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Complement factor H gene associations with end-stage kidney disease in African Americans

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ABSTRACT

Background. Mutations in the complement factor H gene (*CFH*) region associate with renal-limited mesangial proliferative forms of glomerulonephritis including IgA nephropathy (IgAN), dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). Lack of kidney biopsies could lead to under diagnosis of *CFH*-associated end-stage kidney disease (ESKD) in African Americans (AAs), with incorrect

attribution to other causes. A prior genome-wide association study in AAs with non-diabetic ESKD implicated an intronic *CFH* single nucleotide polymorphism (SNP).

Methods. Thirteen *CFH* SNPs (8 exonic, 2 synonymous, 2 3′UTR, and the previously associated intronic variant rs379489) were tested for association with common forms of non-diabetic and type 2 diabetes-associated (T2D) ESKD in 3770 AAs (1705 with non-diabetic ESKD, 1305 with T2D-ESKD, 760 controls). Most cases lacked kidney biopsies; those with known IgAN, DDD or C3GN were excluded.

Results. Adjusting for age, gender, ancestry and apolipoprotein L1 gene risk variants, single SNP analyses detected 6 *CFH* SNPs (5 exonic and the intronic variant) as significantly associated with non-diabetic ESKD (P = 0.002–0.01), three of these SNPs were also associated with T2D-ESKD. Weighted *CFH* locus-wide Sequence Kernel Association Testing (SKAT) in non-diabetic ESKD (P = 0.00053) and T2D-ESKD (P = 0.047) confirmed significant evidence of association.

Conclusions. *CFH* was associated with commonly reported etiologies of ESKD in the AA population. These results suggest that a subset of cases with ESKD clinically ascribed to the effects of hypertension or glomerulosclerosis actually have *CFH*-related forms of mesangial proliferative glomerulonephritis. Genetic testing may prove useful to identify the causes of renal-limited kidney disease in patients with ESKD who lack renal biopsies.

Keywords: African Americans, *CFH*, end-stage kidney disease, genetics, kidney disease

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INTRODUCTION

The complement system is critical to protect hosts from invading pathogens [1]. Dysregulation of this system is associated with susceptibility to infection and autoimmune disorders including systemic lupus erythematosus [2]. The complement factor H gene (*CFH*) and five *CFH*-related genes (*CFHR*) with high-sequence homology are located on chromosome 1q32. This complex genomic region regulates the activity of the alternative pathway of the complement system. *CFH* encodes Factor H protein, a critical inhibitor of the alternative pathway [3]. Loss of function mutations in *CFH* associate with age-related macular degeneration, presumably from microvascular retinal injury due to loss of inhibitory effect on the alternative complement pathway [4].

Several renal-limited forms of mesangial proliferative glomerulonephritis also associate with mutations in the *CFH* and *CFHR* genes [5]. These include IgA nephropathy (IgAN), C3 glomerulonephritis (C3GN) and dense deposit disease (DDD) [6–9]. Mutations in the N-terminal regulatory region of *CFH* associate with complement-mediated C3GN and DDD; both disorders can be progressive and lead to end-stage kidney disease (ESKD). A genome-wide association study (GWAS) in IgAN implicated a single nucleotide polymorphism (SNP) in intron 12 of *CFH* [6]. The intronic SNP that showed the strongest association with IgAN is in high linkage disequilibrium (LD) with copy number variation (CNV) in the adjacent *CFHR1* and *CFHR3* genes. Deletion of these two genes appears to reduce susceptibility to IgAN. Finally, mutations near the C-terminus of *CFH* are associated with atypical hemolytic uremic syndrome (aHUS) [10]. aHUS is a systemic thrombotic disorder manifesting endothelial cell injury and leading to progressive kidney failure. aHUS lacks a mesangial proliferative injury pattern and manifests clinically with thrombocytopenia and intravascular hemolysis.

