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Association of Single-Nucleotide Polymorphisms in *JAK3*, *STAT4*, and *STAT6* With New Cardiovascular Events in Incident Dialysis Patients

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Abstract

Background—Increasing evidence supports a role for cell-mediated immunity in the pathogenesis of cardiovascular disease. Single nucleotide polymorphisms (SNPs) in *JAK3*, *STAT4*, and *STAT6* of the Janus kinase–signal transducer and activator of transcription (Jak-Stat) signal transduction pathway were examined for association with time to new cardiovascular events in incident dialysis patients from the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease study.

Study Design—Prospective cohort study.

Setting & Participants—764 White (n=518) and Black (n=246) participants from 79 dialysis centers.

Predictor—SNPs in *JAK3*, *STAT4*, and *STAT6* selected using a pairwise approach to identify a maximally informative set of tag SNPs for populations of European and African descent.

Outcomes and Measurements—Cox proportional hazards models were used to estimate unadjusted and multivariable adjusted hazard ratios (HR) for incident cardiovascular disease events after dialysis initiation associated with each race-specific SNP.

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Results—Two European tag SNPs (rs3212780 and rs3213409) were associated with new cardiovascular disease events in White patients in *JAK3*, with unadjusted HR 1.92 ($p<0.001$) and 1.82 ($p=0.07$), respectively. One dual-tag SNP (rs3212752) in *JAK3* was associated with new cardiovascular events in White patients with unadjusted HR 2.09 ($p<0.001$) and in Black patients with HR 2.07 ($p=0.007$). SNP rs3213409 codes for a valine to isoleucine change at amino acid 722, a potentially functional mutation. SNPs in *STAT4* and *STAT6* were not associated with cardiovascular events after the initiation of dialysis.

Limitations—This study does not provide direct evidence for the mechanism of increased risk. Replication in independent cohorts is necessary.

Conclusions—Genetic polymorphisms in the Jak-Stat signaling pathway are associated with an increased risk of new cardiovascular events in incident dialysis patients.

Index Words

dialysis; cardiovascular diseases; inflammation; genes

End-stage renal disease (ESRD) carries a high mortality, with 221 deaths per 1000 patient-years among patients receiving dialysis in the United States in 2006.¹ Depending on age, cardiovascular disease (CVD) is 10 – 100 times more common in dialysis patients than in the general population, and cardiovascular mortality accounted for approximately 43% of the roughly 71,000 deaths in 2006^{1,2}. The association of traditional CVD risk factors with mortality may differ between the dialysis and general population, and therapeutic interventions targeting traditional risk factors may not afford the same degree of risk reduction in the dialysis population as in patients with normal renal function^{3,4}. Additional understanding of the mechanisms of atherosclerotic disease in dialysis patients is needed. An increasing body of evidence supports a role for cell-mediated immunity in the pathogenesis of cardiovascular disease. The Janus kinase–signal transducer and activator of transcription (Jak-Stat) pathway is a widely expressed intracellular signal transduction pathway composed of 4 upstream kinases (Jak1–3, Tyk2) and 7 transcription factors (Stat1–4, 5a, 5b, 6) fundamentally important to T lymphocyte differentiation and function^{5–7}. Within the Jak-Stat family, Stat4, Stat6, and Jak3 are of particular relevance to CD4⁺ T cell maturation. T lymphocytes differentiate into several lineages, including CD4⁺ T helper type 1 (T_H1) cells believed to promote atherosclerotic lesions and acute coronary syndromes while T helper type 2 (T_H2) cells likely serve an inhibitory or modulatory role^{8–11}. Differentiation into a T_H1 lineage is mediated by Stat4 after interleukin-12 (IL-12) stimulation, while Stat6 signaling after IL-4 stimulation results in T_H2 differentiation. Unlike Stat4 and Stat6, Jak3 expression [ND1] is restricted only to cells of hematopoietic origin such as T and natural killer (NK) cells, and Jak3 activates multiple Stats including Stat6.

Both human and animal models suggest T_H1 cells are pro-inflammatory and promote atherogenesis, and activated T cells and increased levels of T_H1-produced cytokines such as interferon gamma (IFN- γ) and interleukin 12 (IL-12) are found in atherosclerotic plaques but not in nonatherosclerotic tissue^{12–16}. The role of IL-4 induced T_H2 differentiation in CVD mechanisms may be more complex. While decreased T_H1 and increased T_H2 expression after IL-4 administration can result in smaller atherosclerotic lesion size in mice, IL-4 has also been associated with a pro-atherogenic phenotype and upregulation of vascular cell adhesion molecules^{9,17–19}. Atherosclerosis is a dynamic process, and Jak-Stat signaling may have tissue-specific and, at times, seemingly opposing roles in its pathogenesis. Given the central role of Jak3, Stat4, and Stat6 to T cell function, a better understanding of factors influencing their role in the development of CVD in dialysis patients is useful. This study examined the association between SNPs in *JAK3*, *STAT4*, and *STAT6* with new CVD events after the

initiation of dialysis in incident White and Black dialysis patients drawn from the CHOICE (Choices for Healthy Outcomes in Caring for ESRD) cohort.

