

TCF7L2 Variants Associate with CKD Progression and Renal Function in Population-Based Cohorts

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ABSTRACT

Genetic variants may increase susceptibility to both diabetes and kidney disease. Whether known diabetes-associated variants in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with chronic kidney disease (CKD) progression and markers of kidney function is unknown. Participants of the Atherosclerosis Risk in Communities Study (ARIC; $n = 11,061$ self-identified white and $n = 4014$ black), Framingham Heart Offspring Cohort (FHS; $n = 2468$), and Heredity and Phenotype Intervention Heart Study (HAPI; $n = 861$) were genotyped at five (ARIC) and two (FHS) common *TCF7L2* variants. The diabetes-conferring risk alleles at rs7903146 and rs7901695 were significantly associated with CKD progression among ARIC participants overall and among those without baseline diabetes. The overall adjusted hazard ratios per rs7903146 T allele were 1.17 (95% confidence interval [CI] 1.04 to 1.32) for white individuals and 1.20 (95% CI 1.03 to 1.41) for black individuals. Similarly, the overall hazard ratios per rs7901695 C allele were 1.19 (95% CI 1.06 to 1.34) for white individuals and 1.27 (95% CI 1.09 to 1.48) for black individuals. The FHS cohort supported these results: The rs7903146 T allele was significantly associated with lower estimated GFR ($P = 0.01$) and higher cystatin C ($P = 0.004$) in adjusted analyses overall and among those without diabetes. In the HAPI cohort, the rs7901695 C allele was significantly associated with lower estimated GFR in adjusted analyses ($P = 0.049$), as were several variants upstream and downstream of *TCF7L2* ($P < 0.003$). No identified variant in the ARIC or FHS cohorts was associated with albuminuria. In conclusion, several population-based samples suggest that variants in the *TCF7L2* gene are associated with reduced kidney function or CKD progression, overall and specifically among participants without diabetes.

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Diabetes is a major risk factor for chronic kidney disease (CKD).¹ Both diabetes and kidney function are heritable,^{2–4} and the heritability of kidney disease progression has been studied and confirmed among individuals with polycystic kidney disease.⁵ Inadequate glycemic control over time contributes to the excess risk for renal complications seen among individuals with diabetes; however, many individuals with poorly controlled diabetes never develop CKD, and others with optimal glycemic control develop renal complications,^{6,7} suggesting that it is important to determine whether genetic susceptibility to diabetes also results in increased risk for kidney disease.

Variants in the transcription factor 7-like 2 (*TCF7L2*) gene, specifically single-nucleotide polymorphism (SNP) rs12255372 and rs7901346, have been identified as conferring type 2 diabetes risk and impaired glucose tolerance across many diverse populations.^{8–18} The *TCF7L2* gene product TCF4 is a transcription factor that acts as a downstream effector in the canonical Wnt signaling pathway and may be important in the development of both type 2 diabetes and renal development and disease.^{19,20} It is therefore plausible that mutations in this gene could manifest themselves in reduced kidney function or kidney disease *via* their effects on hyperglycemia as well as independent of this mechanism.

The objective of our study was to examine whether variants in *TCF7L2* are associated with CKD progression and measures of kidney function in population-based studies of individuals with and without diabetes. We specifically studied the intronic SNPs rs12255372, rs7903146, rs7901695, and rs11196205 originally reported to be associated with type 2 diabetes.⁸ Initial analyses were conducted in self-identified white and black participants of the population-based prospective Atherosclerosis

Risk in Communities (ARIC) Study.²¹ Confirmation of results was sought in the Framingham Heart Study Offspring Cohort (FHS)²² and in the cross-sectional Heredity and Phenotype Intervention (HAPI) Heart Study.²³

RESULTS

Study Samples

Table 1 shows the baseline characteristics of the various study samples. Whereas mean estimated GFR (eGFR) was similar across studies, the proportion of people with at least stage 3 CKD (eGFR <60 ml/min per 1.73 m²)²⁴ was 3.0% among ARIC white, 2.8% among ARIC black, 1.5% among HAPI, and 8.2% among FHS participants, the oldest study sample.

Genotype and allele frequencies as well as measures of quality control for all SNPs genotyped in ARIC and FHS and for selected SNPs in HAPI are shown in Supplemental Table 1. Figure 1 shows the correlation of the originally reported *TCF7L2* SNPs⁸ among both white (Figure 1A) and black (Figure 1B) ARIC participants as well as the availability of these SNPs across the various study samples.

SNPs in *TCF7L2* Are Significantly Associated with CKD Progression among ARIC Participants with and without Diabetes

Table 2 summarizes results from the race-stratified multivariate Cox proportional hazards analyses of the association between time to CKD progression and rs12255372, rs7903146, rs7901695, and rs11196205 of *TCF7L2* in white and black ARIC participants. During a median follow-up of 14 yr, there

