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ABSTRACT

Objectives Within the Emirati population, risk factors and genetic predisposition to diabetic kidney disease (DKD) have not yet been investigated. The aim of this research was to determine potential clinical, laboratory and reported genetic loci as risk factors for DKD.

Research design and methods Four hundred and ninety unrelated Emirati nationals with type 2 diabetes mellitus (T2DM) were recruited with and without DKD, and clinical and laboratory data were obtained. Following adjustments for possible confounders, a logistic regression model was developed to test the associations of 63 single nucleotide polymorphisms (SNPs) in 43 genetic loci with DKD (145 patients with DKD and 265 without DKD). Linear regression models, adjusted for age and gender, were then used to study the genetic associations of five renal function traits, including 83 SNPs with albumin-to-creatinine ratio, 92 SNPs with vitamin D (25-OH cholecalciferol), 288 SNPs with estimated glomerular filtration rate (eGFR), 363 SNPs with serum creatinine and 73 SNPs with blood urea.

Results Patients with DKD, as compared with those without the disease, were mostly men (52%vs38% for controls), older (67vs59 years) and had significant rates of hypertension and dyslipidaemia. Furthermore, patients with DKD had T2DM for a longer duration of time (16vs10 years), which in an additive manner was the single factor that significantly contributed to the development of DKD (p=0.02, OR=3.12, 95% CI 1.21 to 8.02). Among the replicated associations of the genetic loci with different renal function traits, the most notable included *SHROOM3* with levels of serum creatinine, eGFR and DKD (P$_{adjusted}$=0.04, OR=1.46); *CASR*, *GC* and *CYP2R1* with vitamin D levels; as well as *WDR72* with serum creatinine and eGFR levels.

Conclusions Associations were found between several genetic loci and risk markers for DKD, which may influence kidney function traits and DKD in a population of Arab ancestry.

Strengths and limitations of this study

► This cross-sectional study to determine the clinical, laboratory and genetic associations of diabetic kidney disease (DKD) and renal function traits in a sample of patients with type 2 diabetes mellitus was the first of its kind in a population of Arab ancestry from the United Arab Emirates.

► A limitation inherent to this study was that the analyses carried out did not include treatment modalities due to patients having multiple conditions and treatments, which made the models largely unstable and difficult to interpret.

► Current analyses of the DKD genetic association had a statistical power of ~57%, suggesting that a larger population sample across the Middle East is required to discover novel clinical and genetic predisposition to DKD in the region with better statistical power.

INTRODUCTION

This overall dramatic worldwide increase in the number of people with diabetes has had a major impact on the increasing incidence and prevalence of diabetic kidney disease (DKD) as one of the most frequent complications of both types of diabetes. Globally, the prevalence of chronic kidney disease (CKD) among adults in the general population is reported to be around 10%.[1] However, 20% of adults with type 2 diabetes mellitus (T2DM) are expected to develop DKD based on estimated glomerular filtration rate (eGFR) measurements ($<60\,\text{mL/min}/1.73\,\text{m}^2$), while the number reaches 30%–50% based on the urinary albumin excretion levels.[1] This considerable variation is due to variances in settings, geographical area and ethnicity.[2] Overall, the risk of DKD in T2DM is approximately 2% per year.[3] In the Arab world, the prevalence of DKD is also highly variable (10.8%–61.2%)% depending on the study design, population, sample selection, race, age, sex, as well as diagnostic criteria among other factors.[4] Meta-analysis has shown that DKD is the leading cause of end-stage renal disease (ESRD) in the Gulf Cooperation Council (GCC) with a prevalence of ~17%.[5] Patients with ESRD have a 20% annual

mortality rate, which is higher than the rate for many solid cancers.[6] In addition to increasing the risk of cardiovascular morbidity and mortality[7 8], DKD is reported to be the single strongest predictor of mortality in patients with diabetes,[9] with a 5-year survival in the range of 30%.[10]

The current trend suggests that the prevalence of DKD will continue to increase worldwide, leading to increased morbidity and mortality and imposing significant socioeconomic burdens on global healthcare systems.[11] A thorough literature search reveals that there remains a wide knowledge gap related to the understanding of risk factors and pathophysiological mechanisms associated with DKD, especially in the GCC and Middle East. Since ESRD can only be treated with highly invasive and costly procedures, such as dialysis or kidney transplantation, better knowledge of genetic, clinical and epidemiological factors associated with DKD is required to allow for timely and more effective treatment options.