Methods

Study Design and Population

Patients were drawn from CHOICE, a previously described, prospective cohort study of 1,041 incident dialysis patients²⁰. This cohort recruited incident dialysis patients a median of 45 days from the start of dialysis between 1995 and 1998 from 79 dialysis centers operated by Dialysis Clinic, Inc. Eligibility criteria were age greater than 17 years and the ability to give informed consent. DNA was available for 806 of the 1,041 participants, and due to sample size considerations, this study was limited to the 764 who self-identified themselves as either “White” (n=518) or “Black” (n=246). The institutional review boards of The Johns Hopkins University School of Medicine and of the participating clinical centers approved the study protocol.

Outcomes

The primary outcome was a new CVD event after dialysis initiation. A CVD event was defined as the occurrence of a new fatal or nonfatal myocardial infarction, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, peripheral arterial bypass, non-traumatic amputation, abdominal aortic aneurysm repair, cerebrovascular accident, or carotid endarterectomy since the initiation of dialysis. Outcome data were obtained from the United States Renal Data System, National Death Index, medical providers, and patient reports. For a subset of patients, medical records were examined and a review committee adjudicated clinical events. CVD status was documented for all patients through December 31, 2004. Patients were censored at the time of renal transplantation or death from a non-CVD cause.

SNP Selection

The primary exposures of interest were SNP genotypes in the *JAK3*, *STAT4*, and *STAT6* genes. Tag SNPs were identified separately for European and African-American populations by using the SeattleSNPs database^{20a} and a pairwise tagging approach [ND2] with an $r^2 > 0.65$ and a minor allele frequency (MAF) $\geq 5\%$ in the referent European and $\geq 10\%$ in the African-American populations²¹. One tag SNP in *STAT6*, eight in *JAK3*, and five in *STAT4* were shared between the two populations. All SNPs are referenced to the forward strand at <http://www.ncbi.nlm.nih.gov/projects/SNP/>. Pairwise correlation coefficients (r^2) between all SNPs were generated with the Haploview software²² by race in each gene and are provided as online supplementary materials (Figures S1–S6; available with this article at <http://www.ajkd.org>). Genotyping was performed with the Illumina BeadArray™ system (Illumina, Inc., San Diego, CA), and quality control was assessed with one negative water control and one Centre d’Etude du Polymorphisme Humain (CEPH) trio from the HapMap project (<http://www.hapmap.org>) per plate. Participants with and without the primary outcome were analyzed on the same plate, and blind replicates from 48 CHOICE participants documented reproducibility with a concordance rate of $>99\%$. Deviation from Hardy-Weinberg equilibrium was evaluated in all SNPs by the Pearson chi-square test. The minor allele of each SNP was designated as the allele present at the lower frequency in the entire cohort.

Exposure Variables

Demographic and medical history data were collected at baseline from all participants via questionnaire, medical record review, and the Center for Medicare and Medicaid Services (CMS) form 2728. The baseline dialysis modality (hemodialysis/peritoneal dialysis) was

identified from clinic records as the technique employed four weeks after enrollment in the study. Severity of comorbid illness was quantified by the previously validated Index of Coexistent Disease (ICED) score, ranging from 0 (no comorbidities) to 3 (greatest degree of comorbid illness)²³. Baseline IL-6 and high-sensitivity C-reactive protein (CRP) concentrations were assayed on specimens stored at -80°C that had been obtained shortly after the initiation of dialysis. CRP was measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) with an intra-assay coefficient of variation of 8.9%, and IL-6 was measured via an ultrasensitive ELISA with an intra-assay coefficient of variation of 7.0%. A measure of percent European ancestry (PEA) was generated for each individual via a maximum-likelihood approach from a panel of 87 ancestry-informative SNPs to account for possible population stratification²⁴.

