Haplotype of Signal Transducer and Activator of Transcription 3 Gene Predicts Cardiovascular Disease in Dialysis Patients

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Signal transducer and activator of transcription 3 (*STAT3*) protein has been linked to cardiovascular disease (CVD) through multiple pathways in experimental and animal studies. *STAT3* gene variation was examined as a predictor of incident CVD in a subcohort of 529 incident white dialysis patients. Fifteen single-nucleotide polymorphisms of the *STAT3* gene were genotyped. Haplotypes were estimated using software PHASE 2.1, and associations with first CVD event were tested using Cox proportional hazards analysis. Adjusted global tests of haplotype association with incident CVD and inflammation markers were performed using permutated *P* value in R-package Haplo.score. An *a priori* specified additive genetic model was assumed for haplotype analysis. Both genotypes (four single nucleotide polymorphisms with $P < 0.001$) and haplotypes ($P = 0.002$ overall) were associated with incident CVD. Two major haplotype blocks, blocks A and C, were identified. Compared with common haplotype A-1, A-3 was associated with a hazard ratio (HR) of 0.70 (95% confidence interval [CI] 0.51 to 0.94) for CVD events after adjustment for covariates including C-reactive protein (CRP) and interleukin 6. Compared with common haplotype C-1, C-3 was associated with an adjusted HR of 2.12 (95% CI 1.25 to 3.57) for CVD events. Associations were independent of inflammation markers, but IL-6 levels were 14% lower (geometric mean ratio 0.86; 95% CI 0.77 to 0.96) per copy of haplotype A-3 compared with haplotype A-1 in block A after adjustment for CRP and other risk factors ($P = 0.008$). Variation in the *STAT3* gene is associated with the risk for CVD among white dialysis patients independent of serum IL-6 and CRP levels.

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The protein signal transducer and activator of transcription 3 (*STAT3*) can both activate and repress transcription level of many genes through the Janus kinase-signal transducer and activator of transcription pathways (1). In particular, laboratory studies have suggested that *STAT3* can affect cardiovascular disease (CVD) risk by modulating transcription of genes in several different pathways, leading to various cardiovascular intermediate phenotypes. *STAT3* has been shown to be the most potent activator of acute-phase gene transcription among the family of *STAT* transcription factors (2). It can either promote or inhibit inflammation through mediating effects of IL-6 or IL-10 (3–5), respectively. For example, S*TAT3* mediates IL-6 effects on angiotensinogen gene expression, which plays a critical role in cardiovascular homeostasis and has significant proinflammatory actions in the vascular wall (6,7). *STAT3* knockout mice have been shown to have an increased production of proinflammatory cytokines (8) and increased fibrosis and susceptibility to inflammation-induced heart damage (9). In addition, *STAT3* affects endothelial function through effects on endothelial production of nitric oxide (10), intercellular adhesion molecule-1 (11), and chemokine (12). *STAT3* also has been shown to play protective roles in myocardial adaptation to stress, such as ischemia injury (13–15). The beneficial action of late ischemic preconditioning on myocardial necrosis is mediated through *STAT3* upregulation of the inducible nitric oxide synthase protein and cyclooxygenase (16,17).

Although experimental and animal studies support the role of *STAT3* in CVD development, no human studies have exam-

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ined the association between *STAT3* gene polymorphisms and CVD risk. Results from laboratory studies may not be generalizable to humans. Dialysis patients are subject to multiple proinflammatory stimuli, which can unmask underlying genetic susceptibilities. We therefore used data from the Choices for Healthy Outcomes in Caring for ESRD (CHOICE) study to examine whether polymorphisms of the *STAT3* gene can predict CVD risk in white incident dialysis patients and whether any observed associations are independent of inflammation markers.