Table 1. Baseline characteristics by study population and race^a

Characteristic	ARIC		FHS	HAPI
	White	Black		
Sample size (n)	11,061 ^b	4014 ^b	2468 ^b	861 ^b
Age (yr; mean [SD])	54.4 (5.7)	53.5 (5.8)	61.0 (9.6)	43.7 (13.9)
Male (%)	47.2	38.2	53.0	53.4
Diabetes (%)	9.1	19.5	11.7	0.58
Fasting glucose (mg/dl; mean [SD])	104.8 (29.1)	112.6 (45.4)	104.4 (26.7)	86.2 (9.4)
BMI (kg/m ² ; mean [SD])	27.0 (4.9)	29.6 (6.1)	28.2 (5.4)	26.6 (4.5)
Waist circumference (cm; mean [SD])	96.2 (13.4)	99.1 (15.2)	99.8 (14.2)	87.3 (10.9)
SBP (mmHg; mean [SD])	118.5 (17.0)	128.6 (21.3)	126.9 (18.7)	121.0 (14.6)
Antihypertensive medication (%)	19.9	40.5	33.6	0.23
Serum creatinine (mg/dl; mean [SD])	0.85 (0.2)	0.93 (0.75)	0.88 (0.28)	0.84 (0.15)
eGFR (ml/min per 1.73 m ² at baseline; mean [SD])	89.6 (17.9)	102.6 (24.8)	84.7 (19.1)	96.0 (18.5)
eGFR <60 at baseline (n [%])	326 (3.0)	109 (2.8)	193 (8.1)	13 (1.5)
eGFR (ml/min per 1.73 m ²) at visit 4 (mean [SD])	80.6 (17.0)	89.4 (22.0)	N/A	N/A
eGFR <60 at visit 4 (n [%])	754 (8.7)	166 (6.9)	N/A	N/A
ACR (mg/g; median [25th, 75th percentiles]) ^c	3.9 (2.0, 7.7)	2.7 (0.9, 8.8)	6.4 (2.8, 14.9)	N/A
Serum cystatin C (mg/L; mean [SD])	N/A	N/A	0.97 (0.24)	N/A

^aBMI, body mass index, SBP, systolic BP; ACR, urinary albumin-to-creatinine ratio.

^bNumber of observations with missing characteristic (ARIC white/ARIC black/FHS/HAPI): Diabetes (18/83/46/0), fasting glucose (250/372/131/395), BMI (7/9/49/0), SBP (6/3/2/0), antihypertensive medication (4/4/2/0), eGFR and serum creatinine (4/85/92/0), ACR (2481/1580/486/N/A), and cystatin C (N/A/N/A/138/N/A). In FHS, 109 (100) participants were missing genotype data at rs12255372 (rs7903146).

^cACR was measured at ARIC study visit 4 and FHS examination cycle 6.

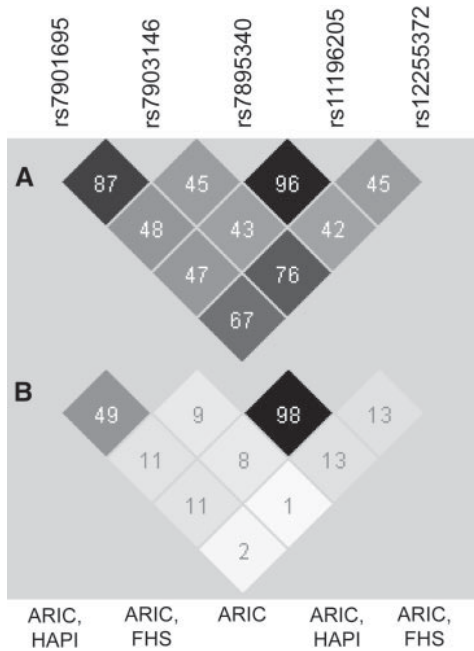


Figure 1. (A and B) LD patterns between SNPs rs7901695, rs7903146, rs7895340, rs11196205, and rs12255372 of *TCF7L2* in white (A) and black (B) ARIC participants. The LD pattern in A is similar to the one observed among the HapMap CEU sample. SNPs are labeled above the LD plots, and the availability of the respective SNP in the various studies is listed below the LD plots.

were 646 cases of CKD progression in white and 355 in black participants.

In white participants, all SNPs, including rs7903146, one of the two main diabetes susceptibility SNPs originally identified,⁸ were significantly associated with CKD progression, even after multivariable adjustment. The risk alleles observed for all SNP were consistent with those previously described as the diabetes risk alleles.^{8,13} In multivariable adjusted analyses stratified by baseline diabetes status, the associations between CKD progression and rs7903146 and rs7901695, a SNP in the same linkage disequilibrium (LD) block as rs7903146, remained significant even among individuals without diabetes (Table 2).

Similarly, in black ARIC participants, both rs7903146 and rs7901695 were significantly associated with CKD progression in multivariable adjusted analyses overall. The association remained significant for rs7901695 among individuals without diabetes at baseline. Among those with baseline diabetes, the risk alleles at rs7903146 and rs7901695 were significantly associated with CKD progression. Tests of statistical interaction between SNP and diabetes status were NS (smallest interaction *P* value 0.14 for rs7903146). Further adjustment for mean percentage of European ancestry did not alter the results observed among black ARIC participants.

The most significant association was observed for rs7901695 ($r^2 = 0.87$ with rs7903146 in ARIC white participants and $r^2 = 0.49$ in black participants; Figure 1) in both white (fully adjusted hazard ratio [HR] 1.19 per C allele; 95%

confidence interval [CI] 1.06 to 1.34; $P = 0.002$) and black ARIC participants (fully adjusted HR 1.27 per C allele; 95% CI 1.09 to 1.48; $P = 0.002$; Table 2).

Role of Hyperglycemia in the Association of *TCF7L2* Variants and CKD Progression in the ARIC Study

For further addressing whether *TCF7L2* variants were associated with CKD progression among ARIC participants without baseline diabetes *via* hyperglycemia, several analyses for rs7903146 and rs7901695 were conducted among ARIC participants without baseline diabetes. First, diabetes and fasting glucose levels during follow-up visits were incorporated as time-dependent variables (Table 3, model 1). For rs7901695, the HR for CKD progression associated with each copy of the C allele remained significant at 1.19 in white participants ($P = 0.010$) and 1.22 in black participants ($P = 0.042$), whereas rs7901346 remained significant in white participants only.