In clinical practice, renal function is assessed using a number of tests that are reported to have high heritability rates.[12] This indicates that genetic factors contribute significantly to interindividual variance in kidney function, and hence, to the susceptibility to CKD and related conditions. Thus far, several genetic loci have been linked to DKD, CKD and renal function traits in adults[13–24] as well as children.[25] However, in comparison with other diseases, including T2DM and other cardiometabolic disorders, studies of kidney disease and kidney function traits are largely insufficient and inconclusive. In spite of efforts to describe novel biomarkers for DKD, currently no tested candidates outperform albumin. A recent report by Saulnier et al suggests that three serum biomarkers (midregional-proadrenomedullin, soluble tumour necrosis factor receptor 1 and N-terminal prohormone brain natriuretic peptide) can improve risk prediction of the loss of renal function in patients with T2DM, in addition to the established risk factors for DKD such as age, sex, diabetes duration, HbA1c, blood pressure, baseline eGFR and albumin-to-creatinine ratio.[26] However, issues such as whether the levels of these markers are affected by genetic variations, and whether the encoding genes contribute to DKD development and progress need further investigation.

The United Arab Emirates (UAE) is among the countries with the highest prevalence rates of T2DM, obesity and cardiovascular disease.[27 28] AlSafar and colleagues have recently reported that approximately 80% of patients with T2DM within UAE present with at least one complication associated with T2DM, including kidney disease (approximately 6%).[29] Furthermore, there is increasing evidence suggesting that the genome structure of individuals of Arabic descent is different from individuals from other populations.[30] Despite the high prevalence rate of DKD in the UAE, there have been no investigations up to date of the genetic associations of chronic kidney conditions and kidney functions, particularly as associated with T2DM. Therefore, the current work aimed to investigate the clinical and laboratory variables linked to DKD in a T2DM Emirati population and to study the associations of the reported genetic loci linked to different renal function tests in CKD and DKD.

MATERIALS AND METHODS
Study type and subjects
This work describes a cross-sectional study of Emirati patients from the city of Abu Dhabi. The demographic information and clinical data for the participants are presented in tables 1 and 2. Four hundred and ninety (n=490) patients with T2DM were included in the study,

Table 1 Baseline data of tested traits and tested SNPs

Trait	Mean±SD or quartiles	N of subjects (male/female)	N of SNPs reported*	N of SNPs tested*	Covariates
ACR *(mg/mmol)*	1.05/3.9/12.7†	115 (56/59)	331	83	Age and gender.
Vitamin D *(ng/mL)*	63.7±27.8	328 (146/182)	442	92	Age and gender.
eGFR *(ml/ min/1.73 m²)*	81.5±28.5	395 (172/223)	1478	288	Age and gender.
Serum creatinine *(µmol/L)*	60/74/95.8†	474 (246/228)	1792	363	Age and gender.
Blood urea *(mmol/L)*	6.4±5.4	450 (263/214)	446	73	Age and gender.
Diabetic kidney disease		With: 145. Without: 265.	288	63	Age, gender, hypertension, T2DM duration and eGFR levels.

*Reported means the total number of SNPs found in the search in the literature and tested means the actual number of SNPs found from the reported SNPs and used for the analyses in this study for each corresponding trait.

†For the albumin:creatinine ratio (ACR) and creatinine analyses, summary statistics indicated by 25th/50th/75th quartiles and not as mean±SD because their distributions are skewed.

eGFR, estimated glomerular filtration rate; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus.

Table 2 Demographic, clinical and laboratory characteristics of T2DM patients with or without kidney disease

