

Evaluation of Genetic Association and Expression Reduction of TRPC1 in the Development of Diabetic Nephropathy

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Key Words

TRPC1 gene · Single-nucleotide polymorphism · Diabetic nephropathy · End-stage renal disease · Diabetes types 1 and 2

Abstract

Background/Aims: The TRPC1 gene on chromosome 3q22–24 resides within the linkage region for diabetic nephropathy (DN) in type 1 (T1D) and type 2 diabetes mellitus (T2D). A recent study has demonstrated that TRPC1 expression is reduced in the kidney of diabetic ZDF- and STZ-treated rats. The present study aimed to evaluate the genetic and functional role of TRPC1 in the development of DN. **Methods:** Genetic association study was performed with two independent cohorts, including 1,177 T1D European Americans with or without DN from GoKinD population and 850 African-American subjects with T2D-associated end-stage renal disease (ESRD), or with hypertensive (non-diabetic) ESRD, and nondiabetic controls. Seven tag SNP markers derived from HapMap data (phase II) were genotyped. TRPC1 gene ex-

pression was examined using real time RT-PCR. **Results:** No significant association of TRPC1 DNA polymorphisms with DN or ESRD was found in GoKinD and African-American populations. TRPC1 gene mRNA expression in kidney was found to be trendily reduced in 12-week and significantly in 26-week-old db/db mice. **Conclusions:** TRPC1 genetic polymorphism may not fundamentally contribute to the development of DN, while reduction of the gene expression in kidney may be a late phenomenon of DN as seen in diabetic animal models.

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Introduction

Diabetic nephropathy develops in approximately 40% of individuals with type 1 and type 2 diabetes, and is the most common cause of end-stage renal disease (ESRD) in the western world [1]. The prevalence and course of diabetic nephropathy are similar in patients with type 1 and type 2 diabetes, when they were matched for disease du-

ration [2]. The development of diabetic nephropathy is likely impacted by both environmental and genetic factors. Genetic analysis of positional candidate genes may provide useful information for understanding the pathogenesis of this complex disease [3, 4].

Several genome-wide scans and linkage analyses indicate that a region of chromosome 3q is linked to diabetic nephropathy [5–10]. In recent years, we have focused on searching the susceptibility genes of diabetic nephropathy in this chromosomal region. Glomerulosclerosis is the major morphological change in patients with diabetic nephropathy [11]. Patients with type 1 and type 2 diabetes appear to progress through similar stages of nephropathy, while the routes they take may differ [12]. Glomerular mesangial cells (MC), located adjacent to glomerular capillaries, appear to play important roles in physiologic regulation of glomerular hemodynamics. The transient receptor potential channel 1 (TRPC1) molecule has been identified in both glomeruli and glomerular MC [13–16]. Importantly, the TRPC1 gene is located in chromosome 3q22–24 within the linkage region to diabetic nephropathy. Thus, TRPC1 represents a strong positional and biological candidate for diabetic nephropathy.

A recent study has demonstrated that significant reduction in TRPC1 gene expression in kidney and liver in diabetic ZDF- and STZ-treated rats appears to result from dysfunction in HNF4 α . Kidney tissue TRPC1 expression at the protein level is also decreased in patients with diabetic nephropathy. It has been proposed that dysregulation of HNF4 α and TRPC1 may underlie susceptibility to diabetic nephropathy [17]. To evaluate the potential role of TRPC1 gene in the development of diabetic nephropathy, in the present study, we have carried out genetic association analyses in two independent cohorts. The first cohort is a collection of 1,286 type 1 diabetes patients with and without diabetic nephropathy from the Genetics of Kidney Diseases in Diabetes (GoKinD) [18]. The second cohort consists of 850 African-American individuals collected from Wake Forest University School of Medicine [19]. They are divided into three groups, i.e. patients with type 2 diabetes-associated ESRD, or with hypertension-associated (nondiabetic) ESRD and nondiabetic subjects (controls). We have also examined TRPC1 gene expression with 12- and 26-week-old db/db mice in order to understand whether the reduction of this gene expression is primary or subsequent defect in the development of diabetic nephropathy.