Next, CKD progression and incident diabetes were treated as competing events. In multivariate Cox regression models including fasting glucose as a time-dependent variable, the HR for CKD progression with each copy of the rs7901695 C allele was 1.16 ($P = 0.047$) in white participants and 1.20 ($P = 0.093$) in black participants (Table 3, model 2). Despite similar magnitude, the significance of the association became marginal as the number of cases of CKD progression was reduced by approximately 20% in this analysis. The association with rs7901346 became insignificant. Supplemental Figure 1 shows the race- and genotype-specific multivariable adjusted cumulative incidence of CKD for rs7901695 among those without baseline or incident diabetes.

Last, a very conservative analysis was performed by additionally excluding individuals with impaired fasting glucose (IFG; fasting glucose ≥ 100 mg/dl) at baseline, which led to an additional reduction of events by approximately 45%. The HR of CKD progression for both rs7901695 and rs7903146 decreased by approximately 40% in white participants while remaining of similar magnitude in black participants but were no longer statistically significant (Table 3, model 3). Further incorporation of time-dependent variables for hypertension and use of angiotensin-converting enzyme inhibitors did not substantially change these results.

Relation of SNPs in *TCF7L2* and eGFR/Albuminuria in the ARIC Study

Supplemental Table 2 shows the association between the most CKD progression-associated *TCF7L2* variants rs7903146 and rs7901695 and both continuous eGFR and the dichotomized outcome of eGFR < 60 ml/min per 1.73 m² at ARIC study visit 4, at which both urinary albumin and creatinine were measured. The association between the variants and either phenotype was not statistically significant. Results of further exploration of the association of rs7901695 and eGFR using a nonparametric lowess graph are shown in Supplemental Figure 2. None of the *TCF7L2* variant risk alleles was associated with increased urinary albumin-to-creatinine ratio (ACR)

Table 2. Multivariable adjusted relative hazard of CKD progression during a median follow-up of 14 yr with *TCF7L2* variants in 10786 white and 3599 black ARIC participants^a

Marker	White					Black				
	HR ^b	95% CI	P	Events	n	HR ^b	95% CI	P	Events	n
rs12255372										
overall	1.13	1.00 to 1.28	0.040	629	10,452	1.07	0.91 to 1.25	0.421	350	3522
no diabetes	1.12	0.98 to 1.29	0.099	474	9553	1.05	0.86 to 1.28	0.649	219	2963
diabetes	1.18	0.93 to 1.49	0.177	155	899	1.10	0.85 to 1.44	0.459	131	559
rs7903146										
overall	1.17	1.04 to 1.32	0.008	635	10,476	1.20	1.03 to 1.41	0.023	349	3537
no diabetes	1.18	1.03 to 1.35	0.019	477	9566	1.10	0.89 to 1.35	0.375	219	2980
diabetes	1.19	0.94 to 1.51	0.150	158	910	1.32	1.03 to 1.71	0.031	130	557
rs7901695										
overall	1.19	1.06 to 1.34	0.002	636	10,527	1.27	1.09 to 1.48	0.002	350	3546
no diabetes	1.20	1.05 to 1.37	0.009	477	9613	1.22	1.01 to 1.48	0.036	220	2987
diabetes	1.21	0.96 to 1.52	0.111	159	914	1.36	1.05 to 1.76	0.018	130	559
rs11196205										
overall	1.15	1.03 to 1.29	0.011	635	10,524	1.06	0.88 to 1.28	0.514	351	3559
no diabetes	1.12	0.99 to 1.28	0.070	477	9608	0.97	0.77 to 1.21	0.779	220	2998
diabetes	1.26	1.00 to 1.58	0.045	158	916	1.21	0.88 to 1.67	0.238	131	561

^aResults obtained from a model adjusting for baseline gender, BMI, SBP, antihypertensive medication intake, fasting glucose, study center, smoking, and diabetes in the overall analyses.

^bHR are for each copy of the known diabetes-susceptibility allele (rs12255372 [T]; rs7903146 [T]; rs7901695 [C]; rs11196205 [C]).

Table 3. Sensitivity analyses of the association of *TCF7L2* variants and CKD progression in white and black ARIC participants to investigate the contribution of incident diabetes and impaired fasting glucose^a

SNP	White ARIC Participants					Black ARIC Participants				
	HR	95% CI	P	Events	n	HR	95% CI	P	Events	n
rs7903146										
model 1 ^b	1.17	1.02 to 1.34	0.024	477	9566	1.09	0.89 to 1.34	0.407	219	2980
model 2 ^b	1.12	0.96 to 1.30	0.138	399	9566	1.02	0.81 to 1.29	0.871	174	2980
model 3 ^b	1.07	0.87 to 1.33	0.505	211	5570	1.04	0.77 to 1.39	0.798	108	1676
rs7901695										
model 1 ^b	1.19	1.04 to 1.36	0.010	477	9613	1.22	1.01 to 1.47	0.042	220	2987
model 2 ^b	1.16	1.00 to 1.34	0.047	400	9613	1.20	0.97 to 1.48	0.093	176	2987
model 3 ^b	1.10	0.90 to 1.35	0.359	210	5624	1.26	0.96 to 1.65	0.090	108	1679

^aData presented for rs7903146 and rs7901695 only because these were the variants showing a significant association in both races.

^bModel 1 evaluates individuals without baseline diabetes and incorporates diabetes and fasting glucose as time-dependent variables into the multivariable adjusted Cox model. Model 2 treats CKD progression and incident diabetes as competing events, removing individuals from the analysis at the date of incident diabetes when this date was before or the same as the date of CKD progression and accounts for time-dependent fasting glucose. Model 3 additionally excludes individuals with baseline fasting glucose ≥ 100 mg/dl.

as a marker of kidney damage at ARIC visit 4 (Supplemental Table 3).

Association between *TCF7L2* SNPs and Kidney Function Is Confirmed in the FHS and HAPI Studies

FHS Offspring Cohort

In FHS, SNPs rs12255372 and rs7901346 were genotyped, which were also available in ARIC. Confirmation was therefore sought of significant associations between rs12255372 and rs7901346 with eGFR and serum cystatin C (cross-sectionally at examination cycle 7), as well as ACR (examination cycle 6). The same risk alleles as observed in ARIC at rs12255372 (T) and rs7903146 (T) were significantly associated with lower eGFR and higher cystatin C, both indicating lower kidney function, in multivariable adjusted analyses (Table 4). Limit-

ing the analyses to participants without diabetes, both SNPs remained significantly associated with cystatin C ($P = 0.02$ for rs12255372 and 0.01 for rs7903146), but only rs7903146 remained marginally associated with eGFR ($P = 0.066$). In sensitivity analyses, the additional exclusion of individuals with hemoglobin A1c $\geq 6.0\%$ and IFG from these analyses reduced the sample size by approximately 20 and 40%, respectively (Table 4). Whereas the association of both SNPs and cystatin C among those with hemoglobin A1c $< 6.0\%$ remained significant ($P < 0.007$), regression coefficients among those with IFG were attenuated by approximately 30% and became NS for both variants ($P < 0.18$).

Estimates from multivariable adjusted logistic regression of eGFR < 60 ml/min per 1.73 m^2 ($n = 193$ in FHS) on rs12255372 and rs7903146 yielded odds ratios of 1.13 (95% CI 0.91 to 1.40) and 1.18 (95% CI 0.98 to 1.41), respectively. The

Table 4. Results from linear regression of ln(eGFR) and ln(cystatin C) and logistic regression of eGFR <60 ml/min per 1.73 m² at the FHS cycle 7 examination on rs12255372 and rs7903146 in FHS participants^a

SNP	Coefficient	SE	P	n
Linear regression of ln(eGFR)				
rs12255372				
overall	-0.015	0.008	0.047	2306
no diabetes	-0.009	0.008	0.261	2041
rs7903146				
overall	-0.018	0.007	0.010	2323
no diabetes	-0.013	0.007	0.066	2056
Linear regression of ln(cystatin C)				
rs12255372				
overall	0.016	0.006	0.006	2263
no diabetes	0.013	0.006	0.015	2004
HbA _{1c} <6.0%	0.015	0.005	0.006	1596
FPG <100 mg/dl	0.010	0.007	0.159	1231
rs7903146				
overall	0.016	0.006	0.004	2279
no diabetes	0.014	0.005	0.010	2018
HbA _{1c} <6.0%	0.014	0.005	0.001	1602
FPG <100 mg/dl	0.009	0.007	0.177	1237
Logistic regression of eGFR <60 ml/min per 1.73 m ²				
	OR	95% CI	P	n
rs12255372				
overall	1.13	0.91 to 1.40	0.253	2306
no diabetes	1.09	0.86 to 1.39	0.472	2041
rs7903146				
overall	1.18	0.98 to 1.41	0.079	2323
no diabetes	1.18	0.96 to 1.46	0.115	2056

^aModel adjusted for age, gender, SBP, antihypertensive medication intake, BMI, fasting glucose, smoking, and diabetes in the overall model. OR, odds ratio; HbA_{1c}, hemoglobin A_{1c}; FPG, fasting plasma glucose.

direction and magnitude of these point estimates were comparable to those for CKD progression from the larger ARIC Study but were not statistically significant (Table 2). In analyses evaluating eGFR as the outcome, adjustment for all covariates but age and gender yielded slightly more significant results. SNP in *TCF7L2* were not associated with the urinary ACR in FHS.

HAPI Heart Study

We further sought to confirm our findings using data from 861 HAPI participants who had clean genotype data available on 347,144 markers across the genome (Affymetrix 500K assay). Two of the SNPs genotyped in ARIC, rs11196205 and rs7901695, were included on the Affymetrix 500K chip. Moreover, 64 other SNP in the 800-kb region around the *TCF7L2* gene were also examined for further mapping of the genetic

architecture of *TCF7L2* variation and kidney phenotypes (Supplemental Table 4).

Table 5 shows that, consistent with the findings for CKD progression in ARIC, the rs7901695 C allele was significantly associated with lower eGFR ($P = 0.049$; $P = 0.013$ after further adjustment for fasting glucose available in only 465 participants). The variant rs11196205 was not significantly associated with eGFR in HAPI ($P = 0.39$; $P = 0.12$ after adjustment for fasting glucose). The strongest associations were observed for rs7918405, rs7096151, rs7070850, and rs7068695 (smallest $P = 0.0003$) all within one LD block approximately 200 kb upstream, and rs1904444 and rs10885436 (smallest $P = 0.002$) in a different LD block approximately 150 kb downstream of the transcribed gene (Table 5, Supplemental Figure 3). The associations remained significant after adjustment for the 14 LD

Table 5. Results from variance components analysis of eGFR in HAPI Heart Study participants^a

Parameter	Physical Position ^b	Coefficient	SE	P ^c	n ^c
rs7918405	114,495,455	-0.44	0.12	0.0003 (0.004)	860 (466)
rs7901695	114,744,078	0.18	0.09	0.0490 (0.013)	858 (464)
rs11196205	114,797,037	0.07	0.08	0.3920 (0.125)	847 (454)
rs1904444	115,067,346	0.38	0.12	0.0020 (0.009)	839 (458)

^aResults are adjusted for age, gender, BMI, SBP, waist circumference, and fasting glucose in a subset of participants. Results are presented for rs7901695 and rs11196205, which were also available in the ARIC Study, and for two of the other 64 SNP examined in the region.

^bPhysical position based on NCBI Build 36.1 (March 2006).

^cP value/n for the entire sample, numbers in parentheses for the subsample with available fasting glucose measurements after further adjustment for fasting glucose.

blocks in the region studied for several markers both upstream and downstream of *TCF7L2* ($P < 0.0036$). Neither of these associated regions was in strong LD with rs7901695.

Because only 13 HAPI participants had eGFR <60 ml/min per 1.73 m², the association between SNPs and this outcome was not examined. Analyses were not restricted to individuals without diabetes or IFG, because only three individuals had diabetes, and $>95\%$ of those in the subsample with available measurements had fasting glucose values of <100 mg/dl (Table 1).

DISCUSSION

We present evidence from three population-based studies that variants in the region of the known diabetes-susceptibility gene *TCF7L2* are significantly associated with CKD progression and markers of kidney function overall and in individuals without known diabetes. In white ARIC participants, carrying the diabetes-susceptibility allele of either rs7906195 (C) or rs7903146 (T) was significantly associated with an approximately 20% increased risk for CKD progression overall and in individuals without baseline diabetes per each copy of the risk allele compared with not carrying the risk allele, even after adjustment for time-dependent fasting glucose and diabetes. Significant associations of similar magnitude were observed among black ARIC participants overall and with baseline diabetes and with rs7906195 among those without diabetes. Moreover, significant associations were observed for rs7903146 (T) and rs7901695 (C) with markers of reduced kidney function in two other, independent, population-based studies, FHS and HAPI.

Only one previous study investigated, as a secondary outcome, the association of rs7903146 and renal function among individuals with diabetes. Individuals homozygous for the risk genotype (TT, $n = 12$) had significantly worse renal function measured by 24-h creatinine clearance.²⁵ This observation is consistent with our findings in ARIC, where white and black individuals with diabetes had 20 to 30% higher risk for CKD progression with each copy of the risk alleles at rs7901346 and rs7901695. Our study extends current knowledge by describing a significant association between *TCF7L2* variants and renal phenotypes not only among individuals with diabetes but also in three population-based samples overall and specifically among those without known diabetes.

Several phenomena could explain the associations we observed: (1) The *TCF7L2* variants lead to increased diabetes risk, and subsequent hyperglycemia causes the renal phenotypes. The observed association among individuals without diabetes is the result of residual confounding by undetected diabetes and impaired glucose metabolism. (2) The diabetes-susceptibility variants increase the risk for CKD not only through their effect on diabetes but also through other renal-specific mechanisms (pleiotropic effect). (3) Other variants in the *TCF7L2*

region in LD with the diabetes-susceptibility variants affect susceptibility to CKD (allelic heterogeneity).

Sensitivity analyses in both ARIC and FHS suggest that the impact of frank diabetes does not fully account for the observed association between *TCF7L2* SNPs and renal phenotypes; however, the role of IFG as a potential mediator is less clear. In white ARIC participants without IFG (fasting glucose <100 mg/dl), free of baseline or incident diabetes, and accounting for time-dependent fasting glucose, we observed an approximately 40% attenuation of the multivariable adjusted HR for both rs7903146 and rs7901695. Similarly, sensitivity analyses in FHS excluding individuals with IFG showed an approximately 30% attenuation of the effect size. Although the sample sizes for these sensitivity analyses in both ARIC and FHS were reduced by almost 50%, this suggests that IFG contributes partly to the significant association between *TCF7L2* variants and kidney function, even among those without known diabetes or those who will not develop diabetes for years.

Conversely, the effect size for rs7901695 among black ARIC participants remained unchanged in these sensitivity analyses. Also, a significant association of rs7901695 and eGFR was observed in HAPI, where $>95\%$ of participants with available measurements had fasting glucose values <100 mg/dl, possibly suggesting an association between rs7901695 and renal phenotypes independent of IFG.

A possible mechanism underlying an association of *TCF7L2* variants and kidney function independent of diabetes could be pleiotropy. Pleiotropy has been reported from previous genetic studies of people with diabetes as one of the phenotypes studied.^{26–28} The *TCF7L2* gene product TCF4 is a downstream effector in the canonical Wnt signaling pathway, which is essential in the developing kidney.²⁹ Mutations in components of this pathway have been identified as the cause of inherited kidney disease.^{19,20} Although our study may suggest a mechanistic link between Wnt signaling and kidney function, the underlying molecular mechanisms clearly need to be further studied. Future genome-wide association studies may help to identify additional important components of this pathway.

Regardless of the causal mechanism, however, the consistently observed significant association between common *TCF7L2* variants and kidney disease and markers of kidney function in three population-based studies among individuals without diagnosed diabetes is of importance. If independently associated, carriers of the risk alleles at rs7901695 and rs7903146, accounting for 30 to 45% of the population-based samples examined here, might be more susceptible to CKD progression or reduced kidney function independent of hyperglycemia. If mediated by IFG, then our study draws attention to the measurable, significant effect that glucose levels between 100 and 125 mg/dl might have on renal phenotypes among individuals without diabetes in the general population. This is of particular importance because an association of *TCF7L2* variants and albuminuria, which usually brings diabetic kidney damage to clinical attention later

during the course of the disease, was not observed in our study. This lack of association between *TCF7L2* variants and albuminuria in ARIC and FHS might be explained by different underlying biologic processes of increased albuminuria and reduced eGFR.

The results presented here must be viewed with several limitations in mind. First, in contrast to both FHS and HAPI, we did not observe a significant cross-sectional association between *TCF7L2* variants and continuous eGFR in ARIC, which might be explained by the apparent U-shape association between allele frequency and eGFR (Supplemental Figure 2). Further research in additional studies therefore seems warranted. The stronger association of *TCF7L2* variants with cystatin C compared with eGFR as markers of kidney function in FHS could be due to better sensitivity of cystatin C to changes in eGFR at higher levels.³⁰

Second, our study could not examine identical variants or identical phenotypes across all three of the studies. Instead, two identical variants were available for both ARIC and FHS with similar, significant associations observed in white ARIC and FHS participants, as well as another two variants in both ARIC and HAPI, one of which was significantly associated with renal phenotypes in both studies. It should be noted, however, that rs7903146 and rs7901695 are in strong LD (pair-wise $r^2 = 0.87$ in white ARIC participants), therefore providing indirect evidence for the expected observation with the variant not genotyped. Despite the differences across the studies, the consistency of the direction and magnitude of effects between the studies lends confidence to the results we observed. Although in FHS and HAPI no information was available for a prospective analysis comparable to ARIC, multivariable adjusted analyses accounted for the same covariates, and the rigorous conduct and covariate collection of the FHS and HAPI studies lends additional confidence to the results. The 95% CI of the odds ratios for eGFR <60 ml/min per 1.73 m² in FHS included the estimates of the HR in ARIC. In fact, the point estimates were almost identical for the two markers typed in both studies, rs1225372 and rs7903146, although the results were not statistically significant in FHS as a result of the smaller sample size. In HAPI, rs7901695 was significantly associated with continuous eGFR, whereas rs11196205 was not. A potential explanation is the limited power in the HAPI sample, allowing only replication of the most significantly associated SNP in ARIC.

Third, our study is further limited because we cannot rule out the possibility that one or more variants in LD with rs7901695 and/or rs7903146 are the causal variant affecting kidney function. Sequencing of all *TCF7L2* exons in a previous study did not reveal any nonsynonymous substitutions.⁸ One previous publication described a SNP upstream of *TCF7L2*, rs10885390, as more strongly associated with diabetes than rs7903146.¹⁵ Because only three HAPI participants had diabetes, we could not assess whether the same variants outside the coding region we observed associated with lower eGFR are also associated with diabetes, which could be addressed in future studies.

Another potential limitation to inferences from association studies is confounding by undetected population stratification. This potential threat is reduced in our study, because we observed the association between *TCF7L2* variants and renal traits in several independent study samples of European ancestry (ARIC white participants, FHS, and HAPI) as well as among black ARIC participants. Moreover, sensitivity analyses among black ARIC participants adjusting for percentage of European ancestry did not change the results we observed. Finally, in previous diabetes studies across multiple populations of European ancestry, associations with variants at the *TCF7L2* locus have not been found to be due to underlying population stratification.¹²

Last, our study is limited by eGFR's being an imperfect measure of kidney function. Serum creatinine levels are strongly influenced by muscle mass, and the estimation of GFR using serum creatinine is most accurate in the low ranges of eGFR.³⁰ In FHS, however, we observed significant associations between *TCF7L2* variants and serum cystatin C, which is not as influenced by muscle mass.

In summary, variants in *TCF7L2* were consistently associated with renal traits in three population-based studies. Risk allele carriers overall as well as among individuals without known diabetes are at increased risk for CKD progression and reduced kidney function. Further studies are needed to replicate our findings and to investigate the underlying mechanisms of this association in more detail.

CONCISE METHODS

Study Populations

ARIC Study.

The ARIC Study is a prospective, population-based, ongoing cohort of 15,792 adults. Study participants aged 45 to 64 yr were recruited from four US communities in 1987 through 1989. Participants underwent four standardized examinations approximately every 3 yr. Since the end of the last examination in 1999, participants are contacted annually by telephone, and hospitalization discharge records from local hospitals and death certificates are obtained. Further details of the study design have been reported previously.²¹ Institutional review boards of participating institutions approved the study protocols. Written informed consent was obtained from participants at each examination. Of 11,478 individuals who self-identified as "white" and 4266 as "black," 669 were excluded from analyses because they did not consent to genetic research or because their genotype at all variants studied was unknown. In addition, participants with baseline serum creatinine values ≥ 1.8 mg/dl (≥ 159 $\mu\text{mol/L}$, women) and ≥ 2.0 mg/dl (≥ 177 $\mu\text{mol/L}$, men; $n = 39$) were excluded from the CKD progression analyses to ensure that the results were not driven by a few individuals with advanced kidney disease.³¹ Individuals with missing covariates were excluded from all regression analyses for a final study sample of 10,786 white and 3599 black participants in these analyses.

Framingham Heart Offspring Study.

The FHS is an observational community-based cohort of cardiovascular disease and its risk factors that was initiated in 1948 with the recruitment of 5209 women and men into the original cohort. In 1971, 5124 children or spouses of the original cohort were enrolled into the offspring cohort; participants were examined approximately every 4 to 8 yr.³² This investigation is composed of offspring participants who attended the seventh examination cycle (1998 through 2001). Of a total of 3537 participants who attended, 2468 had genotype data available for *TCF7L2*, including 2359 for rs12255372 and 2368 for rs7903146. Of these, 2376 had serum creatinine measured, 2330 had cystatin C measured, and 2306 (2323) had a complete covariate profile and made up the final sample size for rs12255372 (rs7903146). Of these participants, 1982 also attended the sixth examination cycle (1995 through 1998) and had a measurement of ACR available. The Framingham offspring study protocol is approved by Boston University Medical Center institutional review board, and all participants signed the informed consent.

HAPI Heart Study.

The HAPI Heart Study consists of 868 related and unrelated Old Order Amish people aged 20 to 80 yr from Lancaster, PA, and is described in detail elsewhere.²³ The Old Order Amish are a genetically homogeneous closed founder population of European descent. This generally healthy population lives an active lifestyle with substantial environmental homogeneity. Most of the HAPI participants were recruited on the basis of their participation in two previous studies.^{33,34} Between 2003 and 2006, HAPI participants underwent two clinic examinations 2 wk apart. For this analysis, data were available for 861 HAPI participants. The protocol was approved by the institutional review board of the University of Maryland, and informed content was obtained.