Type of variables	Variable	DKD	No DKD	P value*
Demographic variables				
	Gender: female†	70 (48.3)	165 (62.3)	0.006
	Age (years)	67.0±10.4	58.6±10.6	<0.0001
Clinical variables				
	Clinical hypertension †	96.6	74.3	<0.0001
	Dyslipidaemia †	95.2	90.9	0.17
	Smoking history †	32.4	24.2	0.07
	Diabetes duration (years)	16.0±9.2	10.1±7.3	<0.0001
	BMI (kg/m^2)	31.3±6.0	32.5±6.3	0.078
Laboratory variables				
Glycaemic indices	Hemoglobin A1c (%)	7.7±1.5	7.8±1.7	0.63
	Fasting plasma glucose (mmol/L)	8.3±3.1	8.9±3.8	0.43
	Random blood glucose (mmol/L)	9.1±3.9	9.5±4.3	0.34
Lipids profile	Total cholesterol (mmol/L)	3.7±1.0	4.0±1.1	0.003
	Triglyceride (mmol/L)	1.5±0.8	1.6±0.8	0.25
	HDL-cholesterol (mmol/L)	1.2±0.6	1.2±0.5	0.45
	LDL-cholesterol (mmol/L)	1.9±0.8	2.1±0.9	0.01
Renal function indices	Albumin:creatinine ratio (mg/mmol) ‡	3.3/10.1/69.6	0.8/2.2/9.0	0.0001
	Vitamin D (nmol/L)	62.9±28.4	65.3±26.8	0.48
	eGFR (mL/min/1.73 m^2)	57.5±27.7	96.1±17.3	<0.0001
	Creatinine (μmol/L) ‡	82/111/146	55/64/76	<0.0001
	Urea (mmol/L)	9.7±8.3	4.7±1.5	<0.0001

*P value for continuous data, calculated using two-sided t-test except for albumin:creatinine ratio and creatinine where it is for Wilcoxon rank-sum (Mann-Whitney) test. P value for percentage data calculated using Pearson χ^2 test with exception of hypertension and dyslipidaemia where Fisher's exact test was used.

† Proportional data shown as number of positive outcome and its percentage. All remaining continuous data shown as mean±SD except albumin:creatinine ratio and creatinine.

‡For the albumin:creatinine ratio and creatinine analyses, summary statistics indicated by 25th/50th/75 quartiles and not as mean±SD because their distributions are highly skewed.

BMI, body mass index; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, high-density lipoprotein; T2DM, type 2 diabetes mellitus.

with 145 diagnosed with DKD. The participants were recruited from Sheikh Khalifa Medical City and Mafraq Hospital, major tertiary hospitals in Abu Dhabi, UAE. All subjects were UAE born and of Arabian descent.

Patient and public involvement

The study was designed because T2DM, along with its multiorgan complications, is a major health challenge in the UAE with increasingly growing public interest. However, patients and the public at large were not involved in defining the research questions, analyses, interpretation or dissemination of the results.

Clinical variables and laboratory data

Various clinical and laboratory measures were collected/assessed during the hospital visits. Blood pressure was taken on two different occasions. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg or if the patients were taking any antihypertensive medications. Dyslipidaemia was

either reported based on clinical records of the participants or diagnosed as previously indicated.[31] The presence of T2DM was confirmed by a qualified physician based on criteria outlined by the WHO.[32] Trained nurses measured the height and weight of each participant using a calibrated wall-mounted stadiometer and a weight scale, respectively. Body mass index was calculated as the weight in kilograms divided by the square of the height in metres (kg/m^2).

Diabetic kidney disease

DKD was defined as either decreased levels of eGFREstimated glomerular filtration rate (<60 mL/min/1.73 m^2) with or without renal damage over a period of at least 3 months,[33] or based on an albumin-to-creatinine ratio ≥30 mg/g, or proteinuria >500 mg over a 24-hour period in the setting of T2DM and/or abnormalities, as assessed by imaging or histology.[34] eGFR was calculated according to CKD-EPI Creatinine Equation.[35]

Accordingly, 145 patients with TD2M were identified with DKD, while 265 were identified as disease free (tables 1 and 2). The remaining 80 patients could not be classified with or without DKD at the time of the study and were excluded from subsequent analyses.

Selection of SNPs

Genetic investigation in this study followed the candidate gene approach.[36] The SNPs tested for each trait are summarised in table 1. These SNPs were selected from a recent Genome Wide Association Study (GWAS) that was intended to determine the genetic associations of T2DM in the UAE population towards establishing the Emirates Family Registry for T2DM.[27] The GWAS was performed for 490 samples with T2DM and 450 healthy controls using the Infinium Omni5ExomeHuman chip (Illumina, San Diego, California, USA). To select the SNPs associated with the kidney function traits included in the study (blood urea, serum creatinine, eGFR values, albumin-to-creatinine ratio and vitamin D levels) as well as DKD, various search engines and data bases including PubMed, Google Scholar, the GWAS catalogue (https://www.ebi.ac.uk/gwas/home), the Phenolyzer database (http://phenolyzer.wglab.org/), the infinome genome interpretation platform (https://www.infino.me/) and the GWAS Central database (http://www.gwascentral.org/) were consulted.