Extra-renal manifestations are strong clues to the presence of aHUS. In contrast, renal-limited IgAN, C3GN and DDD can only be diagnosed with a kidney biopsy. In the absence of biopsy material, subjects with progressive renal-limited kidney disease are often diagnosed as having hypertensive or chronic glomerulosclerosis-associated ESKD [11,12]. A GWAS in African American (AA) cases with non-diabetic etiologies of ESKD implicated the apolipoprotein L1 (*APOL1*) and *CFH* genes [13]. After the profound effect of *APOL1*, intronic *CFH* SNP rs379489 was the most significantly associated variant. The current analyses evaluated this SNP and 12 additional exonic (coding) *CFH* variants to determine whether they were associated with commonly reported forms of non-diabetic and type 2 diabetes-associated (T2D) ESKD in AAs.

MATERIALS AND METHODS

Study subjects

Recruitment and sample collection procedures have previously been reported [13,14]. The study was approved by the Institutional Review Board at Wake Forest School of Medicine (WFSM) and all the participants provided written informed consent. Cases and controls were unrelated and born in North Carolina, South Carolina, Georgia, Tennessee or Virginia (Table 1). DNA was extracted from whole blood using the PureGene system (Gentra Systems, Minneapolis, MN, USA). AA cases with ESKD were recruited from dialysis facilities; cases with non-T2D-ESKD lacked diabetes at the initiation of renal replacement therapy. ESKD was attributed to hypertension (~60%), unspecified glomerular disease or focal segmental

Table 1. Clinical characteristics of African American study samples

	T2D-ESKD cases	Controls	Non-T2D-ESKD cases
n	1305	760	1705
Female (%)	60.7	57.9	43.7
Age (years)	61.3 ± 10.8	48.4 ± 12.7	54.6 ± 14.6
Age at T2D (years)	41.3 ± 12.4	—	—
Duration T2D prior to ESKD (years)	17.1 ± 10.7	—	—
Duration of ESKD (years)	3.66 ± 3.9	—	2.2 ± 1.64
Fasting serum glucose (mg/dL)	—	89.2 ± 13.6	88.6 ± 8.66
BMI (at recruitment; kg/m^2)	30.3 ± 7.2	29.2 ± 7.4	27.2 ± 6.98
BUN (mg/dL)	—	13.3 ± 4.5	—
Serum creatinine (mg/dL)	—	1.03 ± 0.46	—

Categorical data expressed as percentage; continuous data as mean ± SD.

glomerulosclerosis (FSGS) (~30%), HIV-associated nephropathy (~5%) or unknown cause in the absence of a kidney biopsy (~5%); <2% of cases had a kidney biopsy. T2D was diagnosed in cases developing diabetes after age 25 years, without diabetic ketoacidosis or treatment solely with insulin since diagnosis. T2D-ESKD was diagnosed after >5 year T2D duration prior to renal replacement therapy, or with diabetic retinopathy or ≥100 mg/dL proteinuria on urinalysis (when available), in the absence of other causes of nephropathy. Cases with ESKD due to urologic/surgical cause, polycystic kidney disease, aHUS, IgAN, membranous glomerulonephritis, membranoproliferative glomerulonephritis, C3GN, or DDD were not recruited. AA controls without T2D or kidney disease [serum creatinine concentration <1.5 (men) or <1.3 mg/dL (women)] were recruited from the community and WFSM internal medicine clinics. Ethnicity was self-reported and confirmed by genotyping with ancestry informative markers.

Sample preparation, genotyping and quality control

CFH variants were selected from exome sequencing resources (1000 Genomes, Exome Variant Server) in addition to the intronic variant selected from our prior report [13]. SNP selection criteria included allelic discrepancies between European and AA populations (Supplementary data Table S1), PolyPhen2 prediction (http://genetics.bwh.harvard.edu/pph/data/) and minor allele frequency. Owing to the low-predictive value of PolyPhen2, the amino acid changes of exonic variants were considered independently of the PolyPhen2 score in certain circumstances (Supplementary data Table S1). Our intention was to test genetic variants in the *CFH* gene based on the common disease-common variant and the common disease-rare variant hypotheses. This is reflected in Tables 2 and 3 with common single-SNP association testing and in Tables 4 and 5 with the SKAT analysis powered for rare variants.