Materials and Methods

Study Design and Population

The CHOICE study is a multi-center, prospective study to investigate treatment choices and outcomes of dialysis care among patients who begin dialysis. Its rationale and design have been reported (18). Briefly, the CHOICE study recruited 1041 incident dialysis patients from 81 Dialysis Clinic Inc. clinics between 1995 and 1998. Eligibility criteria for CHOICE study included ability to provide informed consent for participation, age older than 17 yr, and ability to speak English or Spanish. Patients were enrolled a median of 45 d from initiation of chronic dialysis (98% within 4 mo); 54% of the cohort had diabetes at baseline, and 51% of the cohort died by December 1, 2001. Because of a limited sample size of black patients ($n = 246$) in the CHOICE cohort for haplotype-based genetic analyses, our analysis is limited to white CHOICE study participants with DNA samples ($n = 529$). All patients provided informed consent. The Johns Hopkins University School of Medicine Institutional Review Board and the review boards for the clinical centers approved the protocol.

Outcomes

The primary outcome was the first fatal or nonfatal atherosclerotic CVD event, including myocardial infarction, cerebrovascular accident, coronary artery bypass graft, percutaneous coronary angioplasty, peripheral artery bypass, nontraumatic amputation, abdominal aortic aneurysm repair, or carotid endarterectomy after initiation of dialysis. Multiple sources, including the Health Care Financing Administration, providers, and patient reports, were used to identify the potential events. Medical records were requested and reviewed preliminarily by a study nurse or a physician. An end point review committee adjudicated events on the basis of criteria that were developed specifically for the dialysis population. Because of a time lag in availability of administrative data, completed data for CVD events were available through November 1, 2000. Serum high-sensitive C-reactive protein (hsCRP) and IL-6 were measured at baseline using high-sensitivity ELISA with an intra-assay coefficient of variation 8.9% and ultrasensitive ELISA with an intra-assay coefficient of variation 7%, respectively.

Exposure Variables

Data on demographics and risk factors were collected by CHOICE study questionnaire at baseline. Major confounding variables included age; gender; smoking; baseline dialysis modality; body mass index; BP; history of diabetes, CVD, and congestive heart failure; serum albumin, total cholesterol, and HDL cholesterol. GFR at baseline was obtained from the Medicaid and Medicare Medical Evidence report. Dialysis modality at baseline was defined as the type of dialysis being used at 4 wk after enrollment in the study and was obtained from clinic records. Peritoneal dialysis included all forms (continuous ambulatory peritoneal dialysis, continuous cycling peritoneal dialysis, and intermittent cycling peritoneal dialysis). The Index of Coexistent Disease was calculated on the basis of assessment of medical records for physical impairment and coexisting disease severity. Scores on this index range from 0 (no comorbid condition) to 3 (highest severity of comorbid conditions). Baseline serum albumin, total cholesterol, and HDL cholesterol were assayed uniformly on all participants with standard methods using baseline specimen that was stored at −80°C.

Single-Nucleotide Polymorphism Selection and Genotyping Methods

Fifteen single-nucleotide polymorphisms (SNP) of the *STAT3* gene were selected on the basis of the following criteria: (*1*) SNP were resequenced and validated by the National Heart, Lung, and Blood Institute Program for Genomic Applications; (*2*) each SNP had a minor allele frequency ≥5%; (*3*) the set of SNP covered major blocks in Europeans identified by HapMap; and (*4*) the ABI TaqMan assay (Applied Biosystems, Foster City, CA) was feasible. SNP were genotyped using the TaqMan system. Assay protocols are available upon request. Water samples were included as negative controls to assess potential cross-contamination between samples, and 47 blind replicates were used to estimate assay reproducibility.

To assess for potential population stratification, we genotyped a panel of 87 ancestry-informative SNP to measure admixture (19). The degree of individual genetic admixture was estimated using a maximum-likelihood approach and characterized as percentage of European ancestry (PEA) (20). PEA was categorized as a result of its highly skewed distribution among white individuals.

