A genome-wide association study for diabetic nephropathy genes in African Americans

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A genome-wide association study was performed using the Affymetrix 6.0 chip to identify genes associated with diabetic nephropathy in African Americans. Association analysis was performed adjusting for admixture in 965 type 2 diabetic African American patients with end-stage renal disease (ESRD) and in 1029 African Americans without type 2 diabetes or kidney disease as controls. The top 724 single nucleotide polymorphisms (SNPs) with evidence of association to diabetic nephropathy were then genotyped in a replication sample of an additional 709 type 2 diabetes-ESRD patients and 690 controls. SNPs with evidence of association in both the original and replication studies were tested in additional African American cohorts consisting of 1246 patients with type 2 diabetes without kidney disease and 1216 with non-diabetic ESRD to differentiate candidate loci for type 2 diabetes-ESRD, type 2 diabetes, and/or all-cause ESRD. Twenty-five SNPs were significantly associated with type 2 diabetes-ESRD in the genome-wide association and initial replication. Although genome-wide significance with type 2 diabetes was not found for any of these 25 SNPs, several genes, including *RPS12*, *LIMK2*, and *SFI1* are strong candidates for diabetic nephropathy. A combined analysis of all 2890 patients with ESRD showed significant association SNPs in *LIMK2* and *SFI1* suggesting that they also contribute to all-cause ESRD. Thus, our results suggest that multiple loci underlie susceptibility to kidney disease in African Americans with type 2 diabetes and some may also contribute to all-cause ESRD.

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Diabetes-associated nephropathy is the most common cause of end-stage renal disease (ESRD) in the United States, accounting for 44.6% of incident cases.[1] African Americans (AAs) have a disproportionately high risk for developing diabetic nephropathy (DN). Compared with Caucasian Americans (CAs), AAs have a 3.7-fold greater incidence rate of developing ESRD and at least a 1.9-fold greater incidence rate than do other racial and ethnic minorities in the United States.[1] Many studies have shown that there is a genetic component to ESRD as reviewed by Bowden.[2] Familial aggregation of DN and diabetic ESRD has been demonstrated in CAs[3–5] and AAs.[6] Clustering occurred in these families without significant differences in glycemic control.[4] However, marked racial and ethnic disparities in familial clustering exist. CAs who have a close relative with ESRD face a 2.7-fold increased risk of developing ESRD,[5] whereas AAs who have a close relative with ESRD have a 9-fold increased risk of developing ESRD.[6] This significant difference in rates of renal complications between CAs and AAs is observed after controlling for differences in socioeconomic status.[5,6]

Several studies have attempted to detect genetic variants influencing the risk of DN and diabetic ESRD. The first genome-wide association study (GWAS) for DN was a low-density (80K single-nucleotide polymorphisms (SNPs)) gene-based study performed in a Japanese population.[7] This

was followed by a study using a DNA pooling method investigating 115K SNPs in Pima Indians with DN.[8] More recently, a GWAS was conducted for type 1 diabetes-associated nephropathy in a CA population,[9] and multiple studies have assessed for association with chronic kidney disease and glomerular filtration rate in nondiabetic populations of European ancestry.[10–12] There have been no previous reports of GWASs in AAs with type 2 diabetes mellitus (T2DM)-ESRD. In this study, we report the first GWAS investigating 832K SNPs for association with T2DM-ESRD in AAs.

RESULTS

Clinical characteristics of study samples

The clinical characteristics of study samples used in the GWAS, replication, and trait discrimination phases are shown in Table 1. The GWAS and replication populations are broadly similar. In both groups, the age at enrollment for T2DM-ESRD subjects is older than that for the control groups. However, the age at enrollment for the control groups in the GWAS and replication phases is older than the age of T2DM diagnosis in T2DM-ESRD and T2DM subjects. All of the case groups with T2DM (T2DM-ESRD and T2DM) have a higher proportion of females, possibly reflecting the increased prevalence of T2DM among AA women,[13] participation bias, and survival. On average, all of the groups were overweight or obese at the time of enrollment. Subjects with ESRD lacking T2DM (non-T2DM-ESRD subjects) had the lowest average body mass index (27.0 kg/m^2, Table 1), and T2DM subjects without nephropathy (T2DM) had the highest average body mass index (33.5 kg/m^2, Table 1).