Material and Methods

Genetic Association Study

Subjects

Clinical material in this study was composed of two independent cohorts. The first cohort consisted of 1,286 type 1 diabetes patients (female 683/male 603) with and without diabetic nephropathy from GoKinD study collected by the Juvenile Diabetes Research Foundation in collaboration with the Joslin Diabetes Centre, George Washington University, and the United States Centres for Diabetes Control and Prevention [18]. Among the GoKinD subjects, 1,177 (91.5%) (622 F/555 M) were of European descent, 109 (8.5%) were African Americans (n = 42), Hispanic Americans (n = 38), Native Indians (n = 7), Asian Americans (n = 4) and 18 unknown. The patients with type 1 diabetes were diagnosed before 31 years of age. Treatment with insulin was instituted within 1 year of diagnosis, and had been uninterrupted since then. Presence of diabetic nephropathy (case) was defined either by persistent proteinuria (urinary albumin excretion rate >300 mg/24 h) in 2 of 3 consecutive measurements (at least 1 month apart), or ESRD (not due to condition other than diabetes). Absence of diabetic nephropathy (control) was considered as persistent normalalbuminuria (<30 mg/24 h) despite duration of type 1 diabetes for at least 15 years. The clinical characteristics of the GoKinD subjects are presented in table 1a.

The second cohort included 850 African Americans (434 F/416 M) collected by the Wake Forest University School of Medicine. This cohort was divided into three groups: (1) 284 (176 F/108 M) with type 2 diabetes-associated ESRD, (2) 284 (158 F/126 M) with hypertension-associated (nondiabetic) ESRD, and (3) 282 (132 F/150 M) nondiabetic controls. In Wake Forest samples, type 2 diabetes-associated and hypertension-associated ESRD were diagnosed as previously reported [19]. Clinical parameters in each group are summarized in table 1b.

Sample collections in both GoKinD and Wake Forest studies were approved by the local ethics committees. Data and material transfer agreements were completed prior to the study, respectively.

Genotyping of SNPs

Examined SNPs (rs953239, rs7638459, rs17624218, rs7621642, rs2033912, rs3821647, rs7610200) were tag markers in the TRPC1 gene selected from the International HapMap Project database (HapMap data release 21a/phaseII Jan 07) [20] and based upon information in the dbSNP database [21]. Haploview (ver 4.0) was employed to visualize linkage disequilibrium (LD) and haplotype block structures between these loci ($r^2 > 0.8$, LOD threshold 3.0). Validation of the polymorphisms in 32 subjects randomly selected from GoKinD was performed and at least 0.016 (1/64 chromosomes) of MAF in the polymorphisms was detected. Assays used for allelic discrimination in genotyping experiments were purchased from Applied Biosystems and performed with TaqMan technique (ABI 7300, Foster City, Calif., USA). For quality control, the subjects were distributed randomly across plates with equal numbers of cases and controls on each PCR plate. Negative controls (Universal-mixture blanks) were included on each plate. Genotyping experiments in approximately 20% of samples were performed in duplicate. Successful genotype calls were ~97% and duplication accuracy ~99%.

Table 1. Clinical characteristics**a** Clinical characteristics of the GoKinD cohort