Statistical Analyses

Baseline characteristics between patients with incident CVD events and patients without CVD events were tested using t test or χ^2 tests as appropriate. Values of hsCRP and IL-6 were log transformed because of their skewed distributions. Allele and genotype frequencies for each SNP were computed and tested for departures from Hardy-Weinberg proportions. Both single SNP and haplotype analyses were performed. For each single SNP, either a dominant or a recessive model was nested within a co-dominant model, and the resulting likelihood ratio tests between nested models were used to identify the best genetic model. Haplotype block structure was identified among the 15 SNP according to linkage disequilibrium structure using the confidence interval (CI) method implemented in Haploview 3.2 (21). Using a Bayesian method implemented in software PHASE 2.1 (22,23), each individual's haplotypes (diplotypes) were estimated using all 15 SNP for analysis of haplotypes across the entire gene or only the four SNP in block A and the five SNP in block C for block-specific analysis. Because of the uncertainty of estimated haplotype pairs from population data, PHASE 2.1 outputs several pairs of haplotype for each individual when there is an uncertainty. Each pair is assigned a posterior probability of certainty. The pair with the highest probability for an individual was called the "most likely pair" and was used (*1*) in Cox proportional hazard models to assess hazard ratios of first CVD event since dialysis for haplotypes and (*2*) in linear regression to examine haplotype associations with serum IL-6 and hsCRP levels. Linear regression models were conducted with log-transformed hsCRP or IL-6 as the dependent variable, and exponentiated coefficients were reported as ratios. Haplotypes with frequencies of <5% were combined. Left truncation technique was used in Cox proportional hazard models to account for the time lag between start of dialysis and time of study enrollment. Robust variance was used in each model to account for possible within-clinic correlations. An *a priori* specified additive genetic model was assumed for haplotype modeling.

To test the robustness of results from survival analysis using the most

likely pair estimates of haplotype and to account for multiple comparison, we conducted linear regression models and logistic regression models for the association of haplotype with inflammation markers and CVD, respectively, using Haplo.glm in R package Haplo.stats (24,25). Adjusted global tests with permutation-derived *P* values implemented in Haplo.score also were conducted. Haplo.glm accounts for haplotype ambiguity by directly modeling an individual's phenotype as a function of each estimated haplotype pair weighted by their estimated probability.

Results

Baseline Characteristics, Minor Allele Frequency, and Haplotype Distribution

A total of 230 CVD cases occurred among 529 dialysis patients who were followed for 3 to 6 yr (median 2 yr). Table 1 presents the distributions of traditional and ESRD-related risk factors for CVD. As expected, participants with incident CVD after dialysis were more likely to be older and had greater prevalence of CVD, congestive heart failure, and diabetes. Furthermore, these individuals had significantly lower serum cholesterol and albumin levels but higher levels of the inflammation markers serum hsCRP and IL-6. Treatment modality did not differ between those with and without incident CVD. Almost all 529 self-reported white individuals in this study had at least 88% European ancestry according to the 87 ancestry-informative markers that were typed (median PEA = 0.99; interquartile range 0.97 to 0.99). Markers of genetic admixture were not associated with CVD events.

A total of 15 SNP in *STAT3*, at an average spacing of 5.7 kb, were genotyped, including one synonymous coding SNP in the nearby *STAT5* gene (6-kb away), two in the 3′-untranslated region, and 12 in introns. The kappa values of the 47 blind replicates ranged from 0.88 to 1.00 (mean 0.96; SD 0.03). All SNP were in Hardy-Weinberg equilibrium. Minor allele frequency and location of each SNP are reported in Table 2. Three blocks were identified using the CI method; the second block consisted of two SNP (Figure 1). There was a high degree of linkage disequilibrium among the SNP. The mean pair-wise D′ was 0.86. Haplotype frequencies from all 15 SNP as well as the first (block A) and the third (block C) blocks are shown by incident CVD status in Table 3.

Single SNP, Inflammation Markers, and CVD

Figure 2 shows *P* values of hazard ratios (HR) of CVD for each SNP after adjustment for other risk factors. Four SNP (rs1135669, rs8081431, rs9912773, and rs17880815) were significantly associated with incident CVD, each with $P < 0.001$. For 15 comparisons, the Bonferroni-corrected critical *P* value is 0.003. However, no significant associations were found between any single SNP and serum level of IL-6 and hsCRP.