Statistical Analysis

Baseline characteristics of patients with and without a new CVD event were compared using t , χ^2 , or Wilcoxon ranksum tests, as appropriate. The association of SNPs with time to new CVD event after dialysis initiation was analyzed in univariate Cox proportional hazards models under additive, dominant, and recessive genetic models with robust variances. The best genetic model was selected based upon the lowest two-sided p -value. As participants were recruited from 79 centers, robust variances were calculated to account for clustering that may occur by clinic site due to site-specific treatment practices. Only the race specifically tagged by the SNP was analyzed. For tag SNPs shared by both races, models combining both races together were performed with adjustment for PEA to account for racial differences in allele frequency. Multivariable Cox proportional hazards modeling, utilizing *a priori*-selected covariates believed to be potential confounders, was performed. These covariates were PEA, sex, age, ICED category (0/1, 2, 3), diabetes status, albumin, IL-6, CRP, cholesterol, high density lipoprotein cholesterol, systolic and diastolic blood pressure, and smoking status. The proportional hazards assumption was confirmed for all Cox regression models through analysis of Schoenfeld residuals. To further confirm the association between SNP genotype and a new CVD event, a competing risk analysis was performed for SNPs identified in the main analysis^{25,26}. The cumulative incidence of new CVD events was examined accounting for the competing risks of other death and renal transplantation/censoring, stratified by genotype. The associations of SNP genotype with IL-6 and CRP levels were quantified with one-way ANOVA. Nominal p -values are given, and Bonferroni-corrected p -values (p') for 26 measured SNPs in Whites, 31 SNPs in Blacks, or 14 dual-tag SNPs for analyses in the combined cohort are also reported when appropriate. All statistical analyses were performed with Stata 9.2 (StataCorp; College Station, TX).

Results

Baseline Characteristics

Table 1 describes the baseline characteristics of the 764 participants stratified by race and new CVD event after the start of dialysis. Follow-up time ranged from 4 days to 8.94 years, with a median time to event of 1.93 years. There were 436 (57.1%) patients with a new CVD event after dialysis initiation and 328 (42.9%) without an event. Of the 518 White participants, 310 (59.8%) had a CVD event after initiation of dialysis, as did 126 (51.2%) of the 246 Black participants. In both Whites and Blacks, patients with a new CVD event were more likely to be older and to have a higher ICED score, history of prior CVD, diagnosis of diabetes, lower diastolic blood pressure, and higher levels of the inflammatory markers IL-6 and CRP. White patients with a new CVD event had lower albumin; this relationship was not seen in Black patients. PEA did not differ between Black patients with and without a new CVD event (15.0% vs. 15.0%), nor between Whites (99.0% vs. 99.0%). A comparison of the baseline characteristics between participants included and not included in the genetic analysis is

available as online supplementary material (Tables S1–S2). Most notably, included White participants were more likely to have longer follow-up and higher blood pressure than the excluded White participants. Included Black and White participants were both more likely to be on hemodialysis, while excluded participants were more commonly on peritoneal dialysis.

Allele Frequencies and Association with New CVD Events

Table 2 summarizes the race-stratified association between SNPs and new CVD events in both univariate and multivariable models for *JAK3*. There were no robust statistical associations between SNPs of *STAT4* and *STAT6* with new CVD events in either race (Table 3). In Whites, three *JAK3* SNPs (two European/one dual-tag) were associated with a new CVD event in dialysis patients. Intronic SNP rs3212780 had an unadjusted HR of 1.92 (95% CI, 1.63–2.27; $p<0.001$) and a multivariable-adjusted HR of 2.29 (95% CI, 1.85–2.84; $p<0.001$) under a recessive model. The non-synonymous coding SNP rs3213409 demonstrated a non-statistically significant unadjusted HR of 1.82 (95% CI, 0.96–1.64; $p=0.07$) and multivariable-adjusted HR of 1.92 (95% CI, 1.18–3.14; $p=0.009$) under a dominant model. Of note, the MAF of this SNP was only 1.7%. A Kaplan-Meier survival curve of *JAK3* haplotype rs3212780/rs3213409 (Figure 1) demonstrated a shorter time to new CVD event after the initiation of dialysis in patients who were both recessive for the rs3212780 minor allele and dominant for rs3213409 ($p<0.001$). Lastly, dual-tag intronic SNP rs3212752 had an unadjusted HR under a recessive model of 2.09 (95% CI, 1.67–2.61; $p<0.001$) and a multivariable-adjusted HR of 2.34 (95% CI, 1.45–3.77; $p=0.001$). SNPs rs3212780, rs3212752, and rs3213409 were not in linkage disequilibrium ($r^2 < 0.03$ for all comparisons).

In Table 2, for Blacks, dual-tag SNP rs3212752 was also associated with an increased risk of a new CVD event with unadjusted HR 2.07 (95% CI, 1.22–3.51, $p=0.007$) and multivariable-adjusted HR 3.31 (95% CI, 1.63–6.72, $p=0.001$) under a recessive model. The unadjusted HR did not remain statistically significant after Bonferroni correction ($p'=0.2$), but the multivariable-adjusted HR did ($p'=0.03$). As rs3212752 was a dual-tag SNP, when analyzed in the entire cohort, the race-adjusted HR was 2.07 (95% CI, 1.42–3.01; $p<0.001$, $p'=0.001$), with multivariable-adjusted HR 2.43 (95% CI, 1.67–3.54; $p<0.001$, $p'=0.001$). In both Whites and Blacks, SNPs rs3212714 and rs3212711 demonstrated a similarly reduced risk of new CVD events, but the association was not statistically significant after Bonferroni correction. When analyzed in the combined cohort, only rs3212711 was statistically significant in a univariate model (HR 0.81; 95% CI, 0.66–0.99, $p=0.04$), and this did not persist after Bonferroni correction ($p'=0.5$). Although tag SNPs were selected in a manner intended to minimize linkage disequilibrium, in Whites, rs3212714 and rs3212711 had an $r^2=0.97$, and in Blacks, $r^2=0.64$.

