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# Genetic variants in Transient Receptor Potential cation channel, subfamily M 1 (*TRPM1*) and their risk of albuminuria-related traits in Mexican Americans

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## Abstract

**Background—**Evidence for linkage of albuminuria to *GABRB3* marker region on chromosome 15q12 was previously reported in Mexican Americans. The objective of this study is to scan a positional candidate gene, Transient Receptor Potential cation channel, subfamily M 1 (*TRPM1*), for genetic variants that may contribute to the variation in albumin-to-creatinine ratio (ACR).

**Methods—**To identify the sequence variants, the exons and 2 kb putative promoter region of *TRPM1* were PCR amplified and sequenced in 32 selected individuals. Identified variants were genotyped in the entire data set (N=670; 39 large families) by TaqMan assays. Association analyses between the sequence variants and ACR, type 2 diabetes (T2DM) and related phenotypes were carried out using a measured genotype approach as implemented in the program SOLAR.

**Results—**Sequencing analysis identified 18 single nucleotide polymorphisms (SNPs) including 8 SNPs in the coding regions, 7 SNPs in the promoter region and 3 SNPs in introns. Of the 8 SNPs identified in the coding regions, 3 were non synonymous [Met(1)Thr, Ser(32)Asn, Val(1395)Ile] and one SNP caused stop codon (Glu1375/*). Of the SNPs examined, none of them exhibited statistically significant association with ACR after accounting for the effect of age, sex, diabetes, duration of diabetes, systolic blood pressure and anti-hypertensive medications. However, a SNP (rs11070811) located in the putative promoter region showed a modest association with triglycerides levels ($P$ = 0.039).

**Conclusion—**The present investigation found no evidence for an association between sequence variation at the *TRPM1* gene and ACR in Mexican Americans, although it appears to have modest influence on T2DM risk factors.



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**Conflict of Interests: NONE**

**Keywords**

*TRPM1*; Type 2 diabetes; Albumin to creatinine ration; polymorphisms; association analysis; Mexican Americans

## 1. Introduction

Albuminuria is a prognostic marker for cardiovascular and renal disease risk in diabetic and nondiabetic subjects and is associated with an increased incidence of cardiovascular disease associated morbidity and mortality [1]. While pathogenesis of albuminuria is multi-factorial, epidemiological as well as animal studies have recognized the potential role of genetic factors in the development of albuminuria. Despite the recent advances in localization of susceptibility genes for various complex diseases, the specific genetic determinants of albuminuria are yet to be identified. In an effort to unravel the genetic architecture of albuminuria, we performed a genome-wide linkage screen in a subset of Mexican American participants in the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) and identified a suggestive evidence for linkage of albuminuria on human chromosome 15q12 at the *GABRB3* marker [2]. We have previously examined Tight Junction Protein 1 *(TJP1)* and Gremlin 1 *(GREM1)* as positional candidate genes for variation in susceptibility to albuminuria or albumin-to-creatinine ratio (ACR) [3,4]. This study screens another plausible positional candidate gene, Transient Receptor Potential cation channel, subfamily M 1 (*TRPM1*), for genetic variants that may be associated with ACR and its related phenotypes.

TRPM1 belongs to a member of the transient receptor potential (TRP) channel super family and melastatin-related TRP (TRPM) subfamily. TRPM proteins consist of six putative transmembrane domains and intracellular N and C termini and forms monovalent-permeable cation channels with variable sensitivity for Ca2+, Mg2+, and other divalent ions. [5]. TRPM is known to induce changes in membrane potential in response to stimulation, and selectively allows cation conductivity through the membrane. TRPM1, the founding member of TRPM family, is a putative tumor suppressor protein expressed in melanocytes. TRPM1 expression levels are inversely correlated to melanoma progression and matastasis [6,7], suggesting a potential role of this protein in cell cycle regulation, proliferation, and migration. TRPM1 proteins are known to be expressed in cells of hematopoietic origin. Expression of TRPM1 in Human B and T lymphocytes, monocytes, and tissues containing hematopoietic cells suggests a potential role in the immune context [8]. It was demonstrated that expression of TRPM1 in HEK293 cells elicited $Ca^{2+}$ influx, indicating that TRPM1 functions as $Ca^{2+}$-permeable channel [9]. Recently it was shown that TRPM1 mediates synaptic transmission and is necessary for the depolarizing light response of ON-bipolar cells [10]. Mutations in *TRPM1* have been associated with complete congenital stationary night blindness [11-13]. Thus, TRPM1 may be potentially involved in various physiological processes and in the pathogenesis of several disorders including those related to renal function.