Targeted genotyping

Targeted genotyping of 13 *CFH* variants was performed utilizing the Sequenom MassArray system (Sequenom, San Diego, CA, USA) in the Center for Genomics and Personalized Medicine Research at WFSM. SNPs were PCR-amplified using primers designed in MassARRAY Assay Design 3.1 (Sequenom) and genotypes were analyzed using MassARRAY Typer (Sequenom). Call rates >97% were achieved for all variants; quality control was ensured using blind duplicates within each cohort of samples (100% concordance rate).

Statistical analysis

Single SNP association testing. Each SNP was tested for departure from Hardy–Weinberg equilibrium (HWE) expectations through Fisher's exact test (HWE $P > 0.05$ in both cases and controls). The overall genotypic test of association and the three genetic models (dominant, additive and recessive) were computed with the test for association between each SNP and each phenotype. Data for all tests of association were adjusted for admixture [15] and *APOL1* G1/G2 risk allele status assuming a recessive model of disease risk [16] (Model 1) or Model 1 + age and gender (Model 2). These tests were computed using the SNPGWA program (http://www.phs.wfubmc.edu/public_bios/sec_gene/downloads.cfm). Large sample test distribution and permutation methods were used to estimate statistical significance. Given *a priori* evidence of the association between *CFH* and nephropathy, we employed a P-value cutoff of <0.05 for statistical significance.

Sequence kernel association testing. Sequence Kernel Association Testing (SKAT) was performed on all 13 *CFH* variants genotyped in non-diabetic ESKD cases, T2D-ESKD cases and non-diabetic non-nephropathy controls [17]. The SKAT Meta package was run on *R 3.0.1* (http://cran.r-project.org/web/views/Genetics.html) using a model weighted for low-frequency

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Table 2. *CFH* single SNP association results in African Americans with non-diabetic ESKD

				Model 1			Model 2		
SNP	BP (hg19)	*n*, case/control[b]	MAF, case/control	P-value	OR	95% CI	P-value	OR	95% CI
rs1061147	196654324	1424/691	0.42/0.46	0.0037	0.82	0.71, 0.94	0.0030	0.81	0.70, 0.93
rs1061170	196659237	1419/691	0.36/0.39	0.021	0.85	0.74, 0.98	0.0091	0.83	0.72, 0.95
rs74443307	196670520	1432/722	0.017/0.012	0.39	1.30	0.71, 2.38	0.52	1.22	0.66, 2.26
rs35453854	196684855	1426/693	0.054/0.065	0.20	0.83	0.62, 1.10	0.44	0.89	0.66, 1.20
rs379489	196693451	1422/688	0.25/0.29	0.012[a]	0.78	0.64, 0.95	0.0041[a]	0.74	0.61, 0.91
rs3753396	196695742	1426/690	0.072/0.057	0.00055[a]	1.70	1.26, 2.31	0.0023[a]	1.62	1.19, 2.21
rs142902005	196696005	1425/691	0.0014/0.00	—	—	—	—	—	—
rs515299	196706677	1423/690	0.19/0.22	0.017	0.82	0.69, 0.96	0.0098	0.80	0.67, 0.95
rs1065489	196709774	1426/693	0.072/0.058	0.00084[a]	1.67	1.23, 2.25	0.0036[a]	1.58	1.16, 2.15
rs34362004	196711098	1425/694	0.011/0.017	0.076	0.58	0.32, 1.06	0.099	0.59	0.32, 1.10
rs35274867	196712596	1422/692	0.026/0.022	0.63	1.12	0.70, 1.79	0.56	1.16	0.71, 1.88
rs34247141	196715063	1427/693	0.13/0.13	0.66	0.95	0.77, 1.18	0.96	0.99	0.80, 1.24
rs371680052	196716472	1419/691	0.0014/0.0014	0.83	1.21	0.21, 7.03	0.93	1.09	0.19, 6.34

Model 1: Additive model adjusted for *APOL1* and admixture.
Model 2: Model 1 + age and gender.
MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
[a]Dominant model (LOF to additive <0.05).
[b]Sample sizes reflect those with complete clinical and genotypic data used in each analysis

Table 3. *CFH* single SNP association results in African Americans with putative type 2 diabetes-associated ESKD

				Model 1			Model 2		
SNP	BP (hg19)	*n*, case/control[b]	MAF, case/control	P-value	OR	95% CI	P-value	OR	95% CI
rs1061147	196654324	1284/691	0.42/0.46	0.015	0.85	0.74, 0.97	0.017	0.83	0.71, 0.97
rs1061170	196659237	1281/691	0.37/0.39	0.17	0.91	0.8, 1.04	0.042	0.85	0.72, 0.99
rs74443307	196670520	1288/722	0.017/0.012	0.20	1.44	0.82, 2.54	0.29	1.42	0.74, 2.71
rs35453854	196684855	1284/693	0.050/0.065	0.029	0.73	0.55, 0.97	0.37	0.85	0.60, 1.21
rs379489	196693451	1285/688	0.26/0.29	0.13	0.89	0.77, 1.04	0.049	0.84	0.7, 1.00
rs3753396	196695742	1284/690	0.050/0.057	0.60	0.93	0.69, 1.23	0.46	0.88	0.63, 1.23
rs142902005	196696005	1284/691	0.0016/0.00	—	—	—	—	—	—
rs515299	196706677	1284/690	0.20/0.22	0.22	0.91	0.77, 1.06	0.17	0.88	0.72, 1.06
rs1065489	196709774	1285/693	0.051/0.058	0.53	0.91	0.69, 1.21	0.35	0.85	0.61, 1.19
rs34362004	196711098	1284/694	0.013/0.017	0.28	0.74	0.43, 1.28	0.49	0.79	0.41, 1.53
rs35274867	196712596	1284/692	0.032/0.022	0.081	1.46	0.95, 2.24	0.011[a]	2.00	1.17, 3.40
rs34247141	196715063	1285/693	0.12/0.13	0.15	0.86	0.7, 1.06	0.63	0.94	0.73, 1.21
rs371680052	196716472	1286/691	0.0023/0.0014	0.55	1.63	0.33, 8.20	0.49	1.84	0.33, 10.17

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
Model 1: Additive model adjusted for *APOL1* and admixture.
Model 2: Model 1 + age and gender.
[a]Dominant model (LOF to additive P < 0.05).
[b]Sample sizes reflect those with complete clinical and genotypic data used in each analysis

Table 4. *CFH* locus-wide SKAT analysis (non-T2D-ESKD)

Model	P-value	*n*, SNPs
Unweighted [1,1]	0.0014	13
Weighted [1,3]	0.00053	13

Covariates: admixture, *APOL1*, age, gender.

Table 5. *CFH* locus-wide SKAT analysis (T2D-ESKD)

Model	P-value	*n*, SNPs
Unweighted [1,1]	0.026	13
Weighted [1,3]	0.047	13

Covariates: admixture, *APOL1*, age, gender.

variants, termed '[c(1,3)]' or an unweighted model termed '[c(1,1)]'. The SKAT analysis provides quality control and support for association. SKAT considers whether a variant confers risk, protection or is neutral (no effect), and it simultaneously considers the influence of all variants and their directions of effect.

