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AN IL-6 HAPLOTYPE ON HUMAN CHROMOSOME 7P21 CONFERS RISK FOR IMPAIRED RENAL FUNCTION IN TYPE 2 DIABETES

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Abstract

Human chromosome 7p21 potentially harbors a major gene for end-stage renal disease (ESRD) and variation in glomerular filtration rate. The pro-inflammatory *IL-6* is a prime positional candidate gene since chronic inflammation has been implicated in diabetic nephropathy. To test this hypothesis, single SNP and haplotype analyses were performed using a case-control study design with 295 cases and 174 normoalbuminuric controls. Cases were subcategorized into those with proteinuria (n=138) or chronic renal failure (CRF)/ESRD (n=157). Tagging SNPs (rs2069827, rs1800795 (-174G>CSNP), rs2069837, rs2069840, rs2069861) were chosen based on linkage disequilibrium patterns and genotyped along with rs1800796 (-634G>C SNP). Single SNP analyses suggested that the rs1800795 may be associated with risk of CRF/ESRD (P=0.028) but this was not significant after Bonferroni correction. While haplotype analyses revealed no association with the presence of proteinuria, there was significant evidence for such an association with CRF/ESRD. Strikingly, there was an excess of the G-G-G-A-G-C haplotype among cases with CRF/ESRD (25.5%) compared to controls (15.4%) (P=0.0016). This association remained statistically significant even after correction for multiple testing (Bonferroni corrected P-value = 0.0224). In conclusion, our study has yielded first evidence for an *IL-6* haplotype that confers risk for impaired renal function in type 2 diabetes.

Keywords

Bonferroni correction; Chronic inflammation; Proteinuria; Chronic renal failure; End-stage renal disease; Genetic susceptibility; Glomerular filtration rate; Genome scan; Haplotype

INTRODUCTION

Diabetic nephropathy and its related traits including end stage renal disease (ESRD) and variation in glomerular filtration rate (GFR) are strongly determined by genes (1). Two recent genome scans revealed evidence for such a gene locus on human chromosome 7p21 (2,3). Ordered subset analysis showed significant evidence for linkage with ESRD (logarithm of odds, LOD = 3.6) in the 37% of African American families with longest duration of type 2 diabetes prior to onset of renal failure (3). In a more recent study on families with type 2 diabetes (2), variation in GFR as estimated by serum cystatin C was also linked to this same locus (LOD

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= 4.0) with the peak of linkage co-localizing almost exactly with that reported by Bowden at al (3). Inspection of the critical region closely underneath the peak of linkage revealed interleukin-6 (*IL-6*) as a prime positional candidate gene.

IL-6 is a key pro-inflammatory cytokine produced by a variety of cell types including glomerular mesangial cells (4,5). Functionally, IL-6 regulates the production of cell adhesion and chemotactic molecules, and mediates the release of other cytokines that amplify the inflammatory response (6,7). Molecular dissection of the *IL-6* promoter has implicated several single nucleotide polymorphisms (SNPs) as having functional importance, particularly -174G>C (rs1800795) and -634G>C (rs1800796) SNPs. In HeLa cells, the rs1800795 C reporter gene construct was found to exhibit lower gene expression compared to the rs1800795 G construct. Furthermore, stimulation with lipopolysaccharide (LPS) or IL-1 upregulated gene expression from the rs1800795 G, but not the C allele construct. Compared to the C allele, the pro-inflammatory rs1800795 G allele was associated with raised plasma levels of IL-6 in healthy subjects (8). The functional importance of rs1800796 has likewise been demonstrated. Carriage of the rs1800796 G allele has been associated with higher IL-6 secretion in peripheral blood mononuclear cells upon stimulated with LPS or advanced glycation end product-bovine serum albumin (9). The control of *IL-6* gene expression is however likely to extend beyond just rs1800796 and rs1800795 and be influenced by the presence of other polymorphisms at this chromosomal locus (10).

The pathogenic role of chronic inflammation in a variety of human diseases is well recognized (11). More recently, this process has been implicated in the etiology of diabetic complications (12). A culprit role for IL-6 in diabetic nephropathy is supported by the observation that serum levels of this cytokine were correlated with the severity of renal disease among both type 1 and type 2 diabetic patients (13,14). Furthermore, macroalbuminuria in type 2 diabetic Japanese patients has been associated with rs1800796 GG genotype (9). Another report of late found that among Asian Indians, the risk of ESRD was associated with the rs1800795 GG genotype, although ESRD was largely attributed to glomerulonephritis (15).