	T1D with DN			T1D without DN		
	all	female	male	all	female	male
Number	578	265	313	599	357	242
Age, years	44 ± 6	44 ± 7	45 ± 6	40 ± 8	40 ± 9	40 ± 8
Duration, years	32 ± 8	32 ± 8	32 ± 8	26 ± 8	26 ± 8	26 ± 8
HbA1c, %	7.4 ± 1.9	7.4 ± 2.1	7.4 ± 1.7	7.5 ± 1.1	7.5 ± 1.1	7.4 ± 1.1
BMI	25.7 ± 5.3	25.2 ± 5.7	26.1 ± 4.9	26.0 ± 4.4	25.7 ± 4.7	26.6 ± 3.7
Creatinine, mg/dl	2.1 ± 1.9	1.9 ± 1.6	2.3 ± 2.0	0.9 ± 0.2	0.8 ± 0.1	1.0 ± 0.1
Cystatin, mg/l	2.2 ± 1.7	2.2 ± 1.6	2.4 ± 1.7	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Cholesterol, mg/dl	186.3 ± 45.9	190.3 ± 47.3	183.0 ± 44.6	185.0 ± 31.3	188.7 ± 30.9	179.7 ± 31.2
HDL, mg/dl	53.6 ± 17.3	59.3 ± 18.2	48.9 ± 15.1	59.1 ± 15.9	64.8 ± 15.5	51.0 ± 12.4
Systolic BP, mm Hg	131 ± 19	130 ± 20	133 ± 18	118 ± 12	116 ± 12	122 ± 12
Diastolic BP, mm Hg	74 ± 11	72 ± 11	75 ± 11	71 ± 8	70 ± 7	74 ± 8

b Clinical characteristics of the African-American cohort

	T2D-ESRD			HTN-ESRD			ND controls		
	all	female	male	all	female	male	all	female	male
Number	284	176	108	284	126	158	282	132	150
Age, years	64 ± 10	65 ± 11	62 ± 9	54 ± 15	56 ± 16	52 ± 14	50 ± 10	50 ± 10	49 ± 10
Age of onset ESRD	60 ± 10	62 ± 11	58 ± 10	48 ± 16	50 ± 17	47 ± 15	–	–	–
Age of onset HTN	–	–	–	33 ± 12	31 ± 12	35 ± 11	–	–	–
Age of onset T2D	43 ± 11	43 ± 11	43 ± 11	–	–	–	–	–	–
BMI	29.3 ± 7.3	29.9 ± 7.8	28.5 ± 6.3	26.5 ± 7.3	27.5 ± 8.4	25.8 ± 6.2	29.3 ± 6.8	31.2 ± 7.6	27.6 ± 5.5

All data are means ± SD.

T2D = Type 2 diabetes; ESRD = end-stage renal disease; HTN = hypertension; ND = nondiabetic controls.

Gene Expression Study

Animals

A total of 29 male homozygous diabetic (db/db) mice and nondiabetic control littermates of the C57BL/KsJ strain were obtained from colonies at Karolinska University Hospital, Stockholm. They were housed 4 per cage in a 12-/12-hour light/dark cycle, water and standard diet was provided ad libitum. Before death, the mice were tested for blood glucose and body weight. The mean blood glucose levels were 21 ± 6 mmol in db/db and 8 ± 1 mmol in db/m mice. All procedures were approved by the North Stockholm's Ethical Committee for Care and Use of Laboratory Animals.

RNA Extraction and Real-Time RT-PCR

Kidney tissues from 12- and 26-week-old db/db and control mice were harvested, and quickly submerged in RNA/later solution (Ambion, Austin, Tex., USA). Tissue samples were placed at 4°C overnight to allow the solution to permeate the cells. The stabilized RNAs were then stored at –80°C until use. Tissue homogenization was prepared according to a protocol developed in our laboratory. Briefly, 30 mg of whole kidney tissue was placed into

a 2-ml microcentrifuge tube containing 0.5 ml of 1-mm diameter glass beads and 0.6 ml of RLT buffer (Qiagen, Hilden, Germany). The tube was then placed in the Mini Beadbeater (BioSpec, Bartlesville, Okla., USA) and shaken twice at 5,000 rpm for 60 s. The supernatant were collected. Total RNAs were extracted by using an RNeasy Mini Kit (Qiagen, Hilden, Germany). The integrity of the RNA was assessed by electrophoresis of 10 µl of each sample through a 1.2% agarose gel. The RNA concentration was determined spectrophotometrically by measuring the A260/A280 ratio. First-strand cDNA was synthesized from 1 µg total RNA from the kidney of each mouse employing random hexamer oligonucleotide in a final volume of 20 µl using TaqMan reverse transcription reagents (Applied Biosystems), according to the manufacturer's protocol. To perform the real time RT-PCR, a specific TaqMan assay (ID No. Mm00441975_m1) was designed (Applied Biosystems) according to mRNA sequence of the TRPC1 gene in mouse (NM_011643.2). The probe was labeled with 6'-carboxy-fluorescein (FAM) as reporter dye and TAMRA as quencher dye. Amplification was performed using the 5'-nuclease TaqMan method with a two-step PCR protocol (95°C for 10 min, followed by 36 cycles of 95°C for 15 s and 60°C for 1 min) in an