Haplotype, Inflammation Markers, and CVD

Using all 15 SNP, six major haplotypes were estimated. Similar to single SNP results, haplotypic variations in *STAT3* were significantly associated with CVD risk in this population. Table 3 shows a shortage of haplotype 3 among those with incident

Table 1. Baseline characteristics by whether patients had an incident CVD event in 529 white CHOICE study participants[a]

Covariates	CVD (n = 230)	Non-CVD (n = 299)	*P*
Age (yr; mean [SD])	62.9 (12.0)	55.9 (15.3)	0.0001
Female (%)	39.6	45.2	0.20
Smoking (%)			
former smoker	55.2	44.8	
current smoker	13.5	17.2	0.07
Baseline hemodialysis (%)	76.5	74.9	0.67
ICED comorbidity score (%)			
level 0/1	21.3	40.9	
level 2	42.2	33.2	
level 3	36.5	25.8	0.0001
Prevalent CVD (%)	67.4	34.6	0.0001
Congestive heart failure (%)	62.2	36.6	0.0001
Diabetes (%)	64.4	44.3	0.0001
BMI (kg/m^2; mean [SD])	26.6 (6.2)	26.7 (6.5)	0.80
SBP (mmHg; mean [SD])	150.8 (18.9)	147.1 (19.4)	0.04
Cholesterol (mg/dl; mean [SD])	185.4 (46.5)	193.7 (50.0)	0.05
HDL cholesterol (mg/dl; mean [SD])	41.0 (13.9)	41.5 (14.7)	0.70
Albumin (g/dl; mean [SD])	3.55 (0.37)	3.68 (0.36)	0.0001
IL-6 (pg/ml; median [IQR])	5.35 (3.19 to 9.54)	3.81 (2.40 to 6.08)	0.0001
CRP (mg/L; median [IQR])	4.71 (2.28 to 14.23)	3.48 (1.60 to 5.87)	0.0001

[a]*P* values from *t* or χ^2 test. BMI, body mass index; CHOICE, Choices for Healthy Outcomes in Caring for ESRD; CRP, C-reactive protein; CVD, cardiovascular disease; ICED, Index of Coexistent Disease; IQR, interquartile range; SBP, systolic BP.

Table 2. Positions and MAF of the 15 SNP in 529 white CHOICE study participants[a]

SNP	rs No.	Cumulative Distance[b]	Polymorphism[c]	MAF (%)[d]
1	rs1135669	0	C/T	16.9
2	rs1053023	5879	A/G	19.3
3	rs1053004	6355	T/C	37.6
4	rs8081431	15143	G/C	17.6
5	rs17881940	16930	G/C	8.2
6	rs2293152	21792	G/C	38.5
7	rs17881320	25502	C/A	8.6
8	rs2306580	31943	C/G	7.6
9	rs3869550	33150	A/G	34.9
10	rs6503695	39796	T/C	33.8
11	rs9912773	50797	C/G	25.1
12	rs744166	54464	T/C	41.9
13	rs957971	60188	C/G	34.9
14	rs7211777	74338	A/G	35.0
15	rs17880815	80088	C/A	26.3

[a]MAF, minor allele frequencies; SNP, single-nucleotide polymorphism.
[b]Distance is expressed in base pairs from the first SNP relative to Genbank sequence.
[c]Minor allele shown in second.
[d]MAF were calculated on the basis of the 529 white CHOICE patients.

