Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 1496 – 1502
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Evaluation of gremlin 1 (*GREM1*) as a candidate susceptibility gene for albuminuria-related traits in Mexican Americans with type 2 diabetes mellitus

Farook Thameem[a,b,*], Sobha Puppala[c], Xin He[a], Nedal H. Arar[a,b,d], Michael P. Stern[e], John Blangero[c], Ravindranath Duggirala[c], Hanna E. Abboud[a,b,d]

[a]*Division of Nephrology, University of Texas Health Science Center, San Antonio, TX 78229, USA*
[b]*George O'Brien Kidney Research Center, University of Texas Health Science Center, San Antonio, TX 78229, USA*
[c]*Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX 78227, USA*
[d]*South Texas Veterans Healthcare System, San Antonio, TX 78229, USA*
[e]*Division of Clinical Epidemiology, Department of Medicine, University of Texas Health Science Center, San Antonio, TX 78229, USA*

Received 21 November 2008; accepted 21 April 2009

Abstract

Several novel genes that are up-regulated in the kidney in diabetes have been identified including *GREM1*, which encodes gremlin 1. *GREM1* maps to human chromosome 15q12, a region previously found to be linked to albumin to creatinine ratio (ACR) in Mexican Americans. The objective of this study is to investigate whether genetic variants in *GREM1*, a positional candidate gene, contribute to variation in ACR. By sequencing 32 individuals for both exons and 2-kilobase putative promoter region of *GREM1*, we identified 19 genetic variants including 5 in the promoter region and 13 in the 3′ untranslated region. Of 19 polymorphisms identified, 13 polymorphisms were genotyped in the entire cohort (N = 670, 39 large families) either by restriction fragment length polymorphism or by TaqMan (Applied Biosystems, Foster City, CA) assays. Association analyses between the genotypes and ACR, type 2 diabetes mellitus, and related phenotypes were carried out using a measured genotype approach as implemented in the variance component analytical tools (SOLAR). Of the variants examined for association, none exhibited statistically significant association with ACR after accounting for the effects of covariates such as age, sex, diabetes, duration of diabetes, systolic blood pressure, and antihypertensive medications. However, 2 novel variants at the 3′ untranslated region showed significant association with estimated glomerular filtration rate ($P = .010$ and $P = .049$) and body mass index ($P = .013$ and $P = .019$) after accounting for trait-specific covariate influences. Furthermore, a novel variant located in the promoter exhibited a significant association with systolic ($P = .038$) and diastolic blood pressure ($P = .005$) after adjusting for the effects of age, sex, diabetes, and antihypertensive medications. In conclusion, the variants examined at *GREM1* are not significant contributors to variation in ACR in Mexican Americans, although they appear to minimally influence risk factors related to ACR.

1. Introduction

Albuminuria is predictive of all-cause and cardiovascular morbidity and mortality in patients with diabetes or hypertension independent of traditional risk factors and in the general population [1]. The pathophysiologic mechanisms underlying the development of albuminuria are multifactorial. Although epidemiologic data indicate that poor glycemic and blood pressure control is undoubtedly involved in the development of albuminuria, there is compelling evidence from twin and family studies that genetic factors make a major contribution to the development and progression of albuminuria [2]. However, the specific genes involved in susceptibility to albuminuria have yet to be identified. During the last decade, a significant amount of research has been devoted to identifying genes potentially involved in the etiology of this common complex trait.

* Corresponding author. Division of Nephrology, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA. Tel.: +1 210 567 4787; fax: +1 210 567 4712.
E-mail address: thameem@uthscsa.edu (F. Thameem).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.04.039

A previous genomewide linkage study in a subset of Mexican American participants in the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) revealed suggestive evidence for linkage of albumin to creatinine ratio (ACR) to a genetic region on human chromosome 15q12 at the *GABRB3* marker [3]. To elucidate the basis for the linkage of ACR in the Mexican Americans, we have previously investigated a positional candidate gene in the 15q12 chromosomal region [4]. This study extends such an effort to investigate another plausible positional candidate gene, *GREM1*, for its association with ACR and its related phenotypes.