Therefore, the purpose of this study is to determine if genetic variants in the *TRPM1* gene are associated with variation in ACR and subclinical measures of cardiovascular disease in Mexican Americans, a population at high risk for type 2 diabetes (T2DM) and its complications including diabetic nephropathy.

## 2. Subjects and Methods

### 2.1 Subjects and Phenotypic data

The recruitment of the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) family members and data collection procedures were reported previously [14]. Briefly, each of the SAFDGS families was ascertained on a proband with T2DM, and the probands were low income Mexican-Americans. All 1st, 2nd, and 3rd degree relatives of probands were invited to participate in the study. Several metabolic, hemodynamic, anthropometric, and demographic variables of about 700 participants from 39 large families were collected at the General Clinical Research Center (GCRC) Laboratory at the South Texas Veterans Health Care System, Audie L. Murphy Memorial Hospital Division, San Antonio, Texas. 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 h after a standardized oral glucose load to measure plasma glucose. Measurement of all these phenotypes including body mass index (BMI) has been described elsewhere [15,16]. Diabetes status was defined by the 1999 criteria of the World Health Organization (i.e., fasting glucose levels ≥ 126 mg/dl and/or 2-hr glucose levels ≥ 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 (ACR), an index of urine albumin excretion rate, was estimated as described previously [3]. Urine samples from the study participants were randomly collected at one time. Urinary albumin excretion was estimated with an immunoturbidimetric method using the COBAS INTEGRA diagnostic reagent system that uses anti-albumin antibody specific to human albumin. Urine creatinine was measured using a kinetic alkaline picrate assay. The ratio of concentration of albumin (mg/dL) to creatinine (mg/dL) in random urine specimen (ACR) was used as an index of urinary albumin excretion (UAE). The ACR values approximate the numeric values of the corresponding albumin excretion rate measured in twenty-four hour urine collection and expressed in g/day. Albuminuria (micro or macro) was defined as an albumin (mg/dl) to creatinine (mg/dl) ratio (ACR) of ≥ 0.03, which is approximately equivalent to an UAE > 30 mg/day. Estimation of glomerular filtration rate (eGFR) using 4-variable Modification of Diet in Renal Disease (MDRD) formula has been previously described [16]. The ACR (*ln* ACR) and triglycerides (*ln* TGL) values were log transformed and used in the association analyses because their raw data were non-normally 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 kb putative promoter region of *TRPM1* gene were PCR amplified and directly sequenced in 32 individuals for DNA sequence variants, who positively contributed to the linkage signal of albuminuria. 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” non-polymorphic control representing a single allelic version of all variants on this chromosome. Genotyping of all the SNPs in the study participants was performed 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 assure accuracy of the genotyping, samples from 50 subjects were replicated 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 (MGA) within the variance components (VC) analytical framework. The VC-based approach accounts for the non-independence among family members. In this approach, VCs are modeled as random effects (e.g. 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 [17,18]. The effect of this measured genotype (i.e., association parameter) together with other covariate effects (e.g., age and sex) and VCs were 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 $\chi^2$ distribution with one degree of freedom. A $p$ value $\leq 0.05$ is considered significant. Given the sample sizes used for this study (minimum = 453 and maximum = 670), genetic effects that account for 1.7% (N = 453) or 1.15% (N = 670) of the total phenotypic variation in a trait could be detected with 80% power at the nominal 0.05 level of significance. Prior to performing MGA, the quantitative transmission disequilibrium test (QTDT) was used to examine hidden population stratification [19]. All statistical techniques described above were implemented in the program SOLAR [18].