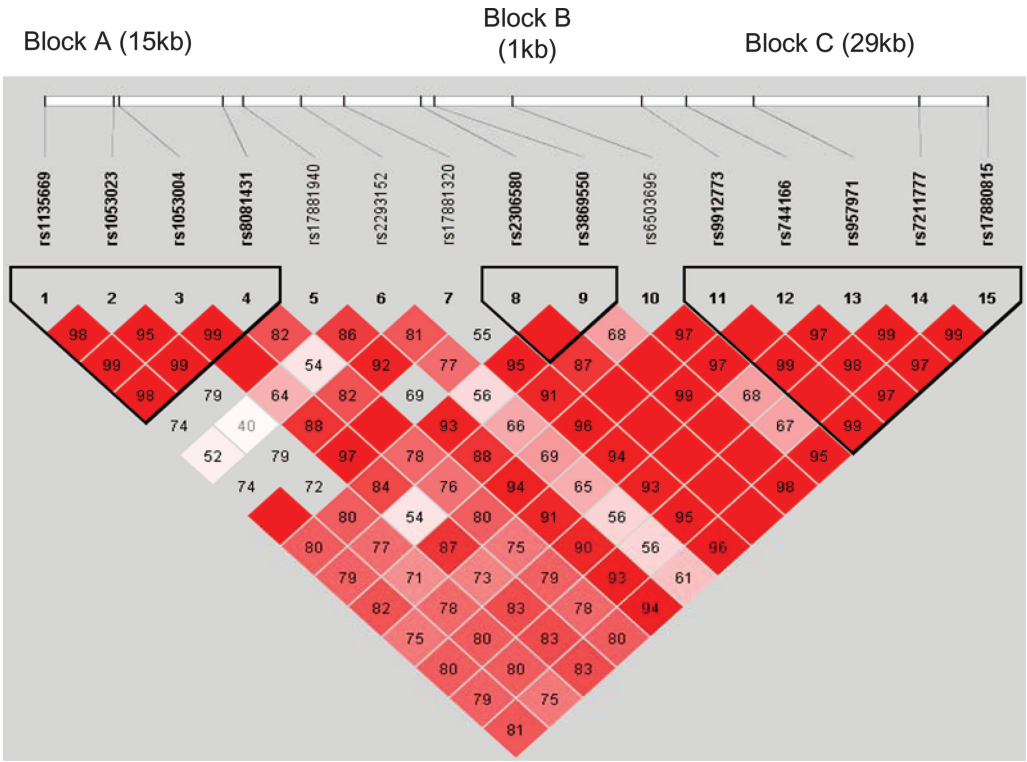

Figure 1. Positions and linkage disequilibrium structure of 15 single-nucleotide polymorphisms (SNP) in the signal transducer and activator of transcription 3 (*STAT3*) gene. Values in squares represent pair-wise D′ values.

CVD ($P = 0.002$ for overall differences in haplotype distribution between CVD and non-CVD). There were 48, 26, 10, 0, 2, and 3 homozygous pairs (diplotypes) for haplotypes 1 through 6, respectively. Compared with haplotype 1, adjusted HR of CVD were 1.10 (95% CI 0.88 to 1.39), 0.75 (95% CI 0.61 to 0.92), and 1.72 (95% CI 1.17 to 2.52) for haplotypes 2, 3, and 6, respectively. Adjusted risk for CVD was decreased from haplotype 6 through haplotypes 1 and 2 to haplotype 3. No significant association was found between prevalent CVD and *STAT3* haplotype variation. Compared with haplotype 1, adjusted

Table 3. Haplotype frequency by whether patients had an incident CVD event since initiation of dialysis

Number	Haplotype			CVD	Non-CVD
	Block A No.	All 15 SNP	Block C No.		
1	A-1	CATG-GCCCAT-CTCAC	C-1	32.6	31.1
2	A-1	CATG-GCCAT-CTCAC	C-1	19.8	17.7
3	A-3	TGCC-GGCCGC-GCGGA	C-2	9.6	14.6
4	A-2	CACG-CGACGC-GCGGA	C-2	7.0	7.2
5	A-1	CATG-GGCCAC-CCCAC	C-4	5.4	8.0
6	A-2	CACG-GGCGGT-CCGGC	C-3	7.2	5.7
Other				18.5	15.7
		Block A			
A-1		CATG		63.3	60.4
A-2		CACG		19.1	18.4
A-3		TGCC		13.9	18.9
A-other				3.7	2.3
		Block C			
C-1		CTCAC		59.6	55.4
C-2		GCGGA		22.4	27.3
C-3		CCGGC		10.2	7.4
C-4		CCCAC		5.4	8.4
C-other				2.4	1.7