Taking into account these intriguing observations as well as the results of linkage and association studies reported to date, we sought to investigate whether susceptibility to advanced diabetic nephropathy including impaired renal function could be explained by genetic variation at the *IL-6* locus. Since gene regulation of *IL-6* is likely to be complex (10), we sought to provide the first haplotype analysis of this locus in relation to risk for this complication.

RESULTS

Patient characteristics

A total of 174 controls and 295 cases were analyzed in this study. Both groups were similar in terms of gender composition, age at diabetes diagnosis, and Hb_{A1c} values (P = NS) (Table 1). Besides being slightly older and having a longer known diabetes duration (both $P < 0.0001$), cases had higher systolic blood pressure ($P < 0.0001$) but slightly lower diastolic blood pressure compared to controls ($P < 0.001$) (Table 1). 53.2% (157/295) of cases had already developed CRF/ESRD by the time of enrolment into this study.

Linkage disequilibrium (LD) and tagSNPs

LD across the *IL-6* locus was assessed using HapMap data for the Caucasian CEU samples. A total of 12 SNPs (rs2068927, rs1800797, rs1800795, rs2069832, rs2069833, rs1474348, rs2069837, rs1474347, rs2069840, rs1554606, rs2069845, rs2069861) were identified to be polymorphic (minor allele frequency >= 0.05). For these, pairwise |D'| values were high and therefore, they were considered to be in a single haplotype block (Supplementary Table 1). To

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capture haplotype diversity, 5 tagSNPs were then selected for genotyping (rs2069827, rs1800795, rs2069837, rs2069840, rs2069861). For completeness, rs1800796 was included although it has a low MAF < 0.05, as it may have functional significance (9).

Single SNP and haplotype analyses

Genotype distributions of all 6 SNPs were in Hardy Weinberg equilibrium when assessed according to individual renal comparison groups except for both rs2069827 (P = 0.0084) and rs1800795 (P = 0.0009) among cases with CRF/ESRD. The P-value for rs1800795 remained significant at 0.0162 after employing Bonferroni's correction for 18 comparisons. Genotype-disease associations were analyzed separately according to whether cases had proteinuria or CRF/ESRD (Table 2). None of the SNPs showed any significant association with the presence of proteinuria. However, rs1800795, which had been reported to be associated with ESRD (15), showed borderline evidence for an association with CRF/ESRD (P = 0.0280) but not proteinuria (P = 0.6477). From a statistically conservative standpoint, this positive association with CRF/ESRD did not remain significant after correction for 12 comparisons (P = 0.3360). Consideration of potentially important covariates including age, gender, blood pressure, glycaemic control and diabetes duration did not affect SNP-disease association (data not shown). There was no significant association between any of the SNPs and advanced diabetic nephropathy (proteinuria and CRF/ESRD combined) with the smallest P-value = 0.1266 (Table 2).

We next performed haplotype analyses using all 6 SNPs (rs2069827, rs1800796, rs1800795, rs2069837, rs2069840 and rs2069861). No haplotype was found to be significantly associated with the combined traits of proteinuria and CRF/ESRD after correction for multiple hypothesis testing (Table 3). As with the single SNP analysis, no association was found with proteinuria alone. On the other hand, there was evidence for an association with CRF/ESRD. Specifically, there was an excess of the G-G-G-A-G-C haplotype among cases with CRF/ESRD (25.5%) compared to controls (15.4%) (P = 0.0016) (Table 3). This risk haplotype is one of three at the *IL-6* locus that bore both rs1800795 G and rs1800796 G pro-inflammatory alleles and its disease association remained statistically significant even after a conservative Bonferroni correction for 14 haplotype-trait comparisons (P = 0.0224).

Additional haplotype analyses were performed after excluding rs1800796 so that only the 5 tagging SNPs (rs2069827, rs1800795, rs2069837, rs2069840 and rs2069861) were studied. In all haplotype-trait comparisons, no significant association was found after correction for multiple hypotheses testing (Supplementary table 2).