## 3. Results

Table 1 shows the clinical characteristics of the genotyped individuals. The mean age was 45 years, and 61% were females. Of the individuals genotyped, the phenotypic data varied from 610 subjects for total cholesterol to 670 subjects for age. Of the individuals examined from 39 families, 29%, 28%, and 14% had hypertension, T2DM, and albuminuria respectively. The eGFR data were available for only 453 subjects. Of the individuals with GFR data, 29% had T2DM.

*TRPM1* (NM_002420) is composed of twenty seven exons (Fig. 1) and encodes for a protein with 1603 aa. To identify DNA polymorphisms in *TRPM1*gene that may contribute to ACR-related traits, exons including their splice sites, and 2 kb upstream from exon 1 were PCR amplified and re-sequenced in 32 individuals. Re-sequencing of *TRPM1* identified 18 single nucleotide polymorphisms (SNPs) including 7 in the putative promoter region, 8 in the coding region and 3 in the introns (Fig. 1). Of the 8 SNPs identified in the coding region, four are synonymous [Thr(413)Thr, Try(769)Tyr, Asn(780)Asn, Asn(825)Asn], three are non-synonymous SNPs [Met(1)Thr, Ser(32)Asn, Val(1395)Ile] and one SNP caused nonsense stop codon. A G to T substitution at codon 1375 changes the codon (GAA) encoding amino acid Glutamic acid into a Ochre codon (TAA) creating a stop codon, which may produce a predicted premature TRPM1 protein.

Based on the re-sequencing data of *TRPM1* in 32 subjects, five promoter variants could be divided into two groups, indicative of distinct linkage disequilibria (LD). These include SNPs 3, 4 (SNP cluster I), and SNPs 5, 6, 7 (SNP cluster II). Therefore, SNPs 4 (cluster I), 6 (cluster II), were selected as representative markers for each unique cluster of variants for further analysis (Fig. 1). The remaining 13 polymorphisms (SNPs-1, 2, 8 to 18) could not be assigned to any group and were analyzed individually (Fig. 1). In total, we genotyped 15

SNPs (SNPs-1, 2, 4, 6, 8 to 18) in the entire data set (N = 670) by TaqMan assays (Table 2). Genotypic data of all the examined polymorphisms were consistent with the Hardy-Weinberg Equilibrium expectations, and there was no evidence for hidden population stratification in the data as tested by QTDT. Before performing statistical association analysis, we estimated the pairwise LD ($r^2$) between all the 15 polymorphisms genotyped. Figure 2 shows the overall pattern of LD as measured by the $r^2$values. As can be seen 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 *TRPM1* SNPs were: SNP-1 – SNP-4 ($r^2$=1.00), SNP-11 – SNP-12 ($r^2$=0.94), and SNP-10 – SNP-11 ($r^2$=0.78).

Association analysis between genotypic and phenotypic data was performed, using the measured genotype approach as implemented in the program SOLAR. In addition to association analysis between *TRPM1* genotypes and ACR in our data, association analyses were also extended to available albuminuria-reated phenotypic data including systolic blood pressure (SBP), diastolic blood pressure (DBP), cholesterol (CHOL), BMI, TGL, HDL-C, eGFR, and T2DM. The location, allele frequencies, and association analyses of 15 SNPs examined are summarized in Table 2. The minor allele frequencies of the polymorphisms ranged from 6.3% (SNP-17) to 42.2% (SNP-1). Of the 15 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 (ACE inhibitors or AT1R antagonists). However, a promoter variant (SNP-6, rs11070811) was found to be significantly associated with TGL ($P = 0.039$) after accounting for the covariate effects of age, sex, diabetes, and lipid medications, which explains only 0.82% of total phenotypic variation in TGL (Table 2). No other examined trait exhibited any statistical evidence for association.