Our search strategy consisted of identifying reported SNPs that cleared the GWAS significance level and were found in our GWAS data. If the original signal SNP was missing from the GWAS data, we searched for a possible proxy SNP using the concept of linkage disequilibrium (LD). This typically indicates a non-random association of alleles at different genetic loci in a given population and their tendency to be inherited as a block (mathematical values r^2 and D'>0.8 indicates high LD). Proxy SNPs were selected using the SNAP database for SNP Annotation and Proxy Search (http://archive.broadinstitute.org/mpg/snap/ldsearch.php).

All SNPs located within and flanking genes that have been previously reported in association with CKD and DKD were included. In total, 43 genetic loci were identified as linked to CKD, DKD or a decline in renal function. Specifically, the gene loci comprised: *ACACB, ACE, ACTN4, ADIPOQ, ADM, AFF3, AGTR1, APOL1, CARS-CNDP1, CNDP2, CPS1, CPVL, CPVL, CHN2, CYBA-ELMO1, ENPP1, ERBB4, FABP2, FRMD3, GLUT1, IRS2, MYO16, LIMK2, MCTP2, MYO16, MYH9, NCALD, NCK1, NOS3, NPHS1, NPHS2, NPPB, PLCE1, PPARγ2, PVT1, RAGE, RGMA, RPS12, SFI1, SHROOM3, TMEM22, TNFRSF1A* and *TRPC6.*

Statistical modelling and analyses

Continuous variables were presented as mean±SD or lower/median and upper quartiles where the distributions were highly skewed. Vitamin D and eGFR levels were normally distributed. However, urea levels, creatinine levels and albumin-to-creatinine ratio data were converted to normal distributions using natural log transformation. The associations of trait values or their natural logs (if transformed) were tested with SNPs using linear regression models, which included age and gender as covariates using PLINK software V.1.07 (http://zzz.bwh.harvard.edu/plink/). The same software was used for counting allele frequencies and testing the quality control (QC) variables. Any SNPs with minor allele frequency (MAF) <0.05, and >5% missing genotype rate, or those that failed the Hardy-Weinberg equilibrium (HWE) test at the 0.001 were excluded. HWE is considered as an important QC test for genetic association studies and assumes that allele and genotype frequencies can be estimated. If the frequencies of the measured genotypes significantly differed from the HWE assumptions, genotyping errors among other possible factors, such as ethnic diversity and high levels of consanguinity in the population, are indicated leading to excluding the SNPs from further analyses. Association with p<0.05 were reported, indicating the replication of previously reported associations.

Statistical analyses for all clinical and laboratory variables were performed using Stata software V.14 . For continuous data, statistical differences were assessed using two-sided t-tests for normally distributed data or the Wilcoxon rank-sum (Mann-Whitney) test for highly skewed data. The Pearson χ^2 test was used for percentage data as well as the Fisher's exact test when the expected frequencies were less than 5. A p value<0.05 was considered as significant. PLINK software was also used for testing the associations between the SNPs and DKD using a case–control logistic regression model, which included age, gender, hypertension status, T2DM duration and eGFR levels as covariates (table 1). The results are presented as p values ($P_{adjusted}$<0.05) and ORs with corresponding 95% CIs. The same approach (patients with DKD vs patients without DKD) was adopted to test the associations between the possible risk factors and the development of the DKD (table 3). However, the logistic model, which included all the associated factors listed in table 4 was validated by the Hosmer-Lemeshow goodness of fit test (p=0.11) to allow for the inclusion of several covariates. Analyses between SNPs and renal function traits were conducted in PLINK using linear regression models that included age and gender as covariates. The results were presented as beta coefficients (regression coefficients for the linear regression model, calculated based on the minor allele), standard errors and p values.

As this was a replication study, we reported all p values <0.05, suggesting possible replications. However, using a Bonferroni correction for multiple testing, the p values of some of the models with statistically significant associations included p<0.00079 for the DKD associations, p<0.0006 for the ACR, p<0.0005 for the vitamin D, p<0.00017 for the eGFR, p<0.00019 for the creatinine and p<0.00068 for associations with urea.