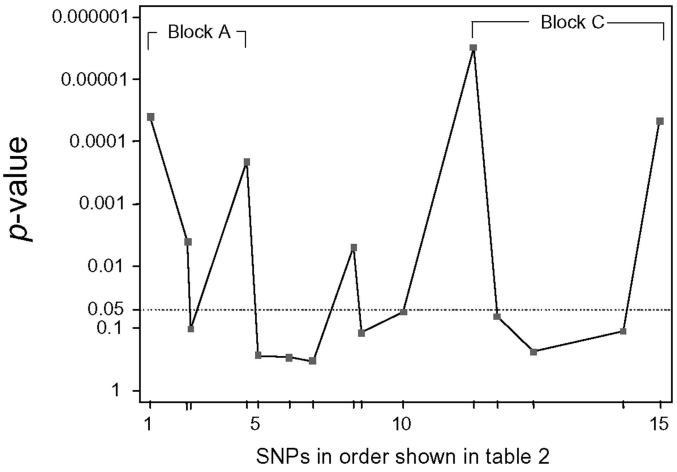

Figure 2. P values of adjusted hazard ratios from Cox proportional model with single SNP. *P* values were obtained from the best genetic model; models were adjusted for age; gender; smoking; baseline dialysis modality; body mass index; systolic BP; comorbidity score; baseline diabetes, CVD, and congestive heart failure; and serum albumin, total cholesterol, HDL cholesterol, C-reactive protein, and IL-6 levels.

odds ratio (OR) of prevalent CVD was 1.32 (95% CI 0.65 to 2.65) for haplotype 3 and 0.93 (95% CI 0.46 to 1.86) for haplotype 6.

To elucidate further the location of the association signal, we constructed haplotypes within blocks A and C. Whereas haplotype A-3 was less common among those who developed CVD events, haplotype C-3 was more common in those individuals (Table 3). Table 4 summarizes results from multivariable Cox proportional hazards regression analyses by blocks. After adjustment for all haplotypes from both blocks as well as other risk factors, compared with common haplotype A-1, A-3 showed a significant protective effect on CVD ($P = 0.02$). Compared with common haplotype C-1, C-3 showed a significant harmful effect on CVD ($P = 0.005$). There were no significant interactions between *STAT3* haplotypes and baseline diabetes or serum levels of IL-6 and CRP on CVD risk.

Median of the probability of the most likely pairs (diploid genotypes) estimated from PHASE 2.1 was 0.99 (interquartile range 0.89 to 1.00) for overall haplotypes and 1.00 (interquartile range 1.00 to 1.00) for both block A and block B haplotypes. The above Cox proportional hazard models were repeated using only haplotypes with a probability of 1, and the results were similar. All regression models were rerun adjusting for genetic admixture, and no significant changes were observed.

The association between *STAT3* gene variation and CVD risk from Cox proportional models became somewhat stronger and more significant after adjustment. Detailed analysis suggested that this was not the result of a single confounder or influential outliers but was a combination of multiple variables, with baseline congestive heart failure being one of the more important. In addition, adjusting for baseline GFR did not change the observed associations. To test further the robustness of the survival analyses using estimated haplotypes, we estimated OR for CVD associated with each haplotype using a two-step iteration process that simultaneously imputes haplotypes and estimates their association with CVD implemented in Haplo.glm. The resulting OR were similar to their corresponding HR (Table 4). Moreover, permutation-derived *P* values of global tests from Haplo.score were 0.02 for haplotypes from all 15 SNP, 0.02 for block A haplotypes, and 0.01 for block C haplotypes. These

Table 4. HR and OR for incident CVD by haplotypes in each block from Cox proportional hazards models and logistic regression models in Haplo.glm, respectively[a]