## 4. Discussion

In recent years, various TRP channels have been discovered and their relationship to kidney function and dysfunction has been brought to light. TRPC3 is thought to facilitate calcium ion influx into the principal cells of the collecting duct in response to vasopressin [20]. In renal epithelial cells, TRPV4 activation results from hypotonic cell swelling suggesting that this channel functions as an osmosensor [21]. TRPP1 and TRPP2 form a complex and function as a mechanosensor in the cilia of renal epithelial cells [22]. A number of kidney diseases have also been linked to dysfunctional activity of TRPs. Mutations in *TRPC6* have been associated with focal segmental glomerosclerosis [23]. Mutations in *TRPP1* and *TRPP2* are linked with autosomal-dominant polycystic kidney disease [24]. TRPV1 dysfunction is implicated in renal hypertension [25]. A link between TRPC1 dysfunction and diabetic nephropathy has also been suggested in an animal model [26]. Animal studies have also implicated a role for TRPV5 in idiopathic hypercalciuria and vitamin D-dependent rickets [27]. Taken together, TRP channel proteins are involved in many pathophysiological processes and have emerged as novel therapeutic targets.

TRPM1, the founding member of TRPM family of TRP super family, was originally characterized as the product of a gene whose expression is decreased in melanomas and is useful as a prognostic marker for metastasis [28]. Expression of TRPM1 in HEK293 cells elicits $Ca^{2+}$ influx [9]. Mutations in *TRPM1* have been associated with complete congenital stationary night blindness [11-13]. Other member of the TRPM family is TRPM2. TRPM2 possesses both ion channel and enzymatic activity as adenosine diphosphoribose (ADPR) hydrolase [29]. Electrophysiological analysis clearly indicates that TRPM2 functions as a plasma membrane nonselective cation channel permeable to calcium. It is activated and regulated by various factors, including cytosolic ADP-ribose and oxidants [29]. In HEK293 cells expressing TRPM2, the intracellular delivery of ADP-ribose increases cytosolic

calcium concentrations [30]. Patients with hypomagnesemia with secondary hypocalcemia, a primary defect in intestinal magnesium absorption, were found to carry mutations in TRPM6 suggesting that TRPM6 is critically involved in transcellular Mg2+ transport in the kidney [31]. A close homologue of TRPM6, TRPM7, is a magnesium and calcium permeable ion channel vital for cellular magnesium homeostasis. TRPM6 specifically interacts with its closest homolog, the Mg(2+)-permeable cation channel TRPM7, resulting in the assembly of functional TRPM6/TRPM7 complexes at the cell surface and forming a kinase domain with homology to the family of atypical alpha kinases [32].

In consideration of the above discussed functional relevance of TRPM family members, in this study, we investigated *TRPM1* as a positional candidate gene for DNA sequence variants based on its location on human chromosome 15q12, a region previously found to be linked with albuminuria in Mexican Americans as well as on the functional relevance of TRPM1 in eliciting Ca2+ influx in vitro. Of the 15 SNPs genotyped in the entire cohort and examined for their association, none of the SNP exhibited statistically significant association with variation in ACR after accounting for the potential covariate effects (Table 2). Therefore, the DNA sequence variants that we identified and examined in *TRPM1* appear not to significantly contribute to variation in urinary albumin excretion in this population. However, these results should be interpreted with caution for the following reasons. It is possible that the genetic variants within unidentified key regulatory elements located either deeper within introns or further upstream or downstream of *TRPM1* could influence gene expression or function thereby contributing to albuminuria. Another limitation is the modest sample size (N = 32) that we used for resequencing and identifying polymorphisms within *TRPM1*, which limits our ability to detect potential rare variants specific to this population.

Our association analyses detected evidence for a modest association between a SNP located in putative promoter region (rs11070811) and TGL (***P*** = 0.039). In consideration of the stated power to detect nominally significant statistical association in our study, this finding is rather suggestive, but may have occurred by chance alone. Additional efforts are to needed to confirm this finding in larger data sets. However, the observed triglycerides association appears to be interesting for the following reasons. The *TRPM1* (15q13.3, 31.3 Mb) is a positional candidate gene within the critical region around the albuminuria linkage peak near the marker *GABRB3* region (at 26.8 Mb) on chromosome 15q12. The albuminuria linkage region strongly overlaps with the significant triglycerides linkage signal that we reported previously in our SAFDGS data which occurred between marker *GABRB3* (5q12, 26.8 Mb) and *D15S165* (15q13.3, 31.3 Mb) regions [15]. In addition, a large genome-wide association meta-analysis was recently performed to localize variants/genes that influence variation in circulating lipid levels and risk of coronary artery disease, using a large initial sample containing individuals of white European descent and two large replication samples [33]. As reported in their supplementary material (Suppl. Tables 4 and 5), some evidence for association of triglycerides was found with a marker (rs4906879, 26.7 Mb, initial study: $P = 5.9 \times 10^{-7}$ and replication study: $P = 2.3 \times 10^{-3}$) near the *GABRB3* region. In another genome-wide association meta-analysis, a modest association was found between rs2676035 (15q14, 33.6 Mb) and HDL-cholesterol in Stage 1 analysis ($P = 9.3 \times 10^{-7}$), although it failed to be significant in Stage 2 analysis [34]. Thus, the 15q12-q14 chromosomal region appears to be promising for further lipid gene discovery activities.

In conclusion, our findings suggest that the genetic variation at *TRPM1* may not be a significant contributor to variation in ACR and its related traits in Mexican Americans. However, our triglycerides association finding together with the known information from both genome-wide linkage and association studies reveals that the *GRBRB3* region and its vicinities may contain loci that potentially influence susceptibility to dyslipidemia in Mexican Americans.

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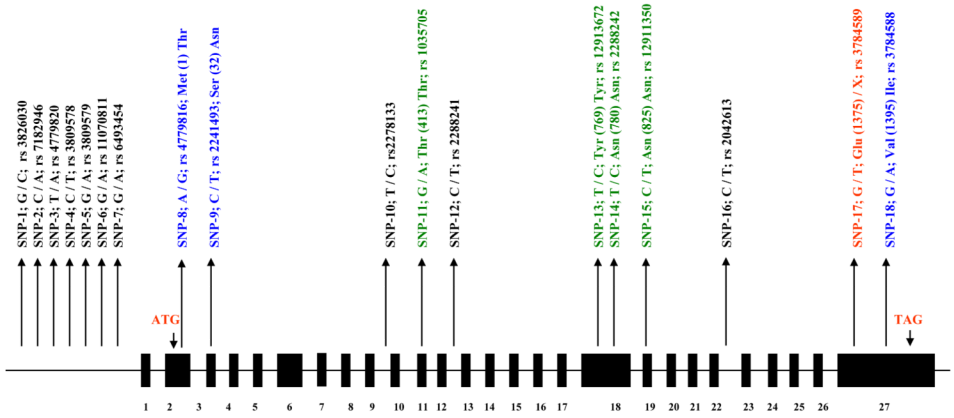


**Fig. 1.**

Schematic diagram of human *TRPM1*(NM_002420) gene structure on 15q13.3 and the location of the polymorphisms identified and genotyped in SAFDGS. The exons of the *TRPM1*are represented by solid box and the intron by a thin line. Polymorphisms with the base change, amino acid substitutions and their reference sequence (rs) numbers are indicated by the vertical arrow.