Genome-wide association study

After the application of SNP and sample quality control metrics, 832,357 autosomal SNPs were analyzed in 965 AA T2DM-ESRD case subjects and in 1029 AA nondiabetic, nonnephropathy controls. A summary of the association results is shown in Figure 1 and the corresponding quantile–quantile plot is shown in Supplementary Figure S1 online. The results shown are adjusted for admixture; however, the primary inferences remain the same adjusting for admixture, age, and gender. The top hit was rs5750250 located on chromosome 22 in the *MYH9* (nonmuscle myosin heavy chain 9) gene ($P = 3.00 \times 10^{-7}$, Figure 1). This gene has been previously associated with both nondiabetic and diabetic forms of ESRD.[14–17] In total, there were 126 SNPs with *P*-values $< 1.0 \times 10^{-4}$ (Figure 1). The flow of the study through the GWAS, replication, combined and trait discrimination phases is outlined in Table 2.

Replication and combined analysis of T2DM-ESRD cases and nondiabetic, nonnephropathy controls

In an effort to replicate the GWAS results, 724 top-scoring SNPs were genotyped in an independent sample of 709 AA T2DM-ESRD cases and 690 AA nondiabetic, nonnephropathy controls (study design, Table 2; Results, Supplementary Table S1 online). The 724 SNPs that were selected for testing in the replication sample were SNPs with the strongest *P*-values for association and with high quality scores for genotyping, that is, missing rate < 0.02 (or missing rate < 0.05, but the missing rate between cases and controls was not significantly different). In addition, *P*-values for Hardy–Weinberg proportions were > 0.0001 for cases and > 0.01 for controls. SNPs were prioritized on significance of the additive genetic model unless there was *a priori* evidence for follow-up, for example, *MYH9*. In this replication analysis, 67 SNPs showed nominal evidence of replication: additive *P*-value < 0.05 with association in the same direction (Supplementary Table S1 online). Table 3 summarizes the association results for 25 SNPs at 19 potential T2DM-ESRD loci. T2DM-ESRD loci were identified during trait discrimination analyses as shown in Table 2 and were based on the following criteria: (1) associated in the replication phase, (2) associated in the T2DM-ESRD versus T2DM, nonnephropathy comparison (Table 4), and (3) showed no association or nominal association ($P > 0.01$) in the T2DM, nonnephropathy versus controls comparison (Table 4). Table 3 shows *P*-values for association in the GWAS, the replication sample, and the combined cohort: 1674 T2DM-ESRD cases and 1719 nondiabetic, nonnephropathy controls. No SNP reached genome-wide significance ($P \leqslant 5 \times 10^{-8}$), *P*-values ranged from 1.24×10^{-4} to 7.04×10^{-7} (Table 3, combined analysis).

The strongest association in the combined analysis (GWAS + replication) was with rs6930576 ($P = 7.04 \times 10^{-7}$, odds ratio (OR) (95% confidence interval (95% CI)) = 1.31 (1.18–1.45); Table 3, Supplementary Figure S2a online). SNP

Table 1 | Clinical characteristics of study samples

	GWAS		Replication		Trait discrimination	
	T2DM-ESRD	Controls	T2DM-ESRD	Controls	T2DM	Non-T2DM-ESRD
n	965	1029	709	690	1246	1216
Female (%)	61.20	57.30	55.70	51.30	64.00	44.70
Age at enrollment (years)	61.6 ± 10.5	49.0 ± 11.9	60.2 ± 10.4	48.5 ± 12.8	57.2 ± 11.7	53.0 ± 14.5
Age at T2D diagnosis (years)	41.6 ± 12.4	—	39.4 ± 12.5	—	46.1 ± 12.6	—
Age at ESRD diagnosis (years)	58.0 ± 10.9	—	56.7 ± 10.9	—	—	47.7 ± 15.5
T2D to ESRD duration (years)	16.2 ± 10.9	—	20.4 ± 10.5	—	—	—
BMI (kg/m^2)	29.7 ± 7.0	30.0 ± 7.0	29.8 ± 6.9	29.4 ± 7.6	33.5 ± 7.6	27.0 ± 7.0

Abbreviations: BMI, body mass index; ESRD, end-stage renal disease; GWAS, genome-wide association study; T2DM, type 2 diabetes mellitus.
Values are presented as trait mean and s.d.