DISCUSSION

Emerging evidence suggests that *IL-6* is important in conferring genetic susceptibility to a host of human diseases including cancer (16), metabolic syndrome (17), systemic-onset juvenile chronic arthritis (8), and osteoporosis (18). In this present study, we have extended these observations by providing evidence that *IL-6* is a susceptibility gene for advanced diabetic nephropathy in type 2 diabetes. Particularly, there was a highly significant finding that pointed to the presence of a specific risk haplotype for impaired renal function in type 2 diabetes (P = 0.0016). With a conservative Bonferroni correction, this genetic association still remained statistically significant. On the other hand, analyses at the level of single SNPs did not revealed any clear evidence for association with advanced diabetic nephropathy even though two of the SNPs (rs1800796 and rs1800795) are thought to have functional significance.

The observation that the genotype distribution of rs1800795 deviated from HWE only in the CRF/ESRD group is consistent with the selective enrichment of the G allele with respect to this phenotype. Indeed, the association with CRF/ESRD did reach nominal significance (P =

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0.028). This SNP is however unlikely to explain the linkage signal for ESRD on human chromosome 7p21 as has been reported in type 2 diabetic African Americans (3) since this SNP is largely monomorphic in this ethnic group. Indeed, a search through dbSNP (http://www.ncbi.nlm.nih.gov/SNP/index.html) quickly revealed that the frequency of the G risk allele ranged from 96 to 100% among African Americans. This preponderance of the G allele is also seen in other human populations such as Chinese (100%), Japanese (100%), Hispanics (80%) and even sub-Saharan Africans (100%). Thus, if the *IL-6* locus does indeed confer susceptibility to impaired renal function among diabetic patients across different human populations, it is likely that a risk haplotype bearing the rs1800795 G_allele is involved in conferring this risk. Although our present study is confined to Caucasians, the positive findings clearly provided a reasonable basis to support this notion. The possibility that this *IL-6* haplotype may help explain the results of a recent genome scan that showed linkage of GFR to 7p21 in Caucasian families with type 2 diabetes is certainly intriguing and could be followed up upon in future studies (2).

Since our study suggested that genetic variation in *IL-6* was associated with impaired renal function rather than type 2 diabetes *per se*, one might have reasonably expected that the frequencies of the rs1800795 GG genotype as well as the GGGAGC haplotype among the proteinuric cases should be intermediate between the normoalbuminuric controls and the CRF/ESRD cases. Indeed, this would appear to be case with the rs1800795 GG genotype frequency rising from 42.9% (CTRL) to 47.7% (PROT) to 54.6% (CRF/ESRD) (Table 2). Similarly, the frequency of the GGGAGC haplotype rose from 15.4% (CTRL) to 16.7% (PROT) to 25.5% (CRF/ESRD) (Table 3). With the largest difference being observed with the CRF/ESRD group, the effect of *IL-6* gene variation seemed primarily to be on the development of CRF/ESRD rather than proteinuria. The intermediate frequency among the PROT group is potentially due to the fact that a limited subset of these cases may subsequently go on to develop CRF/ESRD.

Our present findings are discordant to that of a previous report which found that there was an association of the rs1800796 GG genotype with macroalbuminuria in Japanese patients with type 2 diabetes (9). Interestingly, while this SNP is common in Asians, the reverse is true in Caucasians. The rarity of this SNP does reduce the power of our study to detect a potential association but it should be emphasized that the prevalence of the rs1800796 GG genotype is virtually identical across the renal comparison groups. This suggested that the magnitude of this genotypic effect is absent or miniscule at best in our study population.

A point worth noting is that our most significant findings came from the haplotype analyses that incorporated all 5 tagging SNPs as well as reportedly functional SNP rs1800796. While the latter was not required as a tagging SNP, its fortuitous inclusion turned out to be important. Specifically, this SNP allowed the risk G-GG-A-G-C haplotype to be distinguished from the closely related G-C-G-A-G-C haplotype which itself did not show any disease association (Table 3).