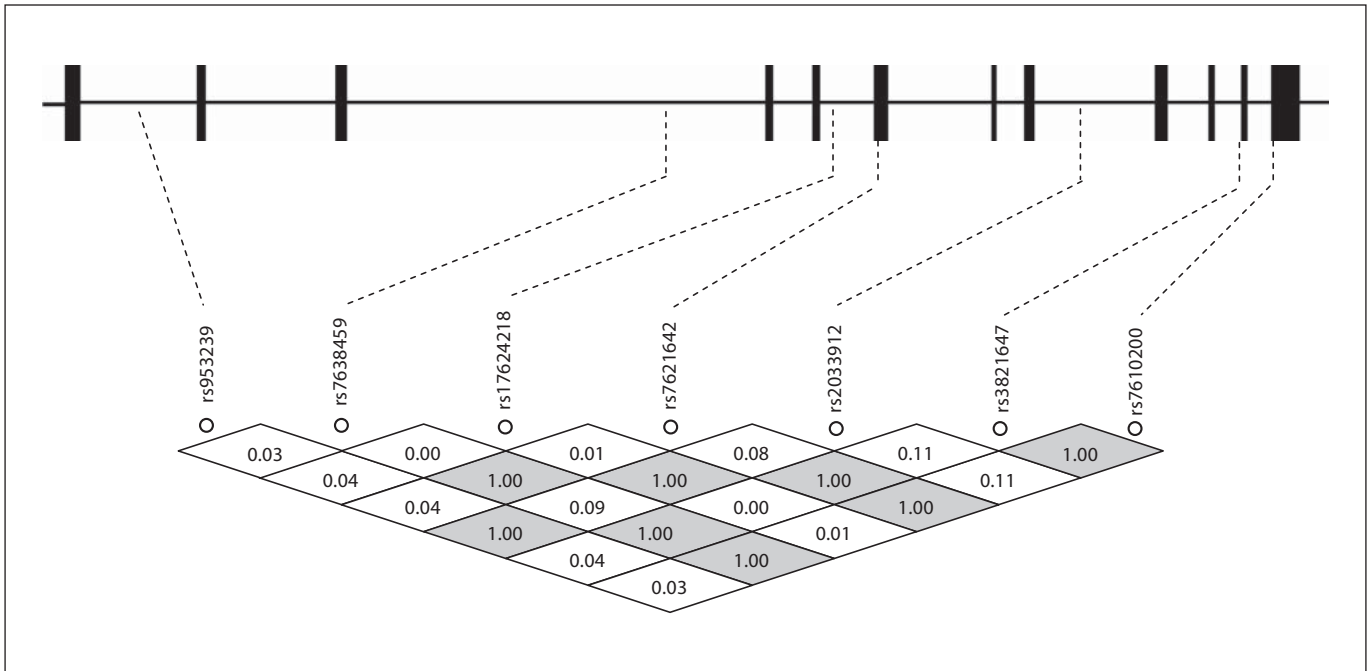


Fig. 1. Tag SNP markers examined in the TRPC1 gene. Seven examined Tag SNP markers and their linkage disequilibrium (LD) matrix along the TRPC1 gene in the GoKinD population. The coding exons in the gene are marked with the blocks. The LD values (D') are shown inside the rhombs.

ABI 7300 real-time PCR system (Applied Biosystems). Experiments were replicated on at least two occasions.

Statistical Analyses

Clinical data are expressed as mean \pm SD. The proportions of genotypes or alleles between the cases and controls were compared using a χ^2 test. Additive model and Armitage's trend tests were also performed. Odds ratios (OR) and 95% CI were calculated to test the relative risk for association. The sample sizes in the GoKinD and African-American populations had $\sim 90\%$ power (at the 5% level) assuming MAFs of 0.15–0.20. Statistical powers were calculated using software of PowerSampleSize (PS version 2.1.31) [22]. Testing for differences in clinical parameters between cases and controls was done, and non-normally distributed traits (plasma cystatin C, serum creatinine, BMI, and cholesterol) were transformed into natural logarithms before analysis. Multivariate logistic regression analysis was also used to examine independent variables associated with diabetic nephropathy between cases and controls in the GoKinD population. The confounders including creatinine, cystatin and systolic blood pressure were applied in the model. LD values between markers were tested using Arlequin programs (ver 2.0) [23] and Haploview (ver 4.0) [24]. Analyses were performed using Statistica (ver 7.1, StatSoft, Tulsa, Okla., USA) and the BioMed Data Program (BMDP ver 1.12, Cork, Ireland). In the animal study, a non-parametric Kruskal-Wallis comparison analysis and/or test for equality of means were done using BMDP (ver 1.12). $p < 0.05$ was considered significant.

Results

In an attempt to refine the possible TRPC1 association with diabetic nephropathy in type 1 diabetes, seven tag SNPs extending across the TRPC1 gene were genotyped in type 1 diabetes patients with and without diabetic nephropathy from the GoKinD samples. In addition to the 1,177 European American (Caucasian) type 1 diabetes patients, this cohort also included 109 non-Caucasian samples (8.5% of the total). To reduce type 1 or type 2 error resulting from population-specific variants, we analyzed the data in European Americans. All examined SNPs were satisfied in Hardy-Weinberg equilibrium assumption. Figure 1 demonstrates the location and LD matrix of examined SNPs in the TRPC1 gene. Pairwise marker correlations suggested strong LD in GoKinD European Americans, in agreement with the haplotype block structure in European Caucasians in HapMap.

Single marker association analysis was conducted on the genotype distribution and allele frequency in the GoKinD population. Detailed information of examined SNPs and their minor allele frequencies (MAFs) in GoKinD European Americans are summarized in table 2. Genotype distribution and the association of the

Table 2. TRPC1 SNPs and MAFs in GoKinD Caucasian type 1 diabetic subjects with and without diabetic nephropathy

dbSNP rsID	SNP type	Position in chr. 3	Genomic/cDNA identity*	Location	Group	n	MAF
rs953239	M = A/C	143928895	g.48941351A>C	intron 1	T1D with DN	576	C 0.419
					T1D without DN	598	C 0.409
rs7638459	Y = C/T	143972841	g.48985297T>C	intron 3	T1D with DN	576	C 0.213
					T1D without DN	599	C 0.191
rs17624218	Y = C/T	143983170	g.48995626T>C	intron 5	T1D with DN	577	C 0.210
					T1D without DN	599	C 0.217
rs7621642	R = A/G	143986295	g.48998751G>A c.1054G>A(S306S)	exon 6	T1D with DN	576	A 0.213
					T1D without DN	596	A 0.191
rs2033912	W = A/T	143999057	g.49011513A>T	intron 8	T1D with DN	578	T 0.213
					T1D without DN	599	T 0.191
rs3821647	R = A/G	144006039	g.49018495G>A c.2065G>A(T643T)	exon 11	T1D with DN	578	A 0.189
					T1D without DN	599	A 0.167
rs7610200	R = A/G	144007548	g.49020004G>A c.2197G>A(R687R)	exon 12	T1D with DN	578	A 0.192
					T1D without DN	599	A 0.168

T1D = Type 1 diabetes; DN = diabetic nephropathy; n = number of samples genotyped; MAF = minor allele frequency.

* References: NT_005612.15, NM_003304 and NP_003295. M, Y, R and W are International Union of Pure and Applied Chemistry (IUPAC) codes used in FASTA sequences at the SNP position.

M = Adenine or cytosine; Y = cytosine or thymine; R = adenine or guanine; W = adenine or thymine.

TRPC1 polymorphisms with diabetic nephropathy in GoKinD European American type 1 diabetes patients are shown in table 3a. The MAF of SNPs rs7621642 and rs2033912 were of borderline significant difference ($p = 0.045$ and 0.047 , respectively) in female European American type 1 diabetes patients with versus without diabetic nephropathy, but not in males ($p = 0.661$ and 0.694 , respectively). The MAF of SNP rs3821647, residing in a haplotype block with rs7610200, trended toward a higher frequency in female type 1 diabetes patients with diabetic nephropathy, compared to those without diabetic nephropathy (0.187 vs. 0.151 , $p = 0.096$). To examine independent variables associated with diabetic nephropathy among female type 1 diabetes patients in the GoKinD population, further multivariate logistic regression analysis was performed. No significant association of SNPs rs7621642 ($p = 0.233$, OR = 1.457, CI 95% 0.785–2.705), rs2033912 ($p = 0.232$, OR = 1.458, CI 95% 0.785–2.707) and rs3821647 ($p = 0.389$, OR = 1.328, CI 95% 0.696–2.532) with diabetic nephropathy among female type 1 diabetic patients with and without diabetic nephropathy was observed.

Tests in the entire GoKinD population were then performed. When African Americans from the GoKinD population were combined with European Americans,

the difference in MAFs of these three polymorphisms were statistically significant in type 1 diabetes patients with versus without diabetic nephropathy ($p = 0.003$, 0.003 and 0.004 , respectively; $P_c = 0.042$, 0.042 and 0.056 , respectively, after Bonferroni correction). In order to understand whether TRPC1 polymorphisms are the population-specific variants or confer the susceptibility to diabetic nephropathy in African Americans, we further genotyped the three SNPs, rs7621642, rs2033912 and rs3821647, in a second cohort of African Americans, including 284 with type 2 diabetes-associated ESRD, 284 with hypertension-associated ESRD and 282 nondiabetic subjects [19]. The genotype distribution and MAF of these polymorphisms in the population of Wake Forest African Americans are listed in table 3b. The genotype distributions of these SNPs were reversed in African Americans, compared to what had been detected in European Americans (table 3a); major alleles of the SNPs in European Americans were minor alleles in African Americans. No significant associations for these three polymorphisms were detected with type 2 diabetes-ESRD or hypertensive-ESRD in African Americans.

We further investigated TRPC1 gene expression at mRNA levels with a mouse model of diabetic nephropathy to determine whether reduced TRPC1 gene expres-

Table 3. Genotype distributions and minor allele frequencies of TRPC1 polymorphisms: American Caucasians (a) and African Americans (b)

a American Caucasians in GoKinD

dbSNP rsID	Group	Gender	Genotype			MAF	p value	OR (95%CI)
rs7621642	T1D with DN	female	GG	GA	AA	A 0.217	0.045*	1.336 (1.005–1.774)
		male	161 (60.8)	93 (35.1)	11 (4.1)	A 0.209		
	T1D without DN	female	195 (62.7)	102 (32.8)	14 (4.5)	A 0.172		
		male	241 (67.9)	106 (29.9)	8 (2.2)	A 0.220		
rs2033912	T1D with DN	female	AA	AT	TT	T 0.217	0.047*	1.331 (1.003–1.768)
		male	161 (60.8)	93 (35.1)	11 (4.1)	T 0.209		
	T1D without DN	female	196 (62.6)	103 (32.9)	14 (4.5)	T 0.173		
		male	242 (67.8)	107 (30.0)	8 (2.2)	T 0.219		
rs3821647	T1D with DN	female	GG	GA	AA	A 0.187	0.096	
		male	172 (64.9)	87 (32.8)	6 (2.3)	A 0.192		
	T1D without DN	female	200 (63.9)	106 (33.9)	7 (2.2)	A 0.151		
		male	256 (71.7)	94 (26.3)	7 (2.0)	A 0.190		

b African Americans from Wake Forest

rs7621642	T2D with ESRD	female	GG	GA	AA	G 0.305	0.847**	
		male	16 (9.2)	74 (42.5)	84 (48.3)	G 0.306		
	HTN with ESRD	female	13 (12.6)	40 (38.8)	55 (50.9)	G 0.312	0.204***	
		male	18 (14.4)	42 (33.6)	65 (52.0)	G 0.271		
	ND	female	11 (7.0)	63 (40.1)	83 (52.9)	G 0.261	0.392***	
		male	9 (6.8)	51 (38.6)	72 (54.6)	G 0.302		
rs2033912	T2D with ESRD	female	AA	AT	TT	A 0.286	0.416**	
		male	14 (8.1)	71 (41.0)	88 (50.9)	A 0.280		
	HTN with ESRD	female	12 (11.2)	36 (33.7)	59 (55.1)	A 0.256	0.722***	
		male	11 (8.8)	42 (33.6)	72 (57.6)	A 0.245		
	ND	female	11 (6.9)	56 (35.2)	92 (57.9)	A 0.242	0.261***	
		male	9 (6.8)	46 (34.9)	77 (58.3)	A 0.285		
rs3821647	T2D with ESRD	female	AA	AG	GG	A 0.207	0.142**	
		male	11 (6.3)	50 (28.8)	113 (64.9)	A 0.259		
	HTN with ESRD	female	9 (8.3)	38 (35.2)	61 (56.5)	A 0.258	0.612***	
		male	10 (7.9)	45 (35.7)	71 (56.4)	A 0.193		
	ND	female	7 (4.4)	47 (29.8)	104 (65.8)	A 0.239	0.033***	
		male	6 (4.6)	51 (38.6)	75 (56.8)	A 0.265		

T1D = Type 1 diabetes; DN = diabetic nephropathy; T2D = type 2 diabetes; ESRD = end-stage renal disease; HTN = hypertension; ND = non-diabetic subjects; MAF = minor allele frequency. Comparison analyses of * T1D with DN vs. T1D without DN, ** T2D with ESRD vs. ND, and *** T2D with DN vs. HTN with ESRD. Figures in parentheses are percentages.

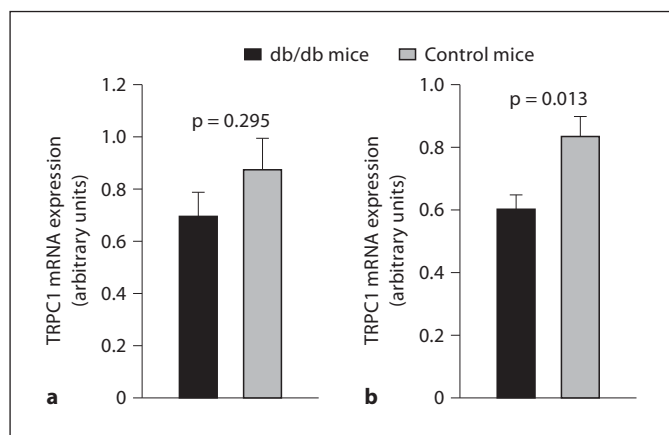


Fig. 2. TRPC1 gene mRNA expression in kidney tissues of db/db mice. TRPC1 gene mRNA expression was examined using real-time RT-PCR in kidney tissue from db/db mice at 12 and 26 weeks of age, respectively. TRPC1 mRNA expression levels between db/db and db/m (control) mice at the age of 12 weeks were not significantly different (mean values 0.874 ± 0.117 SE vs. 0.697 ± 0.061 , $p = 0.295$, **a**). The expression levels of TRPC1 in kidney tissue from 26-week-old db/db mice, however, were significantly decreased compared with the db/m controls (0.836 ± 0.062 vs. 0.601 ± 0.047 , $p = 0.013$, **b**).

sion was a primary or subsequent defect in the development of diabetic nephropathy. TRPC1 gene expression in kidney tissue from db/db mice at 12 and 26 weeks of age, respectively, was examined using a TaqMan real time RT-PCR protocol. Data indicated that TRPC1 mRNA expression levels kidney tissue from db/db mice at the age of 12 weeks compared to the controls were tendency to reduce ($p = 0.295$, fig. 2a), while the expression levels of this gene in 26-week-old db/db mice were significantly decreased ($p = 0.013$, fig. 2b).

Discussion

In the present study, we have conducted a genetic association study to detect TRPC1 genetic susceptibility for diabetic nephropathy in the GoKinD type 1 diabetes subjects. Among this population, a large proportion of subjects are American Caucasian while the rest are African American, Asian, Hispanic and Indian. Evidence has demonstrated that race and ethnicity are related to the progression of kidney dysfunction and clinical outcomes in the patients with diabetic nephropathy. In the United States, the incidence of diabetic nephropathy is higher for African Americans, Native Americans and Asians than

for American Caucasians [25, 26]. We thus performed the analyses for association of TRPC1 genetic polymorphisms and diabetic nephropathy, separately, in American Caucasians and other American ethnic groups. Although single marker association analysis has showed a borderline association between the TRPC1 genetic polymorphisms and diabetic nephropathy in female type 1 diabetes patients of American Caucasians, multivariate logistic regression analysis negates the evidence for the association.

We have tested whether TRPC1 genetic polymorphisms are associated with diabetic nephropathy among African-American diabetic patients or are the ethnic specific variants with the second cohort of African Americans. Data indicate that genotype distribution of TRPC1 polymorphisms in African Americans with type 2 diabetes-ESRD or hypertension and nondiabetic subjects are reversed in comparison with the GoKinD American Caucasians. No significant association of TRPC1 polymorphisms with ESRD in those type 2 diabetes patients is found. The first cohort of GoKinD population is a collection of type 1 diabetes patients with and without diabetic nephropathy, and 70.6% of the patients with diabetic nephropathy have ESRD. In the second cohort of African Americans, the type 2 diabetes patients are associated with EASD. We did not combine the data from American Caucasians' type 1 diabetes with ESRD and African Americans' type 2 diabetes with ESRD together for analysis, because the genotype distributions of TRPC1 polymorphisms between these two cohorts are reversely different. The combined analysis in these two populations may simply cause type 1 or type 2 error. Since there are only 42 African-American type 1 diabetes patients with and without diabetic nephropathy enrolled in the present study, we are not able to exclude the possibility of association between TRPC1 genetic polymorphisms and diabetic nephropathy in African-American type 1 diabetes patients.

Based upon the data from our genetic association study and the recent report [17], we hypothesized that the reduction of TRPC1 gene expression may be the subsequent defect in the development of diabetic nephropathy. To test this hypothesis, we have examined TRPC1 gene expression in kidney tissue from db/db mice at 12 and 26 weeks of age. This animal model has been used for study of the progression of diabetic nephropathy [27]. We found that TRPC1 mRNA expression levels are trendily decreased in 12-week-old and significantly in 26-week-old db/db mice compared to the controls. The results are consistent with the recent report [17] and provide further evidence that TRPC1 expression was not reduced until the later stage of diabetic nephropathy. Moreover, the reduction of TRPC1

expression has been indicated as a result of HNF4 α dysfunction [17]. To detect the susceptibility of the HNF4 α in diabetic nephropathy, a genetic association study of this gene has been taken into our consideration.

In conclusion, the present study provides the first evidence that TRPC1 genetic polymorphisms are lack of association with diabetic nephropathy in either European American subjects with type 1 diabetes from GoKinD or African Americans with type 2 diabetes-associated ESRD. Genetic variation in this gene may not fundamentally contribute to the development of diabetic nephropathy. However, a functional analysis with db/db mice, in consistence with the recent report using STZ-treated rats, indicates that reduction of TRPC1 gene expression is the subsequent effect in the late stage of diabetic nephropathy.

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