European Review for Medical and Pharmacological Sciences 2015; 19: 1388-1397

Role of the transient receptor potential (TRP) channel gene expressions and TRP melastatin (TRPM) channel gene polymorphisms in obesity-related metabolic syndrome

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Abstract. – OBJECTIVE: Metabolic syndrome (MetS) is correlated with increased cardiovascular risk and characterized by several factors, including visceral obesity, hypertension, dyslipidemia, and insulin resistance. The etiology of MetS is complex, and can be influenced by genetic susceptibility. The aim of this study was to investigate a possible association of transient receptor potential (TRP) channels gene expressions and TRP melastatin (TRPM) gene polymorphisms with MetS in a Turkish population.

PATIENTS AND METHODS: A total of 142 patients with obesity-related MetS and 166 healthy controls with similar age and sex were enrolled to this study. For polymorphism studies, genomic DNA from the participants was analyzed by a BioMark 96.96 dynamic array system (Fluidigm, South San Francisco, CA, USA). For gene expression studies, mRNA from blood samples was extracted, and real time polymerase chain reaction on the BioMark HD system was performed.

RESULTS: There was an increase in A allele (64.6% in patients vs. 49.5% in controls) and decrease in G allele frequencies (35.4% in patients vs. 50.5% in control, $p = 0.0019$) of the TRPM5 gene rs4929982 (Arg578Gln) polymorphism. We also observed that the distribution of genotype and allele frequencies of the TRPM8 gene rs12472151 in MetS patients were significantly different from controls ($p < 0.0001$). Although there were marked decreases in TRPC1, TRPC3, TRPM2, TRPM5, TRPV4, TRPV5, TRPV6, MCOLN2 (TRPML2), and MCOLN3 (TRPML3) gene expressions, an augmentation was noted in TRPC6 gene expression.

CONCLUSIONS: Genetic polymorphisms in TRPM5 and TRPM8 genes may modify individual susceptibility to MetS in the Turkish population. This study also revealed that there is a significant relationship between TRP channels gene expressions and MetS.

Key Words:
Expression, Gene variants, Metabolic syndrome, Obesity, Transient receptor potential.

Introduction

Metabolic syndrome (MetS) is characterized by a combination of visceral obesity, hypertension, insulin resistance, dyslipidemia, and impaired glucose tolerance. Experimental, epidemiological, and clinical studies have demonstrated that patients with MetS have significantly elevated cardiovascular morbidity and mortality[1]. The prevalence of MetS is about 30% worldwide[2]. The prevalence of obesity and the MetS is rapidly increasing, leading to increased morbidity and mortality. A survey in Turkey reported a prevalence of 33.9% for the MetS, with a higher prevalence in women (39.6%) than in men (28%)[3]. The prevalence of MetS was also found to be 37.5% in Turkish patients with non-diabetic first acute ST elevation myocardial infarction[4].

The exact cause of MetS is not known. Several studies have found that people with higher calcium intakes have a lower prevalence of MetS and its components[5-7]. Calcium is known to play a key role in blood pressure control and adipocyte metabolism, and thus its effects on MetS could be partially mediated by its effects on body fat, blood pressure, and insulin sensitivity[8].

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Transient receptor potential (TRP) channels are the recently discovered major ion channel superfamily. There are 28 mammalian TRP channels, which are subdivided into six subfamilies based on protein sequence homology: TRPC for "canonical" (TRPC1–7), TRPV for "vanilloid" (TRPV1–6), TRPM for "melastatin" (TRPM1–8), TRPP for "polycystin" (TRPP2, TRPP3, TRPP5), TRPML for "mucolipin" (TRPML1–3), and TRPA for "ankyrin" (TRPA1)[9,10]. TRP channels are non-selective channels permeable to monovalent and divalent cations. Thus, changes in the function or expression of TRP channels could alter intracellular Ca^{2+} levels directly, indirectly or through membrane potential and the regulation of L-type Ca^{2+} channel activity[11].

TRP channels are broadly distributed in several cell types, including adipocytes, vascular smooth muscle cells and endothelial cells, which explains their wide spread function. Thus changes in expression or disturbance of TRP channel function likely result in the development of MetS. Oxidative stress is known to play a major role in the pathogenesis of MetS[12-14]. TRPM channels are also activated by oxidative stress[10]. Our hypothesis is that the TRPM polymorphisms or gene expressions are associated with MetS.