RESULTS

Table 1 contains demographic and laboratory characteristics of all 3010 AA cases with non-diabetic ESKD and T2D-ESKD, as well as the 760 non-nephropathy controls. The percentage of African ancestry in non-diabetic ESKD cases, T2D-ESKD cases and non-nephropathy controls were mean ± standard deviation (SD) 80.01 ± 10.96%, 79.85 ± 11.54%, and 77.7 ± 10.96%, respectively. Contrasting non-T2D-ESKD cases and controls, cases were more often male (P = 0.006), older (P < 0.001), and had lower body mass index (BMI) (P < 0.001). Contrasting T2D-ESKD cases and controls, cases were more likely female (P = 0.03), older (P < 0.001), and had higher BMI (P = 0.004). Supplementary data Table S1 lists the 13 SNPs that were evaluated. All SNPs met HWE expectations in cases and controls.

Table 2 displays single SNP association results in cases with non-diabetic ESKD, relative to controls. Results are reported with an additive genetic model, unless a significant (P < 0.05) lack of fit (LOF) to additivity was observed. Results for Model 1 were adjusted for admixture and *APOL1* and in Model 2 for admixture, *APOL1*, age and gender. The previously associated intronic *CFH* SNP rs379489 remained significantly associated in this larger sample [dominant model, odds ratio (OR) 0.74, 95% confidence interval (CI) 0.61–0.91; P = 0.0041]. In addition, two synonymous and three non-synonymous SNPs were also significantly associated with non-diabetic ESKD after full adjustment (Model 2). Two of these SNPs were associated in a dominant model, rs3753396 and rs1065489 [OR: 1.62 (95% CI: 1.19–2.21) P = 0.0023; and OR: 1.58 (1.16–2.15) P = 0.0036, respectively]. Three SNPs were associated in an additive model, rs1061147, rs1061170 and rs515299 [OR: 0.81 (95% CI: 0.70–0.93) P = 0.0030; OR: 0.83 (95% CI: 0.72–0.95) P = 0.0091 and OR: 0.80 (95% CI: 0.67–0.95) P = 0.0098, respectively]. Variants rs1061147 and rs1061170 are in LD (r^2 = 0.80; data not shown) and likely reflect the same signal. Modest LD was observed for rs1061170 and rs379489 (r^2 = 0.52; data not shown); all other variants in this study had an r^2 value < 0.40.

These same 13 *CFH* SNPs were next evaluated for association with ESKD attributed to T2D in AAs and four were significantly associated (Table 3). Of the four SNPs, three were also associated with non-diabetic ESKD, including the intronic SNP rs379489 [OR: 0.84 (95% CI 0.70–1.00) P = 0.049] and exonic rs1061170 [OR: 0.85 (95% CI: 0.72–0.99) P = 0.042] and rs1061147 [OR: 0.83 (95% CI: 0.71–0.97) P = 0.017]. The fourth SNP (exonic rs35274867) was associated with putative T2D-ESKD [OR 2.00 (95% CI: 1.17–3.40) P = 0.011], but not with non-diabetic ESKD.

Tables 4 and 5 display both unweighted and weighted SKAT results in non-diabetic ESKD cases (Table 4) and T2D-ESKD cases (Table 5). This revealed significant *CFH* association ($P = 0.00053$ adjusted for age, gender, ancestry and *APOL1*) and provides evidence of locus-wide *CFH* association based on all 13 SNPs for non-diabetic ESKD cases. Modest evidence of *CFH* association was also observed with T2D-ESKD in the unweighted and weighted models ($P = 0.026$–0.047).

DISCUSSION

We report a series of *CFH* genetic association analyses in AAs clinically diagnosed with common etiologies of ESKD, specifically excluding those with known IgAN, DDD or C3GN. Consistent and significant evidence of association was observed with several coding *CFH* SNPs in non-diabetic forms of ESKD, and to a lesser extent in T2D-attributed ESKD. *CFH* is associated with several forms of mesangial proliferative glomerulonephritis, all with glomerular complement deposition [5].