Our study has several strengths. Firstly, we used a case-control collection that has been assembled following the sound epidemiological principle that cases and controls should be recruited from the same source population (19). Specifically, all cases and controls were recruited from among patients attending the Joslin Clinic and this reduced the possibility of potential bias or confounding which may arise when cases and controls are collected from separate sites. Although the possibility of population stratification in this patient collection has yet to be fully evaluated by analyzing large numbers of SNPs neutral to the phenotype of interest, we have undertaken practical steps to minimize this potential source of confounding by restricting our study to Caucasians residing in Massachusetts and attending the Clinic. Importantly, it is reassuring that previous positive results obtained using this collection has been confirmed in other independent studies (20). Secondly, our cases consisted of those with

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proteinuria alone as well as those with CRF/ESRD and as such, we were able to test for associations with these sub-phenotypes. Thirdly, we adopted a conservative position seldom taken in genetic association studies by accounting for multiple comparisons using the Bonferroni procedure. This gave us confidence that the association between the G-G-G-A-G-C risk haplotype and CRF/ESRD was likely a true finding.

A few potential limitations should be highlighted. Superficially, the requirement for a minimum known diabetes duration of at least 7 years among controls may seem to be relatively short especially since in our previous studies on type 1 diabetic patients, we had employed a minimal duration of 15 years (21). However, it is well established that the onset of type 2 diabetes is insidious and as such, patients are generally diagnosed only after 5-10 years after the actual onset of disease. Thus, in reality, the actual duration of diabetes in our controls would be closer to 12 years or more. In this connection, it may be highlighted that in a comprehensive meta-analysis of 47 studies comprising 14,727 subjects on the association between the angiotensin-I converting enzyme I/D polymorphism and diabetic nephropathy, no obvious benefit was observed by requiring controls to have a long known diabetes duration (22). This observation is consistent with the fact that most type 2 diabetic patients would already have had the disease for a significant period of time before diagnosis.

A second potential limitation is the possibility that some of our cases may have non-diabetic renal disease since renal biopsies are not routinely performed in clinical practice to diagnose diabetic nephropathy. However, Oslen and Mogensen have earlier concluded in their careful review of their own data as well as nine published studies that non-diabetic renal disease occurs only in a minority of type 2 diabetic patients (23). More recent studies demonstrate that even in the absence of concomitant diabetic retinopathy, the majority (70-74%) of albuminuric type 2 diabetic patients do indeed have diabetic glomerulopathy (24,25). We agree that while it is difficult to completely eradicate disease misclassification in genetic association studies on diabetic nephropathy, our present success in identifying the *IL-6* risk haplotype certainly suggested that misclassification (either in cases or controls) was not a major issue since substantial misclassification will inadvertently have the effect of driving such an association towards the null.

A third limitation is that our sample size is moderate especially when compared for instance, to that needed for genome-wide association studies (GWAS). However, this difference in sample size requirement is related to the fact that up to a million SNPs are tested in GWAS, and each SNP has an extremely low apriori probability of disease association. The large sample size is therefore needed in GWAS to produce very small P values but after taking into account the huge number of hypotheses tested, only a few potential associations will remain nominally significant. Such an extreme situation however does not exist in our current study. Instead, we employed a focused approach by studying select tagging SNPs in a candidate gene that has been implicated in the pathogenesis of diabetic nephropathy. Moreover, this gene is located in a chromosomal region linked to impaired renal function and this may have heightened the aprior probability of a true genetic association. Indeed, our present results would appear to have borne witness to this with strong evidence of a haplotype-disease association that withstood conservative statistical correction. Furthermore, our calculations indicated that this present study has 87% power to detect an association of the magnitude that was observed between the risk haplotype and CRF/ESRD. This suggested that our findings were quite unlikely due to statistical chance despite the modest sample size. Still, as with results from other genetic studies including WGAS, it is imperative to independently replicate our promising findings in other patient collections. Another limitation of our study is that due to its case-control nature, it is not possible to effectively address the potential issue of survival bias. As such, future cohort studies with a substantial number of incident cases of CRF/ESRD will be useful to independently replicate our findings.

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Finally, we currently do not know the functional significance of this G-G-G-A-G-C risk haplotype with regard to controlling *IL-6* gene expression in diabetes. In-vitro studies have suggested that *IL-6* haplotypes may cause differential gene transcription in cell lines of epithelial compared to those of endothelial origin (10). The direct relevance of this finding has unfortunately not been established in the diabetic kidney. It is perhaps noteworthy that the risk haplotype does contain the pro-inflammatory alleles for both -634G>C (rs1800796) and -174G>C (rs1800795) SNPs. This might lead one to speculate that G alleles of both SNPs may be required for the risk of CRF/ESRD. Thus, in human populations (e.g. Japanese, Chinese, African American) that are largely monomorphic for the -174G allele, increased disease risk would appear to be associated with the -634 G allele. In Caucasians with a low frequency of the -634 C allele, it would appear that the -174G>C SNP is potentially more important and that the association was strengthened further in a haplotype analysis that includes both SNPs. One may also postulate that coupled with the presence of other genetic variation at the *IL-6* locus, a localized pro-inflammatory process may occur in certain kidney cell types in diabetes culminating in impaired renal function.

In conclusion, our study has yielded first evidence for an *IL-6* haplotype that confers risk for impaired renal function in type 2 diabetes. This finding may potentially help explain the results of linkage studies for diabetic nephropathy and its related traits that have been previously been localized to chromosome 7p21.

METHODS

Study groups

Since 1998, individuals with type 2 diabetes have been recruited for studies of the genetics of nephropathy from among patients attending the Joslin Clinic in Boston, Massachusetts. Diabetes has been classified as type 2 if it was diagnosed between ages 35 and 64 years and was treated for at least 2 years with diet or oral hypoglycemic agents. Only patients younger than 75 years of age at enrolment are included in the study.

Diagnosis of diabetic nephropathy

Diabetic nephropathy was determined on the basis of the medical records of the Joslin Clinic (supplemented with records of other physicians if necessary), and results of routine urinary analyses, including measurements of the albumin to creatinine ratio (ACR) (26). As the diagnosis of type 2 diabetes is generally established many years after onset of hyperglycaemia, patients were classified as controls if they had type 2 diabetes with known duration of at least 7 years, and the ACR (in mg/g) was < 17 (men) or < 25 (women) in at least 2 out of the last 3 urine specimens spanning at least a 2 year interval. Patients with microalbuminuria or intermittent proteinuria were not included in this study. Patients were considered cases if they had persistent proteinuria or if they had ESRD due to diabetic nephropathy. Persistent proteinuria was defined as 2 out of 3 successive urinalyses positive by either reagent strip (greater than 2+ on Multistix, Bayer Corporation, Diagnostics Division, Elkhart, IN) or an ACR (in mg/g) greater than 250 (men) or greater than 355 (women). Patients with persistent proteinuria and serum creatinine greater than 2.0 mg/dl were considered as cases with CRF. At the time of this study, genomic DNA was available for 295 cases and 174 controls. Only Caucasians were included in this study.

Examination of study participants

All patients selected for the genetic studies were examined at the clinic or at their homes. After consenting to participate in the study, each subject had a standardized physical examination and provided a diabetes history regarding its diagnosis, treatment, and complications. Each individual provided a blood sample for biochemical measurements and DNA extraction.

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Patient medical records were thoroughly reviewed to minimize the possibility of the presence of non-diabetic kidney disease and patients were also directly questioned whether they were ever diagnosed for non-diabetic kidney disease by MDs. The Committee on Human Subjects of the Joslin Diabetes Center approved the protocols and informed consent procedures for our studies.

LD analysis and the selection of tagging SNPs

LD at the *IL-6* locus was assessed based on SNPs genotyped in the Hapmap Caucasian CEU samples (www.hapmap.org). A total of 46 SNPs were genotyped in a 7.4 kb region which encompassed the entire 4.8 kb *IL-6* gene. Of these, 34 SNPs (rs2069824, rs2069826, rs3087224, rs4335044, rs1800796, rs2069857, rs2069829, rs13447445, rs2069830, rs1524107, rs2066992, rs2069839, rs3087234, rs13306434, rs2069834, rs2069835, rs2069836, rs2069838, rs3087235, rs13306433, rs2069842, rs1548216, rs2069843, rs2069844, rs13306435, rs2069860, rs2069849, rs2069850, rs13306436, rs2069851, rs2069852, rs2069853, rs2069854, rs2069855) were not polymorphic or had a low minor allele frequency (MAF< 0.05) among the CEU samples.

Pairwise |D'| and r values was computed for the 12 remaining SNPs (rs2068927, rs1800797, rs1800795, rs2069832, rs2069833, rs1474348, rs2069837, rs1474347, rs2069840, rs1554606, rs2069845, rs2069861). Haplotype blocks were defined as previously described with |D'| >=0.8 being taken as evidence of strong LD (27). Within the haplotype block, tagSNPs (rs2069827, rs1800795, rs2069837, rs2069840, rs2069861) were chosen so that any marker that was not eventually chosen as a tagging marker was already strongly correlated with at least one of the tagging markers with r > 0.9.

Genotyping

Genotyping was performed using restriction fragment length polymorphism (RFLP) or commercially available Taqman-based assays (Applied Biosystems, Foster City, CA) (Online supplementary table 3).

Power calculation

For common SNPs with ~30% MAF (which includes rs2069840 and rs1800795 (-174G>C)), the power of our study exceeded 90%, 80% and 70% when the odds ratio was 1.8, 1.7 and 1.6 respectively. For less common SNPs with ~10% MAF (rs1800796, rs2069837 and rs2069861), power was 80%, 70% and 60% when the odds ratio was 2.0, 1.9 and 1.8 respectively. In haplotype analysis, our study has substantial power (87%) to detect the association between the GGGAGC risk haplotype and CRF/ESRD.

Statistical analysis

Data on the study groups were compared using χ^2 and Student's *t*-tests for categorical and continuous variables, respectively (The SAS® system for windows version 9.1, SAS Institute Inc. Cary, NC). Comparison of means across multiple groups was performed using ANOVA. Conformity of genotype distributions to that expected under Hardy-Weinberg equilibrium was assessed using χ^2 tests for goodness-of-fit. Estimation of haplotype frequencies was performed using the expectation-maximization algorithm (28). Statistical significance of haplotype association with disease was evaluated using a score test based on unphased genotypic data (29). A P-value of <0.05 was considered statistically significant after correction for the number of genotype and haplotype comparisons.

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Supplementary Material

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Table 1

Patient characteristics

Clinical characteristics	Controls	Cases	P-value
Number (n)	174	295	
Gender (male/female)	99/75	179/116	NS
Age at diabetes diagnosis (years)	42 (9)	43 (8)	NS
At the enrollment into the study:			
Age (years)	57 (8)	61 (8)	<0.0001
BMI (kg/m^2)	30 (16)	32 (7)	NS
Known duration of diabetes (years)	13 (7)	17 (8)	<0.0001
Hemoglobin A1c (%)	7.9 (1.4)	8.0 (1.6)	NS
Systolic blood pressure (mmHg)	129 (15)	137 (20)	<0.0001
Diastolic blood pressure (mmHg)	85 (23)	79 (18)	<0.001
Cases with CRF/ESRD (n)	NA	157	

Data are presented as mean (S D). NA, not applicable

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Table 2

Genotype distribution of *IL-6* SNPs according to renal status

SNP	Location	Genotype	CTRL	PROT/CRF/ESRD	PROT	CRF/ESRD	CTRL vs PROT/CRF/ESRD: P-value	CTRL vs PROT: P-value	CTRL vs CRF/ESRD: P-value
rs2069827	5′ flanking region	GG	153 (90.0)	247 (86.1)	112 (83.0)	135 (88.8)	0.2564	0.0706	0.1809
		GT	17 (10.0)	37 (12.9)	23 (17.0)	14 (9.2)			
		TT	0 (0.0)	3 (1.0)	0 (0.0)	3 (2.0)			
			170 (100.0)	287 (100.0)	135 (100.0)	152 (100.0)			
rs1800796	5′ flanking region	CC	4 (2.4)	4 (1.4)	3 (2.2)	1 (0.6)	0.7344	0.9970	0.4414
		CG	24 (14.1)	43 (14.9)	19 (14.1)	24 (15.7)			
		GG	142 (83.5)	241 (83.7)	113 (83.7)	128 (83.7)			
			170 (100.0)	288 (100.0)	135 (100.0)	153 (100.0)			
rs1800795	5′ flanking region	CC	22 (13.1)	38 (13.4)	14 (10.6)	24 (15.8)	0.1535	0.6477	0.0280*
		CG	74 (44.1)	100 (35.2)	55 (41.7)	45 (29.6)			
		GG	72 (42.9)	146 (51.4)	63 (47.7)	83 (54.6)			
			168 (100.0)	284 (100.0)	132 (100.0)	152 (100.0)			
rs2069837	Intron 2	AA	141 (83.4)	232 (81.1)	111 (82.2)	121 (80.1)	0.8247	0.9560	0.7455
		AG	27 (16.0)	52 (18.2)	23 (17.0)	29 (19.2)			
		GG	1 (0.6)	2 (0.7)	1 (0.7)	1 (0.7)			
			169 (100.0)	286 (100.0)	135 (100.0)	151 (100.0)			
rs2069840	Intron 3	CC	24 (14.0)	25 (8.7)	15 (11.2)	10 (6.6)	0.1266	0.0820	0.0904
		GC	60 (35.1)	120 (42.0)	64 (47.8)	56 (36.8)			
		GG	87 (50.9)	141 (49.3)	55 (41.0)	86 (56.6)			
			171 (100.0)	286 (100.0)	134 (100.0)	152 (100.0)			
rs2069861	3′ lanking region	CC	143 (84.1)	256 (89.5)	122 (90.4)	134 (88.7)	0.1487	0.1067	0.2292
		CT	27 (15.9)	29 (10.1)	12 (8.9)	17 (11.3)			
		TT	0 (0.0)	1 (0.4)	1 (0.7)	0 (0.0)			
			170 (100.0)	286 (100.0)	135 (100.0)	151 (100.0)			

CTRL, controls; PROT, cases with proteinuria; CRF/ESRD, cases with chronic renal failure/end-stage renal disease

*P = 0.3360 after Bonferroni correction for 12 comparisons.

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Table 3

IL-6 haplotype distribution according to renal status

	CTRL		PROT+CRF/ESRD		PROT		CRF/ESRD		CTRL vs PROT+CRF/ESRD		CTRL vs PROT		CTRL vs CRF/ESRD	
Haplotype*	**Frequency (%)**	**95%CI**	**Frequency (%)**	**95%CI**	**Frequency (%)**	**95%CI**	**Frequency (%)**	**95%CI**	**Haplotype--specific P-value**	**Global P - value**	**Haplotype -specific P-value**	**Global P - value**	**Haplotype -specific P-value**	**Global P - value**
G-G-C-A-G-C	22.0	17.7-26.4	18.2	15.0-21.3	17.9	13.4-22.5	18.6	14.2-22.9	0.1505	0.1450	0.1953	0.5029	0.2473	0.0397
G-G-C-A-G-T	8.0	5.1-10.8	5.4	3.6-7.3	5.2	2.5-7.8	5.7	3.1-8.3	0.1033		0.1545		0.1947	
G-G-G-A-C-C	31.6	26.7-36.5	29.2	25.5-32.9	33.9	28.2-39.6	25.0	20.2-29.8	0.5225		0.5373		0.1049	
G-G-G-A-G-C	15.4	11.6-19.2	21.4	18.1-24.8	16.7	12.2-21.1	25.5	20.6-30.4	0.0300#		0.6804		0.0016†	
G-G-G-G-G-C	8.6	5.6-11.5	9.1	6.8-11.5	8.0	4.8-11.3	10.2	6.8-13.6	0.8597		0.8801		0.6787	
G-C-G-A-G-C	9.4	6.3-12.5	8.5	6.2-10.8	8.7	5.1-11.8	8.5	5.4-11.6	0.5350		0.6155		0.5897	
T-G-C-A-G-C	5.0	2.7-7.3	6.9	4.8-8.9	7.1	4.1-10.2	6.5	3.8-9.3	0.3413		0.3763		0.4312	
Total	100.0		98.9		97.5		100.0							

CTRL, controls; PROT, cases with proteinuria; CRF/ESRD, cases with chronic renal failure/end-stage renal disease

*Haplotypes with estimated frequencies < 1% are excluded. Haplotypes are formed from rs2069827, rs1800796, rs1800795, rs2069837, rs2069840, and rs2069861.

#Not significant for correction for 7 haplotype-trait comparisons.

†P = 0.0244 after correction for a total of 7 haplotype-PROT and 7 haplotype-CRF/ESRD comparisons