The progression of MetS is influenced by genetic susceptibility and environmental factors, including diet, and the interactions between them[15-17]. Heritability estimates of the MetS range from 13% to 27%[18,19]. Recently, published genome-wide association studies have described candidate genes, including fat mass and obesity associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes, that contribute to MetS and its phenotypes around the world[20,21]. Changes of TRP channel gene expressions or polymorphisms may account for the observed increased cardiovascular risk in MetS patients. A finding of gene-calcium interactions could improve our understanding of the mechanisms of metabolic alterations and could eventually lead to effective, personalized prevention of MetS. Thus, in this study we aimed to investigate whether TRP channel gene expressions and TRPM gene polymorphisms are associated with susceptibility to obesity-related MetS in a Turkish population.

Patients and Methods

Study Populations

A total of 308 unrelated Turkish subjects, 142 with obesity-related MetS and 166 non-MetS controls evaluated at Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey were recruited into this study. The study was approved by the local Ethics Committee, and written informed consent prior to participation in the study was obtained from patients and healthy volunteers according to the Declaration of Helsinki.

MetS was defined by using modified criteria proposed by the Third Report of the National Cholesterol Education Program Adult Treatment Panel[22,23]. A MetS diagnosis was made when a subject fulfilled three of the following five criteria: waist circumference ≥ 102 cm in men and ≥ 88 cm in women, triglyceride (TG) ≥ 150 mg/dl or treatment of dyslipidemia, high density lipoprotein (HDL) cholesterol < 40 mg/dl in men and < 50 mg/dl in women or treatment of dyslipidemia, systolic and diastolic blood pressure ≥ 130 and 85 mm Hg or antihypertensive treatment, and fasting blood glucose ≥ 100 mg/dl or treatment of type 2 diabetes. Patients who had a history of percutaneous coronary intervention within 6 months or coronary artery bypass surgery within 1 year were excluded. Other exclusion criteria included patients who had heart failure, cardiomyopathy, or valvular heart diseases. All patients were in the follow up of the Endocrinology Clinic.

All sex and age-matched controls were healthy and had no symptoms of MetS. The selection criteria of the controls were that all control subjects were from the same geographical area with a similar socioeconomic and ethnic background and were admitted to outpatient clinic. The socioeconomic background was identified by the information obtained from the patient and the controls through a health questionnaire. All the patients and controls were in the middle income status. Exclusion criteria for selecting control subjects were presence of coronary artery disease, peripheral occlusive arterial disease, coagulopathy, vasculitis, autoimmune disease, severe kidney and hepatic diseases, cancer, and pregnancy.

Biochemical Analysis

Venous blood samples were drawn from each subject after ≥ 8 h or overnight fasting. The samples were stored at –80°C until biochemical assay by blinded investigators. All routine chemistry was conducted by the standard laboratory techniques in the Clinical Biochemistry Labora-

tory. Triglycerides were determined enzymatically. High-density lipoprotein cholesterol was measured by the phosphotungstate method. Glucose was enzymatically determined by the hexokinase method.

Blood Samples and DNA Isolation

Peripheral venous blood samples (5 ml) were collected by venipuncture into sterile siliconized Vacutainer tubes with 2 mg/ml disodium ethylenediaminetetraacetic acid. All samples were stored at -20°C until use. Genomic DNA was extracted from whole blood using with salting-out method and stored at -20°C.

Genotyping

The Fluidigm Digital Array is a nanofluidic biochip where digital PCR reactions can be performed with isolated individual DNA template molecules. Polymorphisms were analyzed in genomic DNA using a 96.96 dynamic array on the BioMark HD system (Fluidigm, South San Francisco, CA, USA). The Digital PCR Analysis software (Fluidigm, South San Francisco, CA, USA) was used to process the data after the reaction. Chambers that yielded signals were detected and counted. In the present study, 25 SNPs [rs28441327, rs11070811, rs2241493 (Ser71Asn), rs111649153 (Arg1518His), rs1618355, rs1328142, rs3760663, rs34364959 (Gly900Ser), rs4929982 (Arg578Gln), rs886277 (Asn235Ser), rs34551253 (Ala456Thr), rs3986599 (Val254Ala), rs3750425 (Val1388Ile), rs62569677 (Asn877Asp), rs55924090 (Ile459Thr), rs1016062, rs2362294, rs2362295, rs10490018, rs2052029, rs6431648, rs10803666, rs12472151, rs2215173, rs6740118] were studied for *TRPM1-8* gene polymorphisms.

cDNA Synthesis and Gene Expression

A total of 54 patients with obesity-related MetS, and 41 healthy control subjects with similar age and sex were included to this study. To confirm the expression of TRP proteins in blood, mRNA was isolated from leukocytes by using β-mercaptoethanol, and stored at –80°C until use. cDNA was produced with the Qiagen miScript Reverse Transcription Kit according to manufacturer's protