>
<td>78</td>
</tr>
<tr>
<td>Hispanic Cases (29)</td>
<td>69</td>
<td>21</td>
<td>10</td>
<td>93</td>
</tr>
<tr>
<td>Controls (29)</td>
<td>66</td>
<td>31</td>
<td>3</td>
<td>76</td>
</tr>
<tr>
<td>Asian Cases (60)</td>
<td>85</td>
<td>13</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>Controls (30)</td>
<td>90</td>
<td>10</td>
<td>0</td>
<td>97</td>
</tr>
</tbody>
</table>

Table 3. Hardy-Weinberg Equilibrium at IL-1 VNTR, African Americans, and Caucasians

<table>
<thead>
<tr>
<th>Race</th>
<th>Non2, non2&lt;br&gt;a</th>
<th>Non2,2</th>
<th>2,2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>African American Cases</td>
<td>63</td>
<td>12</td>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>Controls</td>
<td>81</td>
<td>14</td>
<td>3</td>
<td>0.056</td>
</tr>
<tr>
<td>Caucasian Cases</td>
<td>39</td>
<td>13</td>
<td>18</td>
<td>0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>63</td>
<td>29</td>
<td>7</td>
<td>0.231</td>
</tr>
</tbody>
</table>

Table 4. Allelic dosing of IL-1 VNTR genotype, cases versus controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non2, non2&lt;br&gt;a</td>
<td>Referent</td>
<td>–</td>
</tr>
<tr>
<td>Non2,2</td>
<td>0.98</td>
<td>0.87–1.10</td>
</tr>
<tr>
<td>2,2</td>
<td>1.3&lt;br&gt;b</td>
<td>1.12–1.51</td>
</tr>
</tbody>
</table>

Table 5. Association of IL-1RN genotype with ESRD, diabetic versus nondiabetic cases

<table>
<thead>
<tr>
<th>Cause of ESRD</th>
<th>Non2, non2</th>
<th>Non2,2</th>
<th>2,2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>58</td>
<td>24</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>108</td>
<td>16</td>
<td>19</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Logistic regression model for ESRD incorporating genotype and race

<table>
<thead>
<tr>
<th>Variable&lt;br&gt;c</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
<th>Overall P value&lt;br&gt;d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race (Caucasian)</td>
<td>3.43</td>
<td>1.37–8.58</td>
<td>0.001</td>
</tr>
<tr>
<td>African American</td>
<td>12.72</td>
<td>4.22–38.40</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>3.82</td>
<td>0.99–14.77</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2.99</td>
<td>1.50–5.97</td>
<td>0.0005</td>
</tr>
<tr>
<td>IL-1 VNTR (non2, non2 + non2,2)</td>
<td>2.2</td>
<td>1.54–3.09</td>
<td>0.04</td>
</tr>
<tr>
<td>IL-1α -889 (CC)</td>
<td>1.74</td>
<td>0.98–2.66</td>
<td>0.24</td>
</tr>
<tr>
<td>IL-1β -511 (CC)</td>
<td>1.54</td>
<td>1.13–3.09</td>
<td>0.24</td>
</tr>
<tr>
<td>IL-1β -511 x race</td>
<td>1.96</td>
<td>1.66–2.62</td>
<td>0.04</td>
</tr>
</tbody>
</table>

VNTR status was considered as a “2-position” variable: individuals were either allele 2 homozygotes (“2,2”), or were placed into a combined group made up of all others: allele 1, 3, 4, or 5 homozygotes and heterozygotes. No allelic dose effect was noted for any of the other 3 SNPs (data not shown).

We conducted separate analyses on the diabetic and nondiabetic ESRD cases. Results are shown in Table 5. Allele 2 homozygosity was strongly associated with ESRD in both subgroups; there was no difference in the genotype distribution between diabetic and nondiabetic ESRD cases (P = 0.849, Fisher exact test).

A forward logistic regression model was constructed, as shown in Table 6. The wild-type allele is designated “C,” with the variant labeled “T.” As there were larger numbers of Caucasians and African Americans than Asians (P < 0.001), race was incorporated into all models. Each of the 4 polymorphisms was then individually tested for its association with ESRD. Based on the allelic dosing results, IL-1 RN VNTR was coded as 2,2 homozygotes versus others. For the other polymorphisms, a 3-exposure model (homozygous dominant, heterozygous, homozygous recessive) was used. IL-1RN allele 2 homozygosity was associated with ESRD independent of race (P < 0.0005), but none of the other SNPs was significantly associated with ESRD. Race and VNTR allele 2 status were, therefore, incorporated into a base model, and the 3 SNPs were tested for their association with ESRD.
after adjustment for these variables. Although the IL-1α−889 promoter SNP was not significantly associated with ESRD prior to adjustment for IL-1 VNTR allele 2, it was independently associated with ESRD \((P = 0.04)\) after adjustment. IL-1α−889 genotype was then incorporated into the model, and the 2 remaining SNPs tested for associations with ESRD after adjustment for race, VNTR allele 2, and IL-1α−889. No significant associations were found between the 2 remaining SNPs and ESRD.

Each polymorphism was then tested for its interaction with race (i.e., the chance that an individual polymorphism has a differential association with ESRD among races). The IL-1β−511 SNP alone interacted with race \((P = 0.04)\). Specifically, the homozygous wild-type pattern in Caucasians was associated with a decreased risk of ESRD compared to the other 2 genotypes. Finally, to determine whether the association between IL-1α−889 and ESRD differed depending on IL-1 RN VNTR genotype, an interaction term was tested in the model. Because no interaction between IL-1α−889 and VNTR was noted, this term was not added to the final model. The final model therefore included terms for race, IL-1 VNTR genotype, IL-1α−889 genotype, IL-1β−511 genotype, and IL-1β−511 x race interaction.

**DISCUSSION**

In this study, 4 polymorphisms spanning the IL-1 gene cluster were investigated for their association with ESRD. Here we report that 2 of these polymorphisms, the IL-1RN VNTR and the IL-1α−889 SNP, are independently associated with ESRD in a racially heterogeneous dialysis population.

Initially, the 4 polymorphisms were analyzed for the presence of linkage disequilibrium to determine the suitability for a strategy of haplotype analysis. We found that while the IL-1α−889 and IL-1β+3954 SNPs were in LD, there was only weak LD between the last 3 polymorphisms (IL-1β+3954, IL-1β−511, and IL-1RN VNTR). Our results are in concert with those of Bensen et al, who cataloged 95 polymorphisms in the IL gene cluster in a mixed Caucasian and African American population [18]. They found an LD “trough” between IL-1β and IL-1RN, indicating that the receptor antagonist gene was not segregating in concert with genes known to be “upstream” (IL-1α and β).

IL-1RN allele 2 has been associated with diabetic nephropathy [15]. These findings raise the possibility genotype-disease status (in particular, IL-1RN genotype and ESRD) may be confounded by intermediate phenotypes or predisposing diseases such as diabetes. Accordingly, we examined whether the diabetic subgroup within our ESRD cohort was enriched in allele 2 homozygotes; this appears not to be the case, with no significant differences in the genotype distributions.

IL-1RN allele 2 homozygosity was associated with ESRD independent of race in our study. That allele 2 frequencies were not in HWE in our Caucasian cases (i.e., that these alleles were in higher-than-expected frequencies in patients who have advanced to ESRD) implicates this locus in pathogenesis of ESRD. The relationship of the IL-1RN VNTR polymorphism and renal disease has been examined in several previous studies. One study reported an association between IL-1RN allele 2 carriage and ESRD, with both diabetic and nondiabetic ESRD patients having significantly higher allele carriage rate of IL-1RN allele 2 [14], in concordance with our results. Whether homozygosity for allele 2 was a stronger predictor of outcome than merely carriage of the allele was not addressed. The reported association of allele 2 by Blakemore et al added to the body of evidence suggesting that allele 2 has an association with kidney disease [15]. Again, association with allele 2 homozygosity appears not to have been specifically examined. Diabetic nephropathy, rather than ESRD, was also the outcome in an analysis by Loughrey et al, in which no association was found between IL-1RN allele 2 and case status [16].

There are 2 possible explanations for the discordance of findings in the literature. First, disease classification might impact the nature and strength of association between IL-1RN allele 2 and the outcome examined. The true allele 2 association may not be with renal disease, per se, but with inflammation, scarring, and progression to ESRD. If this is the case, studies examining an “intermediate” or “incomplete” phenotype may result in weaker or more variable associations, as observed in the literature. Diabetics with proteinuria, such as those in the Blakemore and Loughrey studies [15, 16] are in a “moderate” stage of disease, and only a minority will progress to ESRD. Our patients, by virtue of having ESRD, are further advanced in the inflammatory progression of renal disease than other study populations. In this context, IL-1RN allele 2 may provide the true link between IL-1RN-mediated inflammation and ESRD, and as such may be a marker of renal disease progression [23].

Second, the true association of disease may be with homozygosity for allele 2. We observed a clear gene-dose effect of allele 2, in which the homozygous state was more strongly associated with disease status than the heterozygous or wild-type homozygous states. In the 2 studies that report an association between allele 2 and outcome, neither specifically examined the issue of allelic dosing, although Blakemore et al reported that allele 2 was associated with diabetic nephropathy when analyzed for allele carriage rate and allele frequency [15].

There is at least one other report of a similar allelic dose effect of IL-1RN allele 2 on a disease condition: in a study of IL-1 polymorphisms in coronary artery disease, Frances et al implicate allele 2 homozygosity as a marker
for vascular disease [11]. In one subgroup, the OR for disease status was substantially increased in allele 2 homozygotes, a finding not noted in heterozygotes.

The observed allelic dosing in the IL-1RN VNTR could be a functional consequence of genotype variation, such as altered production of the gene product [24]. The dose effect of allele 2 on receptor antagonist production has been examined in human cultured endothelial cells [25]. Cells homozygous for the RN allele 2 produced less than half the amount of IL-1 receptor antagonist than wild-type homozygotes (P < 0.05), and tended to produce less than the heterozygous cells. This same group also demonstrated a lower level of endothelial cell population doublings and increased cellular senescence in allele 2 homozygotes as compared to wild-type cells [24]. These authors hypothesized that capacity for endothelial cell regeneration is a determinant of progression of atherosclerosis and vascular disease. Thus, individuals with a lower endothelial replicative phenotype could be at increased risk for vascular disease and, conceivably, progression to ESRD.

IL-1α −889 allele status was also associated with ESRD in our study. Only one other report, to our knowledge, has investigated the IL-1α −889 SNP and its association with renal disease. Loughrey et al found no association of this SNP with diabetic nephropathy [16]. In our analysis, the association of the IL-1α −889 SNP with ESRD became evident only after adjustment for the effect of the VNTR. This, together with the larger P value (P = 0.04 vs. P = 0.0005 for the VNTR) implies that the association of this SNP with ESRD is much weaker than that of the VNTR. There was no interaction between the polymorphisms uncovered in our analysis, demonstrating that one polymorphism does not have a differential association with ESRD dependent on the presence of another. Thus, both polymorphisms may be implicated in true, independent associations with ESRD, underscoring the possible importance of the IL-1 gene cluster as a mediator of inflammation in the progression of chronic kidney disease to ESRD.

At the IL-1β −511 position, there was an association with ESRD that was accounted for by race (P = 0.04); that is, the association of this SNP with ESRD is likely to be the result of a differential effect among races. Specifically, this result was driven by the lack of association of the CC genotype with ESRD in Caucasians. No such association was found in the other races. As this was not a generalized finding, and as the association was marginal, this should be considered a hypothesis-generating finding.

As typical of the dialysis population of Northern California and, increasingly, the dialysis population of the United States, we assembled a cohort with considerable racial diversity. This proved to be both strength and a limitation of our study. Although our overall sample size provided adequate power to assess associations between genotype and ESRD, power was limited to perform race-stratified analyses. Therefore, our control group was assembled to closely match the racial distribution, and our forward logistic regression model was constructed to examine the effects in the different groups in a single analysis, minimizing any loss of power. The racially diverse nature of our cohort was an advantage because it is representative of the United States dialysis population and, thus, increases the generalizability of our results.

CONCLUSION

Specific polymorphisms in the interleukin-1 gene cluster are associated with ESRD in a cohort of dialysis patients. Both the IL-1α −889 and the IL-1RN VNTR polymorphisms predict ESRD status independent of race. For the VNTR polymorphisms, dose of the “deleterious” allele 2 provides a strong association with ESRD. The polymorphisms do not appear to be in linkage disequilibrium, implying that each is independent of each other. For this reason, we posit that logistic regression, rather than haplotype analysis, is the appropriate strategy for detecting polymorphism-disease associations. Logistic regression, applied for the first time to our knowledge in the study of IL-1 genes in ESRD, demonstrates the importance of multiple polymorphisms in the IL-1 gene cluster as determinants of ESRD. Additionally, our approach may clarify inconsistent findings in the literature because examination of independent contributions of several polymorphisms may both increase power and detect true polymorphism-disease associations that are inconsistent or obscured when examined individually. Because our cohort has substantial racial diversity, the results may be more broadly applicable than those of previous findings. 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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