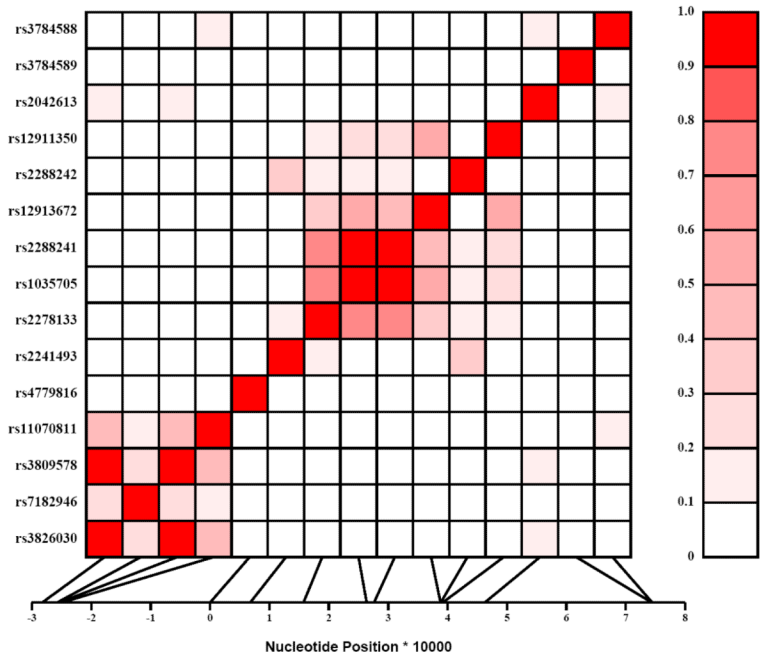


**Fig. 2.**

Linkage disequilibrium (LD) between polymorphisms within the *TRPM1*gene. SNPs are labeled on the y-axis, and the locations (bp) 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. The diagonal represents a comparison of each polymorphism against itself (i.e., $r^2$=1.0).

## Table 1

### Clinical characteristics of the genotyped SAFDGS participants [a]

| Phenotypes [b] | Mean ± SD or % |
|---|---|
| Sex (Females) | 61 |
| Type 2 diabetes | 28 |
| Hypertension | 29 |
| Albuminuria | 14 |
| Age (years) | 44.8 ± 16.2 |
| Systolic blood pressure (mm Hg) | 128.6 ± 18.2 |
| Diastolic blood pressure (mm Hg) | 71.3 ± 9.6 |
| Body mass index (kg/m$^2$) | 30.9 ± 7.0 |
| Waist circumference (mm) | 1001.0 ± 170.6 |
| Total Cholesterol (mg/dl) | 194.0 ± 37.9 |
| High density lipoprotein-Cholesterol (mg/dl) | 46.0 ± 12.0 |
| *ln* Triglycerides | 4.9 ± 0.6 |
| GFR-MDRD (ml/min/1.73 m$^2$) | 88.4 ± 17.9 |
| *ln* Albumin to creatinine ratio | 2.4 ± 0.8 |

[a] Sample size varies from 610 (cholesterol) to 670 (Age) except for glomerular filtration rate (GFR). The sample size for GFR is = 453

[b] GFR-MDRD - GFR estimated by four variable modification of diet in renal disease (MDRD) formula. *ln* = log transformed.