To further confirm the association between SNPs in *JAK3* with new CVD events, a competing risk analysis stratified by genotype was performed. The cumulative incidence of new CVD events remained greater in participants with the 3 *JAK3* risk alleles as compared to those without, after accounting for the competing risks of other death and renal transplantation/censoring (Fig S7–S12; provided as online supplementary material).

There were no robust associations in either race between any SNPs in *JAK3*, *STAT4*, or *STAT6* with IL-6 or CRP concentrations.

Discussion

Two SNPs in *JAK3*, namely, European-tag rs3212780 (genotype C/C) and dual-tag rs3212752 (genotype G/G), were strongly associated with an increased risk for CVD morbidity and mortality in White incident dialysis patients. SNP rs3212752 was a dual-tag SNP and showed a similar association with new CVD events in Black patients. A third European SNP, rs3213409

(genotype A/G), had a low allele frequency but was highly suggestive of a plausible association in White patients. The associations of the *JAK3* SNPs with new CVD events were not appreciably altered by multivariable adjustment for known cardiovascular risk factors. A sensitivity analysis incorporating competing risks of other death and renal transplantation/censoring did not alter the findings.

The Jak3 protein has a C-terminal catalytic domain and a neighboring pseudokinase domain that is believed to regulate activity of the catalytic site^{27,28}. SNP rs3212752, located in intron 12, is 9 base pairs from exon 11 in the pseudokinase domain. The proximity to a splice site suggests a possible functional role, although the SNP may be tagging an as-yet-unidentified functional polymorphism. SNP rs3212752 is also 723 base pairs from the alanine to valine activating mutation at amino acid 572 (p.Ala572Val) described in a cell line derived from a patient with acute megakaryoblastic leukemia²⁹. This mutation in the pseudokinase domain resulted in constitutive phosphorylation and activation of Jak3 and Stat5. The finding of a similar association with new CVD events in both Whites and Blacks provides a measure of internal replication for this SNP. This is important given that this association with new CVD events has not been explored in independent cohorts, and the allele frequency in Whites was relatively low at 6.3%. Additional fine mapping of this region is required to further explore the role of this SNP in CVD pathways.

SNP rs3213409 is a nonsynonymous coding SNP in exon 16 of the pseudokinase domain, resulting in valine to isoleucine substitution at amino acid 722 (p.Val722Ile). This amino acid change has also been previously reported in a patient with acute megakaryoblastic leukemia²⁹. SNP rs3212780 is located in intron 23, and presumably is in linkage disequilibrium with an unknown functional polymorphism. This is further supported by the finding of a greater risk for a new CVD event in patients who carried the rs3212780-rs3213409 C/C-A/G haplotype. These two SNPs likely convey direct risk or are associated with different unmapped polymorphisms.

Jak3 is expressed in cells of hematopoietic origin^{30,31}. It is recognized from diseases such as autosomal recessive severe combined immunodeficiency (SCID) that mutations in *JAK3* can have profound immunologic consequences, such as the absence of mature T cells and severely reduced numbers of NK cells characteristic of SCID³². Animal models have shown a role for NK cells in the pathogenesis of atherosclerotic lesions^{33–35}. In addition, Jak3 activity is important in the development of a third lineage of CD4⁺ T lymphocytes, the CD4⁺/CD25⁺ regulatory T cell (Treg) expressing the transcription factor Foxp3. Foxp3⁺ regulatory T cells help mediate the development of peripheral immunologic tolerance, and lower levels of circulating Tregs have been found in patients with acute coronary syndromes as compared to patients with normal coronary arteries³⁶. Additionally, mice lacking Jak3 do not develop Foxp3⁺ Tregs in the thymus³⁷. Given the immunological contribution to atherosclerosis, pathways that promote tolerance to atherogenic antigens may limit the development of CVD³⁸. It has been suggested that Tregs from hemodialysis patients are more susceptible to oxidized LDL-induced apoptosis, and the increased loss of these regulatory cells may weaken a naturally inhibitory pathway on the development of atherosclerosis in these patients³⁹. Clinically, an inhibitor of Jak3 (CP-690,550) has demonstrated efficacy as an immunosuppressant in solid-organ transplantation, and data suggest inhibition of Jak3 attenuates the development of allograft vasculopathy in a rodent model of aorta transplantation^{40,41}. The inhibitor CP-690,550 also results in a prompt reduction in circulating NK cells in nonhuman primate models^{42,43}. Thus, loss or inhibition of Jak3 activity may result in decreased atherogenesis by reducing T and NK cells, but may potentially promote atherosclerosis through alterations in the Treg population. This suggests the timing and context of Jak3 activation promote a spectrum of pro-inflammatory and inhibitory cell lines, with the endpoint of cardiovascular events dictated by the balance. Since Jak3 expression is restricted

only to hematopoietic cells, a consistent association between genetic polymorphisms and CVD may be more plausible in this gene than in genes expressed in both immune and endothelial cell pathways that are potentially competitive and dynamic over time. *STAT4* and *STAT6* are examples of such genes with broader expression.