Table 3 Risk factors for the development of kidney disease in patients with T2DM from the UAE

Covariate	OR	95% CI	P values
Age	0.99	0.96 to 1.04	0.80
Gender	0.90	0.34 to 2.36	0.83
BMI	1.02	0.97 to 1.08	0.49
Hypertension	2.00	0.60 to 6.74	0.26
HbA1c	0.86	0.69 to 1.07	0.19
Cholesterol	0.59	0.19 to 1.76	0.34
Triglyceride	1.09	0.62 to 1.94	0.76
HDL-cholesterol	1.25	0.64 to 2.45	0.52
LDL-cholesterol	1.38	0.42 to 4.57	0.60
Smoking history	0.80	0.34 to 1.87	0.61
Creatinine	1.03	1.00 to 1.06	**0.03**
Urea	1.13	0.92 to 1.38	0.26
eGFR	0.97	0.94 to 1.00	0.09
Diabetes duration (years)*			
5–10	0.75	0.26 to 2.16	0.59
11–15	1.49	0.56 to 3.91	0.42
16–20	3.35	1.22 to 9.23	**0.02**
>20	3.12	1.21 to 8.02	**0.02**

*Diabetes duration reference is duration ≤5 years
BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; T2DM, type 2 diabetes mellitus; UAE, United Arab Emirates.

Statistical power considerations

The current study had a power ~57%. This is based on the current sample size (145 with DKD vs 265 without DKD), prevalence of DKD ~20% (depending on reference #2), genotype risk (OR)=1.5, significance level of 0.0008 (following the correction of multiple testing using the Bonferroni correction), disease allele frequency=0.5 and multiplicative model. Calculations were verified using the Genetic Association Study Power Calculator (http://csg.sph.umich.edu//abecasis/cats/gas_power_calculator/index.html).

Ethical considerations

Each patient agreed to take part in this study and provided an informed signed consent after a brief session to explain the aims and methods. The study conformed to the ethical principles outlined in the Declaration of Helsinki.

RESULTS

Baseline data of kidney function associated traits and SNPs selection

Of the 490 patients with T2DM that were recruited for this study, 115 patients were tested for genetic associations with the albumin-to-creatinine ratio, 328 for vitamin D levels (measured as 25-OH cholecalciferol), 395 for eGFR levels, 474 for serum creatinine levels and 450 for blood urea levels. Among the 490 patients, 410 had clear classifications for the diagnosis of kidney disease (145 patients with DKD and 265 without DKD) according to the adopted diagnostic criteria (references #33 and #34) and/or patient medical records. The possible covariates that may affect the genetic associations for trait analyses are summarised in table 1.

Clinical and laboratory characteristics of patients with and without DKD

Both patient groups (with or without kidney disease) had poor glycaemic control with blood glucose levels above 8 mmol/L. A comparison of patients with DKD to those without DKD indicated that the majority of patients with DKD were men (52% vs 38% for controls), older in age (67 vs 59 years), had significant rates of comorbidities, such as hypertension and dyslipidaemia, and had a longer T2DM duration (16 vs 10 years). A clear decline in renal function indices was also observed in patients with DKD compared with those without DKD, as indicated by the significantly higher rates of ACR, urea and creatinine, and significantly lower eGFR (table 2). However, patients with DKD tended to have lower low-density lipoprotein-cholesterol results as compared with those without DKD, which suggests that these patients were more likely to have received intensive statin therapy (table 2).

Factors associated with developing DKD in Emirati patients with T2DM

Table 3 shows that T2DM duration was the single factor that significantly contributed to the development of DKD. Increased risk for DKD was significantly associated with the increasing duration of T2DM, cumulatively, up to 20 years of duration. At a T2DM duration ≥20 years, the DKD risk stabilised at approximately 3.12 times higher as compared with patients with duration ≤5 years (p=0.02, 95% CI 1.21 to 8.02). Although the levels of serum creatinine indicated a significant difference between no DKD and DKD patients (p=0.03), the OR of 1.03 showed no increased risk (table 3).

Genetic associations of renal function associated traits in Emirati patients

The results of genetic associations for each tested renal function trait are shown in table 4. In summary, no association passed the Bonferroni correction for multiple testing. However, we report here the most suggestive associations which point to replications of previous reports.

For *blood urea*, the best observed association was with rs11868441 in breast carcinoma-amplified sequence 3 (*BCAS3*) (effect size: −0.038 log per allele A, p=0.014), followed by multiple SNPs in the R-Spondin 3 (*RSPO3*) gene.