Assessment of Baseline Characteristics

ARIC participants provided detailed information on demographic, socioeconomic, health behavior, risk factor control, and medical history variables as described previously.³¹ Racial affiliation was self-reported using the terms “black” or “white” used throughout this report. Details regarding the methods of risk factor measurement have been described previously.^{31,35} Participants were considered as having diabetes at baseline and each follow-up visit when they reported a physician diagnosis of diabetes or current intake of diabetes medication or when they had a fasting glucose of ≥ 126 mg/dl (≥ 7 mmol/L) or nonfasting glucose of ≥ 200 mg/dl (≥ 11.1 mmol/L).

In FHS, covariate data were obtained at the seventh examination cycle, and standard definitions were applied. Details regarding the methods of risk factor measurement have been previously described.³⁶ Participants were considered as having diabetes when they were treated with diabetes medication or when they had a fasting glucose of ≥ 126 mg/dl (≥ 7 mmol/L).

In HAPI, diabetes was defined as a physician-documented diagnosis of diabetes or the current use of antidiabetic medication. Additional clinical measurements were obtained including fasting glucose (available for 466 [54%] of HAPI participants) and BP.

Genotyping

Genotyping of *TCF7L2* polymorphisms rs12255372, rs7903146, rs7901695, rs11196205, and rs7895340 in ARIC was performed separately using the TaqMan assay (Applied Biosystems, Foster City, CA) by the ARIC Central DNA Laboratory. The average call rate of the five SNPs was 93.3%. Hardy-Weinberg expectation was met for all five SNPs among white and black individuals overall (Supplemental Table 1). The κ statistic for at least 705 replicates per SNP was ≥ 0.92 for each SNP. Missing genotype data were nondifferential with respect to the development of CKD progression. Results for SNP rs7895340 were not further investigated in this study because of the strong LD between rs11196205 and rs7895340 ($r^2 = 0.97$ and 0.98 in white and black ARIC participants, respectively).

In FHS, rs12255372 and rs7903146 were genotyped by allele-specific multiplex primer extension of PCR-amplified products with detection by matrix-assisted laser desorption ionization-time of flight mass spectroscopy using the Sequenom iPLEX platform as described previously.³⁷ The call rates for these two SNPs were 98.6 and 98.0%, respectively, and concordance was 100% among 254 individuals genotyped twice. Hardy-Weinberg expectation was evaluated among unrelated individuals ($n = 1689$).

HAPI participants were genotyped using the Affymetrix GeneChip Human Mapping 500K Array. The GeneChip Genotyping Analysis Software (GTYPE 4.0) was used for automated genotype calling, and the GTYPE-generated chip files were re-analyzed using the BRLMM genotype calling algorithm (<http://www.affymetrix.com>). A confidence threshold for call quality of 0.5 was used for this analysis. The resulting mean call rate across of the 861 samples was 97.5%. SNPs that were monomorphic, had a minor allele frequency $< 2\%$, or deviated from Hardy-Weinberg equilibrium ($P < 0.001$) were excluded. The average call rate of the 66 SNPs in the *TCF7L2* region was 98.7%.

Definition of the Study Outcome

Study outcomes were categorized as primary and secondary to address the testing of multiple, albeit correlated, outcomes. For the prospective analysis of the primary outcome in ARIC, CKD progression over an average of 13.2 yr of follow-up was defined as (1) an increase in serum creatinine levels ≥ 0.4 mg/dl above baseline or (2) a hospitalization discharge or death coded for chronic renal disease (*International Classification of Disease, Ninth Revision* [ICD-9] codes 581 to 583 or 585 to 588), hypertensive renal disease (ICD-9 code 403), hypertensive heart and renal disease (ICD-9 code 404), unspecified disorder of kidney and ureter (ICD-9 code 593.9), diabetes with renal manifestations (ICD-9 code 250.4), kidney transplant, renal dialysis or adjustment/fitting of catheter (ICD-9 codes V42.0, V45.1, or V56), or either hemodialysis (ICD-9-Clinical Modification 39.95) or peritoneal dialysis procedure codes (ICD-9-Clinical Modification 54.98) without acute renal failure (ICD-9 codes 584, 586, 788.9, and 958.5).³¹ Assessment of short-term variability within ARIC participants revealed that 0.18 mg/dl was the minimal change in creatinine at which 95% confidence existed that a true change had occurred.³⁸ A rise in serum creatinine was defined as a change of at least twice this amount (0.4 mg/dl).

Secondary kidney traits included eGFR at visits 1 and 4 and the urinary ACR at ARIC Study visit 4. Kidney function was assessed by calculation of eGFR using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation³⁹: $eGFR \text{ (ml/min per } 1.73 \text{ m}^2) = 186.3 * \text{serum creatinine (mg/dl)}^{-1.154} * \text{age}^{-0.203} * 0.742 \text{ (if female)} * 1.21 \text{ (if black)}$. Serum creatinine was measured using a modified kinetic Jaffe reaction, and creatinine values were standardized and calibrated using regression to the Cleveland Clinic laboratory as described previously.^{40,41} Albuminuria was measured as described previously.⁴²

In FHS, serum creatinine was measured by the modified Jaffe method and statistically calibrated as described previously^{40,43} before transformation to eGFR *via* the MDRD equation.²⁴ Stage 3 CKD or higher was defined according to Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines as an eGFR <60 ml/min per 1.73 m².²⁴ Cystatin C concentrations were measured on previously frozen samples (Dade Behring Diagnostic, Marburg Germany); the intra- and interassay coefficients of variation were 2.4 and 3.3%, respectively. Urinary albumin concentrations were measured by immunoturbidimetry (Tina-quant Albumin assay; Roche Diagnostics, Indianapolis, IN) and indexed to urinary creatinine.

In HAPI, a blood sample was drawn, centrifuged, and the serum was frozen and shipped to Quest Diagnostics (Horsham, PA), where creatinine was measured using the modified kinetic Jaffe method. eGFR was calculated using the abbreviated MDRD Study equation.³⁹ The urinary ACR was not measured in this study.

Statistical Analysis

In ARIC, all analyses were conducted separately by self-reported race. Differences in baseline characteristics by genotype were assessed using χ^2 tests and ANOVA, as applicable. The primary analysis evaluated associations of *TCF7L2* variants with time to CKD progression. Follow-up time was defined as time from age 45 when participants became eligible for ARIC enrollment with staggered entries at the baseline ARIC visit date, to the ARIC visit date at which the increase in serum creatinine occurred, date of CKD hospitalization discharge or death date, or the earlier of the date of last contact or January 1, 2003. Variants in *TCF7L2* as independent predictors of CKD incidence were evaluated in Cox proportional hazards models. An additive genetic model specified *a priori* was used to evaluate the genetic effect, and previously described risk variants for diabetes were coded as the risk alleles.^{8,14} The proportionality assumption for Cox models of each variant was assessed by inspection of the complementary $\log(-\log[\text{survival function}])$ curves and was found not to be violated in the analysis of Schoenfeld residuals. The fully multivariable-adjusted Cox model used in analyses included adjustment for baseline CKD risk factors determined *a priori* (gender, diabetes, fasting glucose, systolic BP, antihypertensive medication intake, body mass index, smoking) and study center in white participants as covariates. Sensitivity analyses were conducted among black ARIC participants: A set of 1536 ancestry informative markers genotyped among 3965 black ARIC participants was used to derive mean percentage of European ancestry using the software AncestryMap,⁴⁴ and this variable was included as a covariate in the regression analyses. The role of diabetes as an effect modifier was evaluated by stratifying by baseline diabetes

status and evaluating the statistical interaction between SNP and baseline diabetes status. Sensitivity analyses among participants without baseline diabetes consisted of (1) incorporating diabetes and fasting glucose as time-dependent variables into the multivariable adjusted Cox model; (2) treating CKD progression and incident diabetes as competing events, removing individuals from the analysis at the date of incident diabetes when this date was before or the same as the date of CKD progression and accounting for time-dependent fasting glucose; and (3) additionally excluding individuals with baseline fasting glucose ≥ 100 mg/dl from the previous model. Time to incident diabetes was defined as described previously.⁴⁵ A similar analytical approach was used for the secondary kidney phenotypes of eGFR and ACR (general linear regression) and eGFR <60 ml/min per 1.73 m² (logistic regression). For normalization of the distribution and emphasize differences at lower ranges of eGFR, where the estimation of GFR is more accurate,³⁰ the transformation $\ln(eGFR)$ was analyzed. Similarly, $\ln(ACR)$ was analyzed to normalize the distribution. Analyses of eGFR as the outcome included adjustment for age and gender as covariates for two reasons: (1) To account for the relationship of these covariates to the exposure as well as to the outcome, and (2) to account for the relation of age and gender to glomerular filtration beyond their relation to serum creatinine concentrations. All analyses were conducted using Stata 9.2 statistical software (College Station, TX). The LD plots in Figure 1 were generated using the program Haploview 4.0.⁴⁶ Given the *a priori* study hypothesis, the investigation of candidate variants in *TCF7L2*,⁸ and the moderate to high correlation of the SNP (r^2 range 0.44 to 0.87), $P < 0.05$ for the primary outcomes and $P < 0.01$ for secondary outcomes was considered statistically significant in all studies.

In FHS, analyses were performed using the generalized estimating equation procedure to account for the relatedness of observations among family members. Models were run with the following cross-sectional outcomes: (2) Stage 3 CKD or higher as a dichotomous trait, (2) $\ln(eGFR)$, (3) $\ln(\text{cystatin C})$, (4) $\ln(ACR)$. Primary outcomes were eGFR and cystatin C levels; albuminuria was evaluated as a secondary outcome. Analogous to ARIC, data were analyzed assuming an additive model and adjusting for age, gender, body mass index, systolic BP, hypertension treatment, diabetes, fasting glucose, and smoking. Secondary analyses were performed after exclusion of individuals with diabetes at examination cycle 7. All analyses were conducted using SAS 8.1 (SAS Institute, Cary, NC).

In HAPI, association analyses were performed using variance component analyses to account for relatedness in individuals from large Amish pedigrees using the SOLAR software.⁴⁷ Continuous eGFR was analyzed as a quantitative trait using a measured genotype approach, and parameters estimates were obtained by maximum likelihood methods. Likelihood ratio tests were used to determine the significance of associations under an additive model. Analyses were performed using the residuals of eGFR adjusted for age, gender, systolic BP, body mass index, and waist circumference, as well as for fasting glucose in participants with available fasting glucose measurements. Although a similar analytical approach was taken, continuous eGFR without logarithmic transformation was the primary outcome because of the healthy nature of this group of individuals, and the

multivariate analysis did not account for diabetes because only three individuals had diabetes. Because analyses in HAPI were conducted to confirm an *a priori* hypothesis, statistical significance for candidate gene variants was defined by $P < 0.05$ for rs7901695 and rs11196205 in HAPI participants. Because the remaining 64 variants are not independent of each other, a Bonferroni correction was applied for the 14 LD blocks present in the HAPI data (statistical significance at $P < 0.0036$), even though several of these blocks contain a marker or a proxy of a marker previously described as associated with diabetes.

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