Accordingly, a robust association was not found for *STAT4* or *STAT6* polymorphisms and new CVD events in this cohort. Despite significant published data describing a role for Stat4 and T_H1 lymphocytes in the pathogenesis of CVD, this study did not detect a convincing association. Furthermore, Jak3 phosphorylation of Stat6 in response to IL-4 signaling induces a T_H2 phenotype, a T cell lineage that may both promote and inhibit atherosclerosis. T_H2 cells are known to produce IL-10, a cytokine also characteristic of Tregs that has been associated with protection against atherosclerosis in murine models^{44,45}. In humans, IL-10 genotype and increased serum levels have been associated with a decreased risk of CVD^{46,47}. IL-4, however, also has direct effects upon the vascular endothelium, resulting in increased oxidative stress and upregulation of vascular adhesion molecules—important features in the pathogenesis of atherosclerosis^{17,48,49}. IL-4 via Jak3 signaling leads not only to Stat6 activation, but also to activation of Stat3⁵⁰. Considerable evidence supporting the role of Stat3 in CVD has been published, including the association of *STAT3* haplotypes with an increased risk of CVD events in the CHOICE cohort^{51,52}. Small sample sizes preclude a combined analysis of *STAT3*/*JAK3* risk alleles in CHOICE. Thus, it can be hypothesized that Jak3 pathways other than through Stat6, such as through Stat3, are more important in the development of new CVD events in the ESRD population. This provides additional credence to the hypothesis that functional changes in Jak3 are more important than in Stat4 or Stat6 for CVD risk. It is likely that the cumulative effects of varying immunological processes over time help dictate the clinical manifestation of CVD. A careful understanding of the populations under study will be necessary to help understand likely disparate findings across studies, as well as to best interpret the risk afforded by a genetic polymorphism to an individual at a given point in time.

This study had several limitations. The frequency of rs3213409 was unexpectedly low. It is possible this represents overestimation of the allele frequency in SeattleSNPs' referent population. Additionally, rs3213409 was not in Hardy-Weinberg equilibrium, and this may indicate a survival bias in patients with ESRD. If this SNP also poses an increased hazard for CVD events in the chronic kidney disease population, the death of these individuals prior to reaching dialysis may result in under-representation of the polymorphism in a dialysis cohort such as CHOICE. Similar limitations in the analysis of genetic risk determinants in ESRD cohorts have been described for the association of the malnutrition-inflammation complex in relationship to mortality⁵³. Most patients with chronic kidney disease will not survive to reach ESRD, making survival bias and low allele frequencies a problem for genetic epidemiology studies and, in particular, haplotype analysis in ESRD. Furthermore, this study is not equipped to examine directly the intermediate phenotypes by which these genetic polymorphisms may exercise the increased risk for a new CVD event. Since the Jak-Stat pathway is heterogeneous and responsive to numerous stimuli, identification and examination of intermediate phenotypes (such as T lymphocyte profiling, reporter assays of Jak-Stat protein activity, or quantitation of arterial plaque progression) at different time points will permit better characterization of the genetic effect. In summary, this study provides evidence for an association between genetic polymorphisms in *JAK3* with an increased risk of new CVD events after the initiation of dialysis. These genetic findings need to be replicated in additional cohorts to understand the potential risk among patients both with and without ESRD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Kaplan-Meier survival curve for *JAK3* rs3213409/rs3212780 haplotype in 518 White CHOICE participants.