For *serum creatinine*, two SNPs (rs6999484 and rs1705699) in the intergenic region between *STC1* and *ADAM28* showed the best associations with similar effect sizes (0.03 log per one copy of the corresponding minor

Table 4 Results of genetic association analyses of different renal function indices

SNP	Chr: BP	Gene	A1/A2*	MAF_%	Beta†	SE	P value
Blood urea							
rs11868441	**17:** 59239221	BCAS3	A/G	30.1	−0.038	0.015	0.014
rs1892172	**6:** 127476516	RSPO3	T/C	46.9	0.031	0.014	0.028
rs4644087	**6:** 127481154	RSPO3	C/A	46.8	0.030	0.014	0.03
rs4382293	**6:** 127475433	RSPO3	G/A	47.0	0.030	0.014	0.03
rs2489629	**6:** 127476717	RSPO3	G/A	44.9	−0.029	0.014	0.039
Serum creatinine							
rs6999484	**8:** 23728271	STC1-ADAM28	A/G	23.8	0.033	0.011	0.003
rs1705699	**8:** 23781453	STC1-ADAM28	G/A	24.2	0.031	0.011	0.005
rs2828785	**21:** 25437505	-	A/G	19.9	−0.030	0.011	0.008
rs11227279	**11:** 65495211	KRT8P26-AP5B1	A/G	28.4	−0.024	0.010	0.019
rs7785065	**7:** 32915204	KBTBD2	A/C	43.5	0.022	0.010	0.022
rs4859682	**4:** 77410318	**SHROOM3**	A/C	25.7	0.021	0.010	0.034
rs1031755	**15:** 53951435	WDR72	C/A	11.7	−0.029	0.014	0.038
rs7740534	**6:** 25077179	-	C/A	9.6	−0.032	0.015	0.042
Estimated glomerular filtration rate							
rs2168785	**17:** 37407135	MED1	G/A	28.2	−4.299	1.754	0.015
rs12452509	**17:** 37574722	MED1	G/A	28.1	−4.232	1.757	0.017
rs4776168	**15:** 53936907	WDR72	G/A	10.8	5.828	2.699	0.031
rs10518733	**15:** 53940307	WDR72	C/A	10.8	5.828	2.699	0.031
rs7541937	**1:** 35341982	DLGAP3	C/A	44.0	3.590	1.693	0.035
rs10032549	**4:** 77398015	**SHROOM3**	G/A	32.2	−3.655	1.750	0.037
rs2484639	**1:** 243462367	SDCCAG8	A/G	43.5	3.378	1.656	0.042
Vitamin D(25-OH cholecalciferol)							
rs1801725	**3:** 122003757	CASR	A/C	22.6	−6.923	2.584	0.008
rs1155563	**4:** 72643488	GC	G/A	18.9	−6.951	2.608	0.008
rs12794714	**11:** 14913575	CYP2R1	A/G	41.5	−5.110	2.099	0.015
rs10500804	**11:** 14910273	CYP2R1	C/A	42.2	−5.004	2.060	0.016
Albumin:creatinine ratio							
rs4528660	**18:** 3043516	LPIN2-MYOM1	G/A	17.8	−0.323	0.144	0.027

*A1/A2: minor to major alleles.
†Beta: regression coefficient for the linear regression model, calculated based on A1 (the minor allele).
BP, base pair position; Chr, chromosome; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

allele), followed by SNP rs2828785 (p=0.008, effect size=−0.030 log per one copy of allele A), which is located in the non-coding gene area on chromosome 21. In addition, multiple genetic areas were also indicated, although with less significant associations.

eGFR levels were significantly associated with SNPs within the *MED1* gene, rs2168785 with effect size of −4.299 per allele G and rs12452509 with effect size of −4.232 per allele G and p=0.015 and 0.017, respectively. In addition, the SNPs in two genetic regions, *WDR72* and SHROOM3, which are associated with serum creatinine levels, were also associated with eGFR levels, indicating a strong link to renal function.

Vitamin D levels were associated with three genetic regions including rs1801725 in *CARS* (effect size: −6.923 per allele A, p=0.0078), rs1155563 in *GC* (effect size: −6.951, p=0.0081) and two SNPs, rs12794714 and

rs10500804, in cytochrome P450 family 2 subfamily R member 1 (*CYP2R1*) with effect sizes of approximately −5.0 and p values of 0.015 and 0.016, respectively.