**Table 2**

**Association analysis between the TRPM1 polymorphisms and ACR related traits**

| SNPs | Gene location | Major/Minor Frequencies (%) | Trait[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | BMI[b] | CHOL[c] | HDL[c] | TGL[c] | eGFR[d] | SBP[e] | DBP[e] | ACR[f] | T2DM[g] |
| | | | P-value | | | | | | | | |
| **SNP-1 (rs3826030)** | Promoter | G (57.8) / C (42.2) | 0.1169 | 0.6895 | 0.2712 | 0.5310 | 0.8404 | 0.2219 | 0.6988 | 0.8187 | 0.9468 |
| **SNP-2 (rs7182946)** | Promoter | T (75.8) / G (24.2) | 0.4707 | 0.9909 | 0.9933 | 0.0716 | 0.4584 | 0.8458 | 0.7984 | 0.4416 | 0.7617 |
| **SNP-4 (rs3809578)** | Promoter | C (57.8) / T (42.2) | 0.1169 | 0.6895 | 0.2712 | 0.5310 | 0.8404 | 0.2219 | 0.6988 | 0.8187 | 0.9468 |
| **SNP-6 (rs11070811)** | Promoter | C (66.2) / T (33.8) | 0.3302 | 0.5796 | 0.2973 | **0.0392** | 0.7144 | 0.2427 | 0.4370 | 0.3413 | 0.7620 |
| **SNP-8 (rs4779816)** | Met(1)Thr | G (89.7) / A (10.3) | 0.2302 | 0.6330 | 0.2044 | 0.9231 | 0.6382 | 0.7640 | 0.6413 | 0.5222 | 0.5857 |
| **SNP-9 (rs2241493)** | Ser(32)Asn | T (91.2) / C (8.8) | 0.9889 | 0.5801 | 0.2899 | 0.6854 | 0.8715 | 0.2733 | 0.5011 | 0.7377 | 0.6346 |
| **SNP-10 (rs2278133)** | Intron-9 | G (62.3) / A (37.7) | 0.1807 | 0.6262 | 0.2454 | 0.7851 | 0.6555 | 0.6258 | 0.3385 | 0.9656 | 0.3497 |
| **SNP-11 (rs1035705)** | Thr(413)Thr | A (60.6) / G (39.4) | 0.4193 | 0.5892 | 0.6367 | 0.9023 | 0.6620 | 0.6411 | 0.2913 | 0.7687 | 0.6047 |
| **SNP-12 (rs2288241)** | Intron-12 | T (59.1) / C (40.9) | 0.5513 | 0.2720 | 0.4861 | 0.7875 | 0.7077 | 0.8211 | 0.3088 | 0.6446 | 0.6259 |
| **SNP-13 (rs12913672)** | Tyr(769)Tyr | T (75.0) / C (25.0) | 0.3782 | 0.3642 | 0.7672 | 0.6246 | 0.7721 | 0.6284 | 0.8443 | 0.7800 | 0.4861 |
| **SNP-14 (rs2288242)** | Asn(780)Asn | G (88.6) / A (11.4) | 0.3932 | 0.5965 | 0.6129 | 0.6662 | 0.2795 | 0.7872 | 0.8719 | 0.9029 | 0.4672 |
| **SNP-15 (rs12911350)** | Asn(825)Asn | G (85.1) / A (14.9) | 0.9899 | 0.8943 | 0.9674 | 0.7872 | 0.4372 | 0.8160 | 0.1738 | 0.2873 | 0.9349 |
| **SNP-16 (rs2042613)** | Intron-22 | T (68.4) / C (31.6) | 0.7439 | 0.3119 | 0.8490 | 0.6725 | 0.3430 | 0.3014 | 0.6980 | 0.7397 | 0.8831 |
| **SNP-17 (rs3784589)** | Glu(1375) / * | C (93.7) / A (6.3) | 0.5654 | 0.6928 | 0.5396 | 0.7571 | 0.5199 | 0.3608 | 0.5331 | 0.8311 | 0.3120 |
| **SNP-18 (rs3784588)** | Val(1395)Ile | C (84.6) / T (15.4) | 0.6442 | 0.5531 | 0.1807 | 0.1421 | 0.8522 | 0.9757 | 0.9635 | 0.9520 | 0.4602 |

[a] Traits; BMI = Body mass index, CHOL = Cholesterol, HDL = High-density lipoprotein cholesterol, TGL = Triglycerides, eGFR = Estimated Glomerular filtration rate, SBP = Systolic blood pressure, DBP = diastolic blood pressure, ACR = Albumin to creatinine ratio, T2DM = Type 2 diabetes

[b] adjusted for age and sex terms, diabetes

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

[d] adjusted for age and sex terms, diabetes, duration of diabetes, systolic blood pressure, bmi and anti hypertensive treatment

[e] adjusted for age and sex terms, diabetes and anti hypertensive treatment

[f] adjusted for age and sex terms, diabetes, duration of diabetes, systolic blood pressure and anti hypertensive treatment

[g] adjusted for age and sex terms

* - stop codon