Table 1
Baseline characteristics in 518 White and 246 Black CHOICE participants*

	White Participants n=518 (67.8%)		p	Black Participants n=246 (32.2%)		p
	New CVD Event n=310 (59.8%)	No Event n=208 (40.2%)		New CVD Event n=126 (51.2%)	No Event n=120 (48.8%)	
Age (yr)	62.8 ± 22.7	53.3 ± 31.6	<0.001	60.1 ± 18.0	47.9 ± 22.9	<0.001
Percent European Ancestry	99 (97–99)	99 (97–99)	0.9	15 (8–24)	15 (7–22)	0.5
Men (%)	184 (59.4)	110 (52.9)	0.2	55 (43.7)	57 (47.5)	0.6
ICED (%) [†]			<0.001			0.01
Low	73 (23.6)	95 (45.9)		33 (26.2)	52 (43.3)	
Intermediate	134 (43.2)	59 (28.5)		59 (46.8)	37 (30.8)	
High	103 (33.2)	53 (25.6)		34 (27.0)	31 (25.8)	
Prior history of CVD (%)	188 (60.7)	65 (31.4)	<0.001	55 (43.7)	24 (20.2)	<0.001
Diabetes (%)	197 (63.6)	77 (37.2)	<0.001	90 (71.4)	48 (40.0)	<0.001
Smoking (%)			0.7			0.9
Never	110 (36.7)	69 (34.2)		54 (43.6)	52 (45.2)	
Former	147 (49.0)	99 (49.0)		49 (39.5)	42 (36.5)	
Current	43 (14.3)	34 (16.8)		21 (16.9)	21 (18.3)	
Dialysis modality (%)			0.08			0.04
Hemodialysis	243 (78.4)	149 (71.6)		117 (92.9)	101 (84.2)	
Peritoneal dialysis	67 (21.6)	59 (28.4)		9 (7.1)	19 (15.8)	
SBP (mmHg)	150.3 ± 40.0	146.0 ± 40.6	0.06	152.0 ± 35.1	154.2 ± 26.4	0.5
DBP (mmHg)	76.7 ± 19.9	79.5 ± 21.5	0.003	79.5 ± 16.4	85.8 ± 16.0	<0.001
BMI (kg/m ²)	25.6 (22.3–29.9)	25.2 (22.1–29.1)	0.7	26.8 (23.7–31.0)	25.1 (22.0–29.4)	0.02
IL-6 (pg/mL)	4.8 (3.0–9.0)	3.8 (2.3–6.7)	<0.001	4.5 (2.7–7.0)	3.3 (2.0–4.8)	<0.001
CRP (mg/L)	4.3 (2.2–11.4)	3.4 (1.5–6.3)	0.01	3.7 (1.6–13.1)	3.1 (1.4–5.1)	0.04
Fibrinogen (mg/dL)	383.7 ± 8.8	378.1 ± 12.6	0.6	366.1 ± 12.0	347.9 ± 10.6	0.3
Albumin (g/dL)	3.57 ± 0.017	3.70 ± 0.034	0.003	3.63 ± 0.02	3.63 ± 0.03	0.8
Total Cholesterol (mg/dL)	181 (155–216)	189 (160–223)	0.08	185.5 (157.0–215.5)	178.0 (148.0–207.0)	0.2
HDL cholesterol (mg/dL)	40.0 (32.0–49.0)	37.5 (31.0–49.0)	0.6	46 (38–54)	43 (33–57)	0.2

Note: Age, SBP, DBP, fibrinogen, and albumin are reported as mean ± standard deviation. Percent European ancestry, BMI, IL-6, CRP, total cholesterol, and HDL are reported as median (interquartile range). Conversion factors for units: fibrinogen in mg/dL to mmol/L, x 0.01; albumin in g/dL to g/L, x 10; total cholesterol and HDL cholesterol in mg/dL to mmol/L, x0.026.

Abbreviations: ICED, index of co-existent disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; IL-6, interleukin 6; CRP, C-reactive protein; HDL, high-density lipoprotein.

* Some variables may not sum to 764 due to missing data.

[†] Index of Coexistent Disease (ICED), systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), C-reactive protein (CRP), high density lipoprotein (HDL).

Table 2
JAK3 SNPs and *p*-value of association with new CVD events, stratified by race