Gremlin 1, a member of cysteine knot protein family, regulates diverse processes including growth, differentiation, and development by antagonizing the activity of bone morphogenetic proteins (BMPs) 2, 4, and 7 [5]. The binding of gremlin to selective BMPs prevents ligand-receptor interaction and subsequent downstream signaling. A primary role for gremlin in kidney organogenesis recently demonstrated that *GREM1*-deficient mice die shortly after birth because of complete renal agenesis [6]. *GREM1*-mediated reduction of BMP4 activity in the mesenchyme around the nascent ureteric bud was shown to be essential to initiate ureteric bud outgrowth and invasion of the metanephric mesenchyme [7]. Gremlin 1 promotes vascular smooth muscle cell proliferation and migration [7]. Furthermore, the recent finding that gremlin expression is up-regulated in experimental models of diabetic nephropathy (DN) in vitro and in vivo, coupled with its enhanced expression in response to transforming growth factor–β (TGF-β) and its potential to interact with other important signaling pathways, suggests that gremlin may play an important role in mediating some of the pathologic effects of TGF-β on mesangial cell proliferation and matrix production in the diabetic milieu [8].

GREM1 therefore represents a potential candidate gene for further analysis, coupled with its localization on chromosome 15q12, a region previously linked with ACR. The aim of this study was to examine whether DNA polymorphisms in *GREM1* contribute to variation in susceptibility to ACR and its correlated traits in Mexican Americans, a population at high risk for type 2 diabetes mellitus (T2DM) and its complications including DN.

2. Subjects and methods

2.1. Subjects and phenotypic data

The SAFDGS family members' recruitment and data collection procedures of about 700 participants from 39 large families were reported previously [9]. Briefly, probands were low-income Mexican-Americans with T2DM; and all first-, second-, and third-degree relatives of probands were invited to participate in the study. A variety of metabolic, hemodynamic, anthropometric, and demographic variables were collected at the General Clinical Research Center Laboratory at the South Texas Veterans Health Care System, Audie L Murphy Memorial Hospital Division, San Antonio, TX, using standard procedures. Blood samples were obtained after a 12-hour fast for assessment of various phenotypes including glucose, total cholesterol, triglycerides (TGL), and high-density lipoprotein cholesterol (HDL-C); and they were collected again 2 hours after a standardized oral glucose load to measure plasma glucose. Measurement of all these phenotypes including body mass index (BMI) has been described elsewhere [9,10]. *Diabetes status* was defined by the 1999 criteria of the World Health Organization (ie, fasting glucose levels $\geq$126 mg/dL and/or 2-hour glucose levels $\geq$200 mg/dL). Participants who did not meet these criteria but reported to be under treatment with either oral antidiabetic agents or insulin and who gave a history of diabetes were also considered to have T2DM.

Albumin to creatinine ratio, an index of urine albumin excretion rate, was estimated as described previously [4]. Study participants' urine samples were randomly collected at one time. Urinary albumin excretion (UAE) was estimated with an immunoturbidimetric method using the COBAS INTEGRA diagnostic reagent system that uses antialbumin antibody specific to human albumin. Urine creatinine was measured using a kinetic alkaline picrate assay. The ratio of concentration of albumin (in milligrams per deciliter) to creatinine (in milligrams to deciliter) in random urine specimen (ACR) was used as an index of UAE. The ACR values approximate the numeric values of the corresponding albumin excretion rate measured in 24-hour urine collection and expressed in grams per day. *Albuminuria* (micro or macro) was defined as an albumin (in milligrams per deciliter) to creatinine (in milligrams per deciliter) ratio of at least 0.03, which is approximately equivalent to a UAE greater than 30 mg/d. Estimation of glomerular filtration rate (eGFR) using 4-variable Modification of Diet in Renal Disease (MDRD) formula has been previously described [11]. The quantitative traits values were log transformed for ACR (*ln* ACR) and TGL (*ln* TGL) and used in the association analyses because their raw data were nonnormally distributed. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave informed consent.

2.2. Molecular variants identification and genotyping

The exons and 2-kilobase (kb) putative promoter region of *GREM1* gene were polymerase chain reaction (PCR) amplified and directly sequenced in 32 individuals for DNA sequence variants. DNA sequencing was performed using ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and a capillary sequencer (Model 3730xl, Applied Biosystems). Human chromosome 15–specific somatic cell hybrid DNA (NA11418; Coriell Cell Repositories, Camden, NJ) was

used as a "hemizygous" nonpolymorphic control representing a single allelic version of all variants on this chromosome. Genotyping of all the single nucleotide polymorphisms (SNPs) was performed either by restriction fragment length polymorphism assay or by TaqMan assay (Applied Biosystems), which was carried out on a GeneAmp PCR system 9700 (Applied Biosystems); and fluorescent signals were detected on an ABI PRISM 7700 sequence detector (Applied Biosystems). To ensure accuracy of the genotyping, coded blind replicate samples from 50 subjects were included in each genotyping assay.

2.3. Statistical genetic analysis

The genotypic data were checked for mendelian pedigree inconsistencies using the program INFER and GENTEST as implemented in PEDSYS. Allele frequencies were estimated using maximum-likelihood techniques, which account for the pedigree structure. All polymorphisms were tested for Hardy-Weinberg equilibrium. Linkage disequilibrium (LD) between SNPs was estimated using the r^2 values. Association analysis in our family data was carried out using the measured genotype approach within the variance components (VCs) analytical framework. The VC-based approach accounts for the nonindependence among family members. In this approach, VCs are modeled as random effects (eg, additive genetic effects and random environmental effects), whereas the effects of measured covariates such as age and sex are modeled as fixed effects on the trait mean. The marker genotypes were incorporated in the mean effects model as a measured covariate, assuming additivity of allelic effects [12,13]. The effect of this measured genotype (ie, association parameter) together with other covariate effects (eg, age and sex) and VCs was estimated by maximum-likelihood techniques. The hypothesis of no association is tested by comparing the likelihood of a model in which the effect of the measured genotype is estimated with a model where the effect of the measured genotype was fixed at zero. Twice the difference in the log-likelihoods of these models yields a test statistic that is asymptotically distributed, approximating a χ^2 distribution with 1 *df*. A P value $\leq .05$ is considered significant. Before performing the measured genotype approach, the quantitative transmission disequilibrium test was used to examine hidden population stratification [14]. All statistical techniques described above were implemented in the program SOLAR [13].

3. Results

The clinical characteristics of the genotyped individuals are shown in Table 1. Of the individuals genotyped, the phenotypic data varied from 610 subjects for total cholesterol to 670 subjects for age. Of the examined individuals from 39 families, 29%, 28%, and 14% of them had hypertension, T2DM, and albuminuria, respectively. The eGFR data were available for only 453 subjects.

Table 1
Clinical characteristics of the genotyped SAFDGS participants used for the present study

Phenotypes	Mean ± SD or %
Sex (female)	61
T2DM	28
Hypertension	29
Albuminuria	14
Age (y)	44.8 ± 16.2
SBP (mm Hg)	128.6 ± 18.2
DBP (mm Hg)	71.3 ± 9.6
BMI (kg/m^2)	30.9 ± 7.0
Waist circumference (mm)	1001.0 ± 170.6
Total cholesterol (mg/dL)	194.0 ± 37.9
HDL-C (mg/dL)	46.0 ± 12.0
ln TGL	4.9 ± 0.6
GFR MDRD (mL/[min 1.73 m^2])	88.4 ± 17.9
ln ACR	2.4 ± 0.8

Sample size varies from 610 (cholesterol) to 670 (age) except for GFR. The sample size for GFR is 453.

GREM1 (NM_013372) is composed of 2 exons, and exon 2 encodes for the full-length protein (Fig. 1). Both exons including their splice sites and 2 kb upstream from exon 1 were amplified by PCR and sequenced in 32 individuals who positively contributed to the linkage of ACR. This analysis identified 19 diallelic variants including 5 in the putative promoter region and 14 in the 3′ untranslated region (UTR) (Fig. 1). Our sequence analysis performed in 32 subjects identified from a minimum of 2 heterozygotes (SNP-17) to maximum of 15 heterozygotes (SNP-9). Of the 19 variants identified, 18 are single nucleotide polymorphisms (SNPs); and one is an insertion/deletion polymorphism (IDP). Furthermore, our analysis failed to identify any sequence variation in the coding region. Of the polymorphisms identified, 7 SNPs are novel in this population; and 12 of them have already been deposited in the SNP database (Fig. 1).

Based on an initial genotyping in the 32 subjects, half of the variants could be divided into 3 groups, indicative of distinct LDs. These include SNP-1, -4, -10, -11, and -17 (SNP cluster I); SNP-6 and -7 (SNP cluster II); and SNP-8 and -9 (SNP cluster III). Therefore, SNP-17 (cluster I), -7 (cluster II), and -9 (cluster III) were selected as representative markers for each unique cluster of variants for further analysis. The remaining 10 polymorphisms (IDP-1 and SNP-2, -3, -5, -12, -13, -14, -15, -16, and -18) could not be assigned to any group and were analyzed individually (Fig. 1). In total, we genotyped 13 variants (IDP-1 and SNP-2, -3, -5, -7, -9, -12, -13, -14, -15, -16, -17, and -18) in the entire data set (N = 670; 39 large families) either by restriction fragment length polymorphism or TaqMan assays. Genotypic data of all the genotyped polymorphisms were consistent with the Hardy-Weinberg equilibrium expectations, and there was no evidence for hidden population stratification in the data as tested by quantitative transmission disequilibrium test. Based on the genotypic data of the

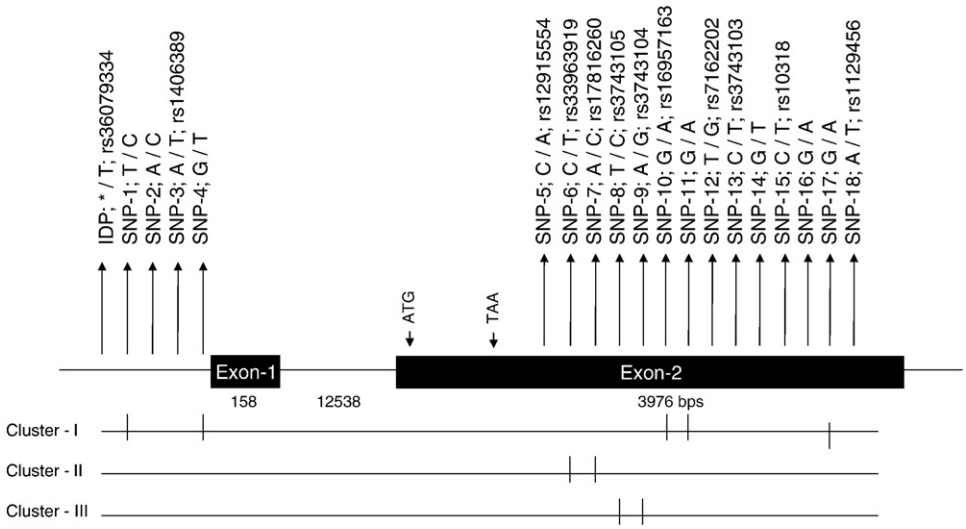

Fig. 1. Schematic diagram of human *GREMI* (NM_013372) gene structure on 15q12 and the location of the polymorphisms identified and genotyped in SAFDGS. The exons of the *GREMI* are represented by solid box; and the intron, by a thin line. Polymorphisms with the base change or deletion and their reference sequence numbers are indicated by the vertical arrow. Clusters of markers in LD (I, II, and III) are indicated below the gene structure. Rs indicates reference sequence.

13 SNPs, SNP-17 (representative of cluster I) was excluded from further analysis because the minor allele frequencies of SNP-17 were less than 0.5% (Fig. 1). Before performing statistical association analysis, we estimated the pairwise LD (r^2) between all the 12 variants. Fig. 2 shows the overall pattern of LD as measured by the r^2 values. As can be seen

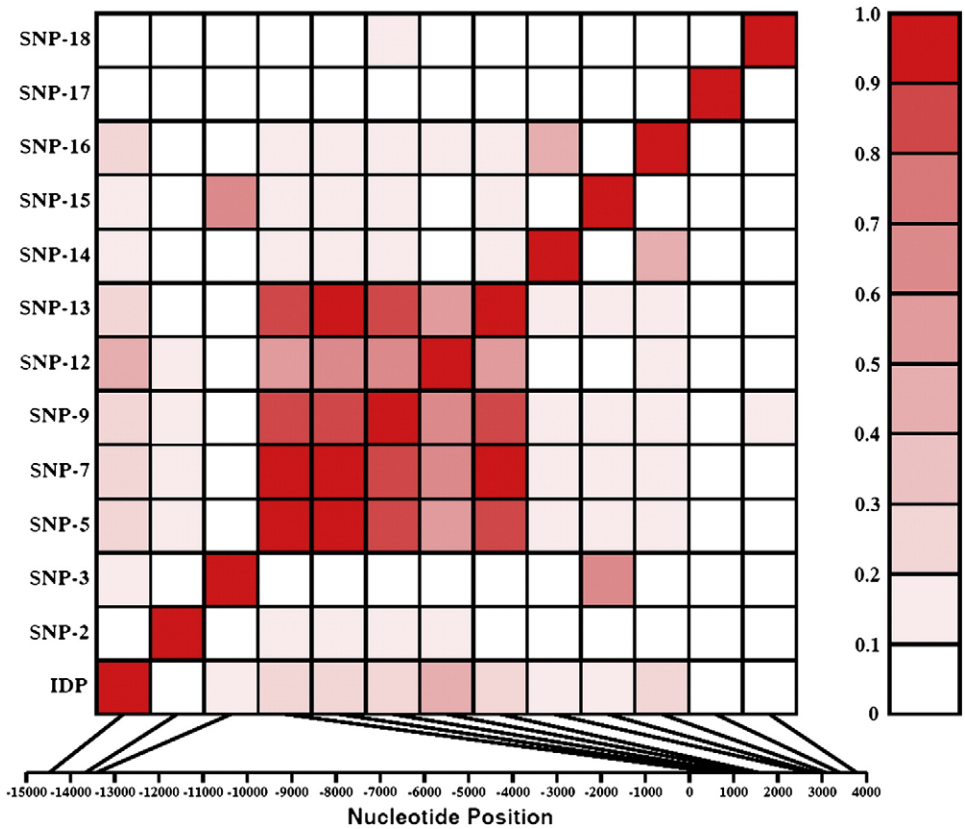

Fig. 2. Linkage disequilibrium between polymorphisms within the *GREMI* gene. Polymorphisms are labeled on the y-axis, and the locations (base pair) within the gene are shown on the x-axis. Pairwise LD is estimated using r^2 values and depicted in the figure by the color intensity of the shaded box, as shown in the legend. The diagonal represents a comparison of each polymorphism against itself (ie, $r^2 = 1.0$).

from Fig. 2, the pairwise LD between variants ranged from 0 to 0.99; and the highest pairwise LD ($r^2 > 0.8$) found among the *GREM1* SNPs were as follows: rs12915554- rs17816260 ($r^2 = 0.99$), rs17816260-rs3743103 ($r^2 = 0.91$), rs12915554-rs3743103 ($r^2 = 0.89$), rs17816260-rs3743104 ($r^2 = 0.87$), rs12915554-rs3743104 ($r^2 = 0.86$), and rs3743104-rs3743103 ($r^2 = 0.81$).

In addition to association analysis between *GREM1* genotypic and ACR data in our pedigree, association analyses were also extended to available albuminuria-related phenotypic data including systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, TGL, CHOL, HDL-C, eGFR, and T2DM. The location, allele frequencies, and association analyses of 12 variants examined are summarized in Table 2. The minor allele frequencies of the polymorphisms ranged from 10.0% (SNP-2) to 48.1% (SNP-7). Of the 12 variants examined for association, none of the variants exhibited statistically significant association with ACR after accounting for the potential covariate effects of age, sex, diabetes, duration of diabetes, SBP, and antihypertensive treatment (angiotensin-converting enzyme inhibitors or AT1R antagonists). Association analyses, however, indicated that the 2 novel SNPs located in the 3′ UTR (SNP-14 and SNP-16) were significantly associated with eGFR ($P = .01$ and $P = .049$, respectively) and BMI ($P = .013$ and $P = .019$, respectively) after accounting for the trait-specific covariate effects (Table 2). In addition, a novel promoter variant (SNP-2) with minor allele frequency of 10% was found to be associated with SBP ($P = .038$) and DBP ($P = .005$) after adjusting for the covariates age, sex, diabetes, and antihypertensive treatment (Table 2).

4. Discussion

Identification and characterization of susceptibility genes for albuminuria and related traits in diabetic patients have been a major focus of current biomedical research efforts. Many genes that play a role in embryonic development are recapitulated in adult disease including DN. Prominent among these is gremlin 1, a member of the BMP antagonists family [5]. Several observations suggest a role for gremlin 1 as a modulator of cell turnover and extracellular matrix production in DN. Gremlin 1 expression can be induced in mesangial cells in response to high glucose, TGF-β, and cyclic mechanical strain [15]. In addition, its expression is modulated by the mitogen-activated protein kinase and reactive oxygen species, all of which are reported to have a role in the pathogenesis of diabetic retinopathy. Gremlin 1 coexpression with BMPs is thought to antagonize their actions in the diabetic milieu. Gremlin 1 modulates the effects of platelet-derived growth factor and cytokines on mesangial cell proliferation and matrix production [15]. The concomitant increased expression of multiple profibrotic genes and gremlin in retinal pericytes exposed to high glucose and the regulation of gremlin expression by profibrotic cytokine suggest a role for gremlin in fibrogenesis [16]. A pathogenic role may be attributed to gremlin in the context of pathogenesis of DN, as overexpression of gremlin induces transdifferentiation of cultured tubular epithelial cells to a more fibroblast-like phenotype [17]. Taken together, these data suggest a pathogenic role for gremlin in DN and identify gremlin as a potential therapeutic target [18].

Table 2
Association analysis between the *GREM1* polymorphisms and ACR-related traits

Variants	SNP ID	Gene location	Position (bp)[a]	Major/minor allele (%)	Traits								
					BMI[b]	CHOL[c]	HDL-C[c]	TGL[c]	GFR[d]	SBP[e]	DBP[e]	ACR[f]	T2DM[g]
					P values								
IDP	rs36079334	Promoter	−14479	T (57.2)/A (42.8)	.198	.913	.298	.338	.434	.518	.973	.532	.186
SNP-2	–	Promoter	−13661	A (90.0)/C (10.0)	.730	.648	.153	.218	.281	**.038**	**.005**	.971	.745
SNP-3	rs1406389	Promoter	−13414	A (78.4)/T (21.6)	.688	.915	.541	.176	.589	.535	.862	.559	.723
SNP-5	rs12915554	3′ UTR	595	C (52.0)/A (48.0)	.203	.526	.903	.397	.150	.296	.598	.869	.454
SNP-7	rs17816260	3′ UTR	794	C (51.9)/A (48.1)	.214	.501	.658	.498	.164	.329	.529	.855	.489
SNP-9	rs3743104	3′ UTR	1094	G (53.4)/A (46.6)	.138	.806	.580	.433	.238	.559	.915	.823	.900
SNP-12	rs7162202	3′ UTR	1454	A (63.3)/C (36.7)	.412	.541	.904	.209	.637	.993	.877	.520	.305
SNP-13	rs3743103	3′ UTR	1564	C (52.2)/T (47.8)	.277	.563	.473	.989	.256	.297	.454	.936	.762
SNP-14	–	3′ UTR	2736	G (89.5)/T (10.5)	**.013**	.344	.570	.374	**.010**	.379	.767	.389	.761
SNP-15	rs10318	3′ UTR	2852	C (83.3)/T (16.7)	.939	.264	.533	.857	.369	.457	.541	.291	.689
SNP-16	–	3′ UTR	3088	G (81.4)/A (18.6)	**.019**	.438	.674	.833	**.049**	.240	.484	.681	.962
SNP-18	rs1129456	3′ UTR	3776	A (90.1)/T (9.9)	.657	.084	.477	.426	.904	.260	.858	.869	.802

CHOL indicates total cholesterol.

[a] Position relative to the A of the start codon (ATG), which is +1.

[b] Adjusted for age and sex terms and diabetes.

[c] Adjusted for age and sex terms, diabetes, and lipid medication.

[d] Adjusted for age and sex terms, diabetes, duration of diabetes, SBP, and antihypertensive treatment.

[e] Adjusted for age and sex terms, diabetes, and antihypertensive treatment.

[f] Adjusted for age and sex terms, diabetes, duration of diabetes, SBP, and antihypertensive treatment.

[g] Adjusted for age and sex terms.

Based on the functional significance of *GREM1* and its location on chromosome 15q12, a region previously found to be linked with ACR in Mexican Americans [3,4], we investigated *GREM1* as a positional candidate gene containing sequence variants that may contribute to albuminuria and its related traits in Mexican Americans. Of the 12 genetic variants examined for association, none of the variants revealed statistically significant association with ACR after accounting for covariate influences (Table 2). Therefore, it is unlikely that DNA sequence variants that we examined in this gene significantly contribute to the risk for variation in albuminuria in this population. However, these results should be interpreted with caution because genetic variants within unidentified key regulatory elements located either deeper within introns or further upstream or downstream of *GREM1* may be present and affect gene expression or function, thereby contributing to albuminuria. In addition, the development of albuminuria may be influenced by interacting genetic and environmental risk factors; and the genetic variants that we have identified in the *GREM1* gene may be seen to confer risk only when accounting for the modifying factors [4]. Our association analyses however indicated the presence of significant association between 2 novel 3′ UTR SNPs (SNP-14 and SNP-16) and GFR and BMI, respectively. Furthermore, a promoter variant was significantly associated with SBP and DBP measures. The functional significance of these associations needs to be elucidated, although the contribution of these novel SNPs to variation in albuminuria-related traits appears to be minor.

To characterize whether the variants identified in the promoter region on *GREM1* could disrupt the *cis*-acting putative transcription factor binding sites, the sequence extending 2.0 kb upstream of exon 1 of the *GREM1* was analyzed by a computer-assisted Trans-Fac program [19]. Analysis of the 5′ upstream region predicted clustering of several potential *cis*-acting binding elements for multiple transcription regulators including AP1, GATA-1, and USF. However, none of the variants that we identified disrupted any of the predicted transcription regulators.

Our study has potential limitations. Given the sample size (32 subjects) used for resequencing *GREM1*, it is possible that we might have missed rare variants specific to this population. Another limitation to be acknowledged is that the GFR estimated by MDRD formula has not been validated in Mexican Americans. Whereas the time-consuming and expensive direct measurement of GFR is difficult to perform (eg, inulin, iothalamate clearance) in large-scale studies, the eGFR using the abbreviated equations is currently one of the best validated means for transforming serum creatinine measurements into GFR in adults [20]. Given attention to such limitations, we have opted to estimate the GFR using the MDRD commonly used in genetic studies [21,22]. Finally, it should be noted that, once the issue of multiple testing is considered, the significant association found for some albuminuria-related traits becomes statistically insignificant.

In conclusion, we report for the first time that the polymorphisms identified in *GREM1* show no compelling evidence of association with ACR in Mexican American families with T2DM. Although this study found some evidence for contribution of genetic variants in *GREM1* to the phenotypic variation in some albuminuria-related traits, the magnitude of the genetic effects appears to be minimal. Identification of variants in this study may make it possible to explore this locus for its possible contribution to subclinical cardiovascular- and renal-related risk factors in other populations.

Acknowledgment

We thank members of the SAFDGS for their participation and cooperation. This study was supported by a Scientist Development Grant from the American Heart Association (FT), Diabetes Action Research and Education Foundation (FT), Satellite HealthCare (FT), George O'Brien Kidney Research Center (P50 DK061597; HEA, FT), VA-Merit Review (HEA, NHA), and grants from the National Institute of Diabetes, Digestive, and Kidney Diseases (MPS: DK42273, DK47482; RD: DK53889). This work was also supported by the National Center for Research Resources contracts UL1 RR025767 and KL2 RR025766 for the Institute for Integration of Medicine and Science. We also thank the General Clinical Research Center, South Texas Healthcare System.

References

[1] Weir MR. Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol 2007;2:581-90.
[2] Freedman BI, Bostrom M, Daeihagh P, et al. Genetic factors in diabetic nephropathy. Clin J Am Soc Nephrol 2007;2:1306-16.
[3] Bhandari K, Duggirala R, Kawalitt I, et al. Genome-wide search for albuminuria susceptibility gene in Mexican Americans. Diabetes 2001; 50:A240-1.
[4] Lehman DM, Leach RJ, Johnson-Pais T, et al. Evaluation of tight junction protein 1 encoding zona occludens 1 as a candidate gene for albuminuria in a Mexican American population. Exp Clin Endocrinol Diabetes 2006;114:432-7.
[5] Merino R, Rodriguez-Leon J, Macias D, et al. The BMP antagonist gremlin regulates outgrowth, chondrogenesis and programmed cell death in the developing limb. Development 1999;126:5515-22.
[6] Michos O, Panman L, Vintersten K, et al. Gremlin-mediated BMP antagonism induces the epithelial-mesenchymal feedback signaling controlling metanephric kidney and limb organogenesis. Development 2004;131:3401-10.
[7] Maciel TT, Melo RS, Schor N, et al. Gremlin promotes vascular smooth muscle cell proliferation and migration. J Mol Cell Cardiol 2008;44:370-9.
[8] Walsh DW, Roxburgh SA, McGettigan P, et al. Co-regulation of gremlin and notch signalling in diabetic nephropathy. Biochim Biophys Acta 2008;1782:10-21.
[9] Puppala S, Dodd GD, Fowler S, et al. A genomewide search finds major susceptibility loci for gallbladder disease on chromosome 1 in Mexican Americans. Am J Hum Genet 2006;78:377-92.
[10] Duggirala R, Blangero J, Almasy L, et al. A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. Am J Hum Genet 2000;66:1237-45.

[11] Puppala S, Arya R, Thameem F, et al. Genotype by diabetes interaction effects on the detection of linkage of glomerular filtration rate to a region on chromosome 2q in Mexican Americans. Diabetes 2007;56: 2818-28.
[12] Boerwinkle E, Chakraborty R, Sing CF. The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. Ann Hum Genet 1986;50: 181-94.
[13] Almasy L, Blangero J. Exploring positional candidate genes: linkage conditional on measured genotype. Behav Genet 2004;34:173-7.
[14] Abecasis GR, Cookson WOC, Cardon LR. Pedigree tests of transmission disequilibrium. Eur J Hum Genet 2000;8:545-51.
[15] McMahon R, Murphy M, Clarkson M, et al. IHG-2, a mesangial cell gene induced by high glucose, is human gremlin. Regulation by extracellular glucose concentration, cyclic mechanical strain, and transforming growth factor–beta1. J Biol Chem 2000;275:9901-4.
[16] Kane R, Stevenson L, Godson C, et al. Gremlin gene expression in bovine retinal pericytes exposed to elevated glucose. Br J Ophthalmol 2005;89:1638-42.
[17] Murphy M, McMahon R, Lappin DW, et al. Gremlins: is this what renal fibrogenesis has come to? Exp Nephrol 2002;10:241-4.
[18] Lappin DW, McMahon R, Murphy M, et al. Gremlin: an example of the re-emergence of developmental programmes in diabetic nephropathy. Nephrol Dial Transplant 2002;17:65-7.
[19] Wingender E, Chen X, Hehl R, et al. TRANSFAC: an integrated system for gene expression regulation. Nucleic Acids Res 2000;28:316-9.
[20] Lamb EJ, Tomson CR, Roderick PJ. Clinical Sciences Reviews Committee of the Association for Clinical Biochemistry: estimating kidney function in adults using formulae. Ann Clin Biochem 2005;42: 321-45.
[21] Schelling JR, Abboud HE, Nicholas SB, et al. Family Investigation of Nephropathy and Diabetes Research Group: genome-wide scan for estimated glomerular filtration rate in multi-ethnic diabetic populations: the Family Investigation of Nephropathy and Diabetes (FIND). Diabetes 2008;57:235-43.
[22] Freedman BI, Bowden DW, Rich SS, et al. Genome-wide linkage scans for renal function and albuminuria in type 2 diabetes mellitus: the Diabetes Heart Study. Diabet Med 2008;25:268-76.