One SNP, rs4528660, which is located in the intergenic region between *LPIN2* and *MYOM1* (effect size: −0.323 log per allele A, p=0.027) was strongly associated with the *albumin-to-creatinine ratio*.

These data demonstrated that renal function traits are linked to several loci in the UAE population and that some loci (eg, *WDR27* and *SHROOM3*) are linked to more than one trait.

Genetic associations of DKD in Emirati patients

Sixty-three SNPs in 43 genetic loci, which have previously been linked to CKD or DKD, were included in our genetic analyses of DKD (145 patients with DKD versus 265 without DKD). A logistic regression model including

Table 5 Association between SNPs linked to CKD and DKD patients from the UAE

SNP	Chr: BP	Gene	A1/A2*	MAF		P$_{unadjusted}$	P$_{adjusted}$	OR (95% CI)
				DKD (n=145)	No DKD (n=265)			
rs7422339	2: 211540507	CPS1	A/C	35.8%	27.9%	0.019	0.20	1.25 (0.89 to 1.75)
rs4859682	4: 77410318	**SHROOM3**	A/C	29.0%	21.9%	0.024	**0.04**	1.46 (1.01 to 2.10)

*A1/A2: minor to major alleles.

BP, base pair position; Chr, chromosome; CKD, chronic kidney disease; CPS1, carbamoyl-phosphate synthase; DKD, diabetic kidney disease; MAF, minor allele frequency; SHROOM3, shroom family member 3; SNPs, single nucleotide polymorphisms; UAE, United Arab Emirates.

five possible covariates that may affect the development of kidney disease was applied (table 1). As depicted in table 5, the unadjusted analyses indicates two associations in *CPS1* (rs7422339, p=0.019) and *SHROOM3* (rs4859682, p=0.024), respectively. Following the adjustment for possible covariates, only the *SHROOM3* rs4859682 remained significant (p=0.04, OR=1.46). This confirms that *SHROOM3* is a string risk locus for DKD, considering similar associations with serum creatinine and eGFR levels.

DISCUSSION

A combination of environmental and clinical factors in genetically predisposed individuals have been suggested to be involved in DKD, including persistent hyperglycaemia, arterial hypertension and/or dyslipidaemia.[37] In addition, familial aggregation of nephropathy in T2DM has been reported in several populations.[38] Therefore, understanding the complex multifactorial interactions between genetic, clinical and traditional kidney disease risk factors can provide insight into novel drugs and treatment strategies for DKD towards reducing the likelihood of developing ESRD. In this study, we investigated whether the genetic markers that correspond to DKD and renal function traits reported for different populations are similar to the Arab population.

The current Emirati population sample indicated that most patients with T2DM who developed DKD were males, about 10 years older, had more frequent comorbidities, specifically hypertension and showed a marked decline in their renal function profiles, as compared with those who did not develop the disease. However, patients with T2DM who did not develop DKD still had higher rates of comorbidities and poor diabetic control, in agreement with our previous results.[29] We also found that the duration of diabetes was the single factor that significantly contributed to the development of kidney disease. Specifically, the risk of developing DKD became significant when the duration of T2DM reached the 15-year mark. This is in alignment with previous reports, which suggest that patients with T2DM who do not develop signs of kidney disease by 15 years' duration of diabetes seem to be protected, most likely due to genetic factors.[39]

The most notable finding of this study was the association of shroom family member 3 (*SHROOM3*) with serum creatinine, eGFR and DKD. The minor allele for the SNP rs4859682 (A) was observed to increase the serum creatinine (0.021 log increase per one copy) and also to increase the risk for DKD (OR=1.46). *SHROOM3* was first reported to be associated with eGFR levels in patients with DKD,[13] then with serum creatinine[40] and serum magnesium levels.[41] This association was further replicated in different ethnicities.[19 42] The *SHROOM3* gene product is expressed in the human kidney and is reported to play an important role in epithelial cell shape regulation,[43] as well as the maintenance of the glomerular filtration barrier integrity.[44] Defective Shroom3 protein leads to decreased actin organisation and affects the mechanical characteristics and integrity of the glomerular podocyte resulting in thinning of the glomerular filtration membrane.[44] Additionally, *Shroom3* heterozygous (*Shroom3*$^{Gt/+}$) mice showed developmental irregularities that manifested as adult-onset glomerulosclerosis and proteinuria.[45] Furthermore, genetic variants (such as the intronic variant rs17319721) were found to contribute to kidney allograft injury and the development of fibrosis through a mechanism involving transforming growth factor beta (TGF-β) signalling.[46] Although the variant rs17319721 was not found in our dataset, it is highly linked to the SNP rs4859682 (r^2=0.85, D'=1), which was reported in this study to increase the risk for DKD and affect the levels of serum creatinine. Overall, this suggests that *SHROOM3* may be considered as a multiethnic risk gene for DKD and associated kidney function traits in various populations, including the Arabs who inhabit the UAE.