SNP	Tag SNP	Minor Allele	Region [†]	MAF [‡] (%)	Control HWE $p^§$	Hazard Ratio		p (Bonferroni-corrected p)	
						Unadjusted (95% C.I.)	Adjusted// (95% C.I.)	Unadjusted	Adjusted
White Participants									
rs3213415	E	G _[ND3]	Ig	0.5	**	1.13 (0.49,2.61)	1.6 (0.51,5.01)	0.8	0.4
rs3212798	A/E	G	I	17.2	0.4	0.90 (0.76,1.08)	0.89 (0.72, 1.1)	0.3	0.3
rs3212780	E	C	I	27.9	0.2	1.92 (1.63,2.27)	2.29 (1.85,2.84)	<0.001 (<0.001)	<0.001 (<0.001)
rs3213409	E	G	E	1.7	**	1.82 (0.96,1.64)	1.92 (1.18,3.14)	0.07	0.009 (0.2)
rs2072496	E	G	I	11.0	0.7	0.84 (0.67,1.05)	0.83 (0.61, 1.11)	0.1	0.2
rs3212762	A/E	C	I	37.4	0.2	0.92 (0.73,1.16)	1.09 (0.9,1.32)	0.5	0.4
rs3212760	A/E	C	I	39.2	0.2	1.29 (0.92,1.8)	1.37 (0.93,2.03)	0.1	0.1
rs3212752	A/E	G	I	6.3	**	2.09 (1.67,2.61)	2.34 (1.45,3.77)	<0.001 (<0.001)	0.001 (0.03)
rs3212741	E	C	I	21.3	0.8	0.88 (0.74,1.05)	0.92 (0.7,1.2)	0.2	0.5
rs3212714	A/E	C	I	27.6	0.3	0.70 (0.54,0.91)	0.69 (0.46,1.03)	0.007 (0.2)	0.07
rs3212711	A/E	C	I	27.7	0.2	0.71 (0.55,0.90)	0.67(0.46,0.99)	0.006 (0.1)	0.04 (0.7)
rs3212701	A/E	G	I	19.9	0.8	1.24 (0.83,1.84)	1.61 (1.06,2.44)	0.3	0.03 (0.5)
rs2110586	A/E	C	I	20.3	0.5	1.09 (0.86,1.38)	1.01 (0.74,1.39)	0.5	0.9
Black Participants									
rs3212798	A/E	G	I	39.6	1.0	0.79 (0.56,1.11)	0.95 (0.58,1.55)	0.2	0.8
rs2302603	A	C	I	42.9	0.5	0.81 (0.47,1.42)	0.88 (0.49,1.58)	0.5	0.7
rs1122385	A	C	I	60.9	0.1	0.96 (0.68,1.35)	0.94 (0.77,1.14)	0.8	0.5
rs3212762	A/E	C	I	52.7	0.3	0.95 (0.67,1.33)	1.88 (1.31,2.7)	0.8	0.001 (0.03)
rs3212760	A/E	C	I	36.1	0.02	0.83 (0.58,1.19)	0.91 (0.63,1.32)	0.3	0.6
rs3212756	A	C	I	14.3	1.0	0.89 (0.59,1.34)	1.18 (0.84,1.67)	0.6	0.3
rs3212752	A/E	G	I	23.8	0.8	2.07 (1.22,3.51)	3.31 (1.63,6.72)	0.007 (0.2)	0.001 (0.03)
rs3212745	A	G	I	13.1	1.0	1.09 (0.76,1.54)	1.57 (0.98,2.53)	0.7	0.06
rs3212723	A	C	E	7.8	**	0.94 (0.53,1.67)	0.76 (0.39,1.5)	0.8	0.4
rs3212714	A/E	C	I	48.8	0.9	0.93 (0.66,1.3)	0.84 (0.61,1.17)	0.7	0.3

SNP	Tag SNP [*]	Minor Allele	Region [†]	MAF [‡] (%)	Control HWE <i>p</i> [§]	Hazard Ratio		<i>p</i> (Bonferroni-corrected <i>p</i>)	
						Unadjusted (95% C.I.)	Adjusted ^{//} (95% C.I.)	Unadjusted	Adjusted
rs3212711	A/E	C	I	52.9	0.9	0.83 (0.69,1.0)	0.79 (0.63,0.99)	0.05	0.04 (0.7)
rs3212701	A/E	G	I	56.7	0.8	0.61 (0.42,0.89)	0.7 (0.46,1.07)	0.01 (0.3)	0.1
rs2110586	A/E	C	I	8.0	**	0.88 (0.58,1.32)	0.75 (0.5,1.12)	0.5	0.2

Abbreviations: SNP, single-nucleotide polymorphism

^{*} (E)European, (A)ffrican-American

[†] Ig = intragenic ; I = intron ; E = non-synonymous exon

[‡] MAF = minor allele frequency

[§] *p*-value for deviation from Hardy Weinberg equilibrium (HWE) in Control participants (no new CVD event)

^{//} Adjusted for PEA, age, sex, albumin, ICED score, IL-6, CRP, SBP, DBP, smoking status, diabetes, cholesterol, and HDL

^{**} HWE does not exist as no patients without a new CVD event were homozygous for the minor allele

Table 3

STAT4 and STAT6 SNPs and *p*-value of association with new CVD events, stratified by race