These analyses were initiated to follow-up an intronic *CFH* variant associated with non-diabetic ESKD in a GWAS [13]. The present study included a larger sample of AAs presumably with common forms of non-diabetic ESKD and T2D-associated ESKD who typically lacked renal biopsies. Two synonymous, two 3′ untranslated region, and eight coding exonic variants were assessed; four are predicted to be deleterious (Supplementary data Table S1). Lack of renal histologic material in AAs (and many patients with ESKD) hinders diagnostic accuracy in renal-limited forms of glomerulonephritis. Many of these patients develop progressive nephropathy with resultant secondary hypertension [11, 12]. In the absence of a kidney biopsy their disease is often attributed to the effects of systemic hypertension, coded as glomerulosclerosis, or as unknown cause [18].

Genetic analyses have the potential to dissect subsets of patients with related or specific etiologies of ESKD from heterogeneous samples [19] IgAN is the most common form of *CFH*-associated glomerular disease and it occurs significantly less often in AAs than in populations of Asian and European ancestry [20, 21]. Much of this disparity is genetically mediated and geographic differences exist in risk allele frequencies [6]. However, IgAN, as well as the rare disorders DDD and C3GN, would be under diagnosed in the absence of a kidney biopsy. AAs and ethnic minorities generally have poorer access to healthcare relative to European Americans [22]. Our data support that cases of IgAN, DDD and C3GN may be missed in AAs. It is widely appreciated that many AAs clinically diagnosed with T2D-associated ESKD have non-diabetic forms of nephropathy [19, 23].

The renal literature frequently lacks kidney biopsy material necessary to diagnose renal-limited disease. Many of these AA cases likely had renal-limited forms of mesangial proliferative disorders related to *CFH* and *CFHR*, although it cannot be proven histologically. It is possible that *CFH* associates with progressive FSGS and/or focal global glomerulosclerosis or that alternative pathway complement activation as seen in IgAN, DDD and C3GN were present in our cases. Although power tends to be a limitation for many genetic studies, we do not believe that it adversely affected our results. We estimate 80% power to detect non-T2D-ESKD associations for common variants (rs1061147 and rs1061170) and >90% power to detect associations among low-frequency variants (rs3753396 and rs1065489) at $\alpha = 0.01$ (CaTs Power Calculator, University of Michigan; data not shown). We were unable to directly assess whether the observed variants are in LD with CNV in *CFHR3*/*CFHR1* since the non-T2D-ESKD cases lack individually genotyped GWAS data (DNA was pooled). One would not expect low frequency and rare variants to be in LD with *CFHR* CNVs, especially given the distance (i.e. 50 kb). We therefore used common SNPs which were associated with ESKD as a proxy search (using the SNAP program from the Broad Institute) to investigate whether they were in LD ($r^2 > 0.8$) with SNPs located within exons of *CFHR3*/*CFHR1* using published CNV methodology in these genes [24, 25]. Common *CFH* SNPs that we found to be associated with ESKD were not in LD with CNV-associated SNPs in *CFHR3*/*CFHR1*. Moreover, we assessed whether rs6677604, the intronic *CFH* SNP previously associated with IgAN which is in strong LD with (and thus a proxy for) the CNV *CFHR1,3*Δ (as reported by Gharavi *et al.*) [6], was in LD with any of the variants identified in this study. Again, no variants were found to be in LD.

In conclusion, we replicated association of an intronic SNP in *CFH* with clinically diagnosed non-diabetic and T2D-associated etiologies of ESKD in AAs. We further demonstrate that multiple exonic or coding SNPs in *CFH* are associated with common complex forms of ESKD in populations of recent African ancestry. This supports a higher frequency of mesangial proliferative forms of glomerulonephritis in the AA population with ESKD than previously appreciated or that the complement system plays a role in progressive glomerulosclerosis. It also demonstrates how genetic methodologies can be applied to dissect-related kidney disorders from large and heterogeneous sample sets.

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CONFLICT OF INTEREST STATEMENT

None declared.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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