Haplotype	HR (95% CI)		OR (95% CI)	
	Model 1	Model 2	Model 1	Model 2
Block A				
A-1	1.00	1.00	1.00	1.00
A-2	0.86 (0.54 to 1.35)	0.78 (0.48 to 1.25)	1.16 (0.76 to 1.79)	1.07 (0.69 to 1.67)
A-3	0.80 (0.52 to 1.23)	0.70 (0.51 to 0.94)[b]	0.60 (0.39 to 0.92)[b]	0.53 (0.34 to 0.83)[c]
A-other	1.26 (0.61 to 2.61)	1.06 (0.50 to 2.27)	1.86 (0.72 to 4.84)	1.68 (0.62 to 4.59)
Block C				
C-1	1.00	1.00	1.00	1.00
C-2	0.89 (0.53 to 1.51)	0.91 (0.64 to 1.30)	0.67 (0.47 to 0.98)[b]	0.64 (0.43 to 0.94)[b]
C-3	2.06 (1.28 to 3.32)[c]	2.12 (1.25 to 3.57)[c]	2.16 (1.20 to 3.91)[c]	1.96 (1.05 to 3.66)[b]
C-4	0.80 (0.54 to 1.20)	0.75 (0.48 to 1.16)	0.63 (0.34 to 1.16)	0.62 (0.33 to 1.18)
C-other	1.89 (1.17 to 3.07)	1.59 (0.89 to 2.86)	1.69 (0.50 to 5.71)	1.62 (0.46 to 5.66)

[a]Model 1 was adjusted for all haplotypes; age; gender; smoking; baseline dialysis modality; BMI; SBP; comorbidity score; diabetes, baseline CVD, and congestive heart failure; and serum albumin, total cholesterol, HDL cholesterol. Model 2 was adjusted for model 1 plus IL-6 and CRP. For Cox regression models, haplotypes from both blocks were included; for logistic regression models, haplotypes from one block were included. CI, confidence interval; HR, hazard ratio; OR, odds ratio.
[b]$P < 0.05$.
[c]$P < 0.01$.

associations remained significant even after adjustment for inflammation markers (Table 4, model 2). Associations from single SNP analyses were in the same direction as those from haplotype analyses.

Compared with haplotypes 1 and A-1, haplotypes 3 and A-3 were associated with 8% lower (geometric mean ratio 0.92; 95% CI 0.79 to 1.06) and 14% lower (geometric mean ratio 0.86; 95% CI 0.77 to 0.96), respectively, IL-6 levels after adjustment for hsCRP and other covariates that were used in model 1 of Table 4 in a linear regression model that used the most likely pairs of haplotype for each patient. A linear model in Haplo.glm showed similar results. The HR for incident CVD was 1.22 (95% CI 1.11 to 1.34) per doubling of IL-6 and 1.24 (95% CI 1.14 to 1.35) per doubling of hsCRP level after adjustment for all haplotypes and other risk factors. These HR were similar to those obtained from models without adjustment for haplotypes. Variations in *STAT3* were not associated with hsCRP levels. The observed association between inflammation markers and *STAT3* gene variation remained after adjustment for baseline use of lipid-lowering medication and angiotensin-converting enzyme inhibitors.

Haplotype and Mortality

A total of 281 deaths occurred during 1427 person-yr of follow-up. Haplotype 6, consisting of A-2 and C-3, was the only haplotype that was significantly associated with all-cause mortality (HR 1.54; 95% CI 1.14 to 2.08; $P = 0.005$).

Discussion

In our cohort, *STAT3* gene variation was associated with CVD risk in white dialysis patients. Three major blocks were identified in 529 white dialysis patients. Compared with common haplotypes A-1 and C-1, respectively, block A haplotype A-3 marks decreased CVD risk and block C haplotype C-3 marks increased CVD risk. Furthermore, associations between *STAT3* variation and CVD risk could not be explained by the association of *STAT3* variation with serum IL-6 levels, suggesting alternative pathways through which *STAT3* may influence CVD risk.

To our knowledge, this is the first study to examine the associations between *STAT3* polymorphisms and CVD risk in humans. The rich information from the CHOICE study enabled us to examine the independent association between *STAT3* gene variation and CVD risk adjusting for traditional and ESRD-related CVD risk factors. The use of incident dialysis patients minimized bias that is caused by the use of prevalent cases. All 15 SNP were genotyped and validated, and together they cover major haplotype blocks of the gene; therefore, these SNP adequately represent major haplotype variation in *STAT3*. We used a combination of single SNP and haplotype-based methods to investigate the association of variation in *STAT3* with CVD risk.