SNP	Tag SNP*	Minor Allele	Region [†]	MAF [‡] (%)	Control HWE <i>p</i> [§]	Hazard Ratio			(Bonferroni-corrected <i>p</i>)	
						Unadjusted (95% C.I.)	Adjusted (95% C.I.)	Unadjusted	Adjusted	
White Participants										
STAT4										
rs3024919	A/E	C	Ig	17.1	0.02	1.05(0.88,1.25)	1.16(0.97,1.39)	0.6	0.1	
rs3024912	A/E	G	Ig	23.9	0.4	0.85(0.67,1.07)	0.98(0.79,1.20)	0.2	0.8	
rs3024904	E	A	I	11.1	0.03	1.1(0.83,1.45)	1.14(0.85,1.53)	0.5	0.4	
rs3024886	A/E	C	I	18.1	0.5	1.09(0.92,1.28)	1.22(1.06,1.40)	0.3	0.005 (0.1)	
rs3024851	A/E	T	I	5.1	0.4	1.22(0.84,1.78)	1.31(0.82,2.09)	0.3	0.3	
rs932169	E	G	I	7.8	0.1	1.05(0.82,1.34)	0.89(0.68,1.18)	0.7	0.4	
rs3024847	E	T	I	59.7	0.7	0.98(0.83,1.17)	0.84(0.74,0.95)	0.9	0.004 (0.1)	
rs1400656	A/E	G	I	5.2	0.4	1.26(0.89,1.81)	1.36(0.9,2.08)	0.2	0.2	
STAT6										
rs3024979	E	A	I	10.2	1.0	1.26(0.99,1.62)	1.39(1.04,1.84)	0.07	0.03 (0.5)	
rs841718	A/E	C	I	40.6	0.4	0.75(0.58,0.96)	0.9(0.68,1.19)	0.03 (0.5)	0.5	
rs3024971	E	C	I	11.2	0.7	1.18(0.9,1.54)	1.10(0.83,1.46)	0.2	0.5	
rs3024980	E	G	I	1.7	**	1.57(1.07,2.30)	1.35(0.81,2.24)	0.02 (0.4)	0.2	
rs2598483	E	C	I	10.3	1.0	1.15(0.90,1.47)	1.30(0.97,1.73)	0.3	0.08	
Black Participants										
STAT4										
rs3024919	A/E	C	Ig	25.8	0.5	0.86(0.58,1.27)	0.84(0.63,1.10)	0.5	0.2	
rs3024912	A/E	G	Ig	40.7	0.2	0.82(0.56,1.21)	0.82(0.61,1.09)	0.3	0.2	
rs3024895	A	C	I	7.1	0.5	1.09 (0.71,1.68)	0.86 (0.5,1.47)	0.7	0.6	
rs925847	A	C	I	39.3	0.01	0.71 (0.51,1.00)	0.84 (0.59,1.20)	0.05 (0.8)	0.3	
rs3024894	A	C	I	15.6	0.5	1.23 (0.86,1.74)	1.69 (1.20,2.38)	0.3	0.003 (0.09)	
rs3024886	A/E	C	I	27.8	0.001	0.77 (0.51,1.17)	1.09 (0.68,1.76)	0.2	0.7	
rs3024879	A	C	I	36.9	0.3	1.68 (1.12,2.52)	1.38 (0.77,2.46)	0.01 (0.3)	0.3	
rs3024873	A	G	I	11.0	1.0	1.10 (0.76,1.58)	1.29 (0.81,2.07)	0.6	0.3	
rs3024861	A	A	I	81.1	0.2	1.01 (0.74,1.37)	1.22 (0.82,1.81)	0.9	0.3	

SNP	Tag SNP*	Minor Allele	Region [†]	MAF [‡] (%)	Control HWE $p^{\S}$	Hazard Ratio		(Bonferroni-corrected p)	
						Unadjusted (95% C.I.)	Adjusted [¶] (95% C.I.)	Unadjusted	Adjusted
rs3024858	A	G	I	24.2	0.3	0.94 (0.42,2.11)	1.05 (0.35,3.17)	0.9	0.9
rs3024851	A/E	T	I	12.0	**	1.17 (0.85,1.61)	1.09 (0.71,1.67)	0.3	0.7
rs1517352	A	C	I	10.4	0.3	1.18 (0.73,1.91)	1.18 (0.73,1.91)	0.5	0.4
rs1400656	A/E	G	I	5.5	**	1.19 (0.83,1.72)	1.19 (0.83,1.72)	0.4	0.5
STAT6									
rs4559	A	G	Ig	44.9	1.0	0.91 (0.63,1.32)	0.77 (0.44,1.33)	0.6	0.3
rs841718	A/E	C	I	68.9	0.7	0.71 (0.4,1.26)	0.57 (0.35,0.93)	0.2	0.03 (0.6)
rs3024967	A	G	I	7.6	0.2	0.85 (0.53,1.37)	1.06 (0.63,1.78)	0.5	0.8
rs3024955	A	C	I	15.3	1.0	0.94 (0.64,1.38)	1.05 (0.74,1.48)	0.8	0.8
rs3024954	A	C	I	22.7	0.8	1.25 (0.66,2.38)	1.80 (0.89,3.65)	0.5	0.1

Abbreviations: SNP, single-nucleotide polymorphism

[‡](E)uropean, (A)ffrican-American

[†]Ig = intragenic ; I = intron ; E = non-synonymous exon

[‡]MAF = minor allele frequency

[§] p -value for deviation from Hardy Weinberg equilibrium (HWE) in Control participants (no new CVD event)

[¶]Adjusted for PEA, age, sex, albumin, ICED score, IL-6, CRP, SBP, DBP, smoking status, diabetes, cholesterol, and HDL

^{*}HWE does not exist as no patients without a new CVD event were homozygous for the minor allele