Similarly, the two loci, WD repeat domain 72 (*WDR72*), associated with eGFR and serum creatinine levels), and *BCAS3*, associated with blood urea levels), are also well-known transethnic renal function traits loci.[47] *WDR72*, in particular, has been well studied in association with kidney function traits and pathologies. For instance, *WDR72* has been reported to be associated with creatinine production or secretion,[48] as well as signal transduction, cell cycle regulation and vesicular trafficking that affects podocyte activity, reduced eGFR and progression of CKD.[49] In addition, *RSPO3* has been previously reported to be associated with blood urea nitrogen concentration, in line with the results of the current study.[42] The association of rs4528660 near *MYOM1* (Myomesin 1) with the albumin-to-creatinine ratio is also in agreement with previous work, which links this locus to albuminuria in patients with diabetes.[50] The current analyses also replicated the associations of calcium-sensing receptor (*CASR*), group-specific component (*GC*) (vitamin D-binding protein, also known

as GC-globulin) and *CYP2R1* with levels of vitamin D.[51] These genes encode proteins that are involved in vitamin D function, including activation by hydroxylation (CYP2R1), transportation (GC) and serum calcium level sensing (CASR).[51] They have also been associated with calcium–vitamin D physiology and pathology, such as serum calcium levels, familial hypocalciuric hypercalcaemia, tertiary hyperparathyroidism and vitamin D deficiency presenting as rickets (see OMIM entries: CARS: 601199; CYP2R1: 608713; and GC: 139200). For instance, CASR protein is expressed in the kidney among other tissue and regulates ion metabolism including calcium and magnesium. Mutations in *CASR* lead to abnormalities in the regulation of the parathyroid gland and renal function causing hypercalcaemia and increased blood pressure, which in turn may affect kidney function.[52] Similarly, GC proteins bind actin and work as actin scavengers (as such GC may play a role in podocyte integrity), as well as a binding site for vitamin D. The GC protein is the precursor to the Gc-macrophage activating factor, a macrophage activator and suggests, together with vitamin D, that GC has an important role in the immune function and pathogenesis of CKD.[53] Vitamin D is hydroxylated at the C25 position by specific hydroxylase coded by the *CYP2R1* gene to 25-hydroxyvitamin D, which is the main circulating form of vitamin D. The low levels of vitamin D observed in CKD, due to reduced CYP2R1 production by the liver or due to a mutation in the gene, disturb calcium balance and lead to hyperparathyroidism. Conversely, the loss of renal protection caused by vitamin D and the increase in the renin–angiotensin pathway leads to hypertension that further advances kidney disease.[54] Furthermore, these genes have recently been shown to influence the outcome of vitamin D_3 supplementation which, in the Arab context, is an important finding of our study.[55]

In summary, this work presents the first study to investigate the clinical and genetic factors influencing kidney function traits and DKD in a population of Arab ancestry. The results demonstrate that the duration of T2DM is the single most important risk factor for DKD development in patients with T2DM in the UAE. Our study highlights that several genetic loci, which have been previously linked to renal function associated traits, are shared between diverse ethnic groups. As such, we have replicated previous findings of the association of *SHROOM3* with DKD. The logistic analyses performed here did not include treatment modalities since most of the patients had multiple conditions and underwent multiple treatments, which makes logistic regression largely unstable and difficult to interpret. A larger population sample across the Middle East is now being considered to confirm the extent of the shared genetic predisposition reported in the current study. Considering the high prevalence of T2DM in this population and the recent evidence of genomic structure variations among different ethnic groups, more genetic-driven population studies are warranted towards effective genetically guided personalised medicine.

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