Studies on animal or human tissues have shown several biologic pathways through which *STAT3* affects CVD development. *STAT3* has been shown to regulate both proinflammatory and anti-inflammatory processes through targeting the promoter regions of IL-6 and IL-10 genes (3–5), respectively. Negative feedback exists among *STAT3*, IL-6, and IL-10 through a family of inhibitor proteins that are referred to as suppressor of cytokine signaling (1). Our study showed that block A of the *STAT3* gene is associated with IL-6 level. However, the association between the *STAT3* gene and inflammatory markers may be affected by the anti-inflammatory effects of *STAT3*.

In addition to influencing CVD risk by regulating inflamma-

tion, *STAT3* may affect CVD risk through its action on vascular endothelial function (10–12), angiotensinogen gene expression (6,7), regulation of the inducible nitric oxide synthase protein and cyclooxygenase (16,17), and myocardial adaptation to stress (13–15). Associations between *STAT3* and CVD remained after adjustment for inflammatory markers, suggesting that *STAT3* affects CVD risk through pathways other than inflammation.

Because STAT3 protein cannot be measured except at the cellular level, we are unable to test whether the observed association is through transcription and expression of the *STAT3* gene. However, many studies have shown in *vitro* that mutation of the *STAT3* gene is associated with *STAT3* transcription and expression. For example, mutations of the *STAT3* promoter in murine myeloid leukemic cells were associated with *STAT3* mRNA changes (26). Study further suggested that minimal mutations in the murine *STAT3* gene alone may alter dramatically the ratio of *STAT3α* to *STAT3β* mRNA in cells (27). Murine cardiac myocytes that were transfected with wild-type or mutated-type *STAT3* cDNA were associated with the amount of STAT3 protein (28). A mutation of the *STAT3* gene in human macrophages was associated with the amount of STAT3 protein (4).

The findings that there was no or an opposite association between *STAT3* haplotype and prevalent CVD may be due to survival bias in this cohort. For example, compared with haplotype 1, patients with haplotype 6 are at higher risk for CVD and therefore have higher risk for death. This would result in lower frequency of haplotype 6 among individuals who had prevalent CVD and survived to be included in our study, which would lead to an attenuated or even reversed association between haplotype 6 and prevalent CVD. However, in a prospective study of incident CVD, such as CHOICE, this survival bias would be minimized because we have captured all deaths as a result of CVD in our study. Our findings are unlikely due to population stratification, because adjusting for genetic admixture did not change our findings. The survival analysis did not take into account haplotype ambiguity, which can magnify statistical significance. However, the logistic regression implemented in Haplo.glm accounted for the haplotype ambiguity by weighting the probability of each haplotype, although it was not able to utilize fully the time-to-event data of CHOICE. Therefore, we performed both analyses and found consistent results. In addition, accounting for multiple comparisons in both single SNP analysis and the haplotype analysis did not alter the results.

Our study has several limitations. Because of the relatively small number of black CHOICE participants and the large number of haplotypes estimated in *STAT3*, our analysis was limited to only white CHOICE participants. Although allele frequencies of the 15 SNP were similar as reported by other sources, including HapMap, Perlegen, and National Heart, Lung, and Blood Institute Program for Genomic Applications, findings from this study require replication in other large cohorts, including other races. The observed associations may be due to unmeasured confounders. The lack of association between *STAT3* gene variations and hsCRP levels may be the result of misclassification because of nongenetic influences. Serum hsCRP was measured only at one point, and this problem may be compounded by the high intraindividual variation of serum CRP in the dialysis population (29). In addition, we did not investigate less common SNP in this gene (minor allele frequency $<5\%$).

Conclusion

We provide evidence for association between *STAT3* polymorphisms and serum IL-6 level and incident CVD risk in white dialysis patients. The associations between *STAT3* and CVD were independent of inflammatory markers. These findings need replication and further characterization of the *STAT3* gene to identify causal variation that may code for an alternative pathway, not mediated by inflammation, between *STAT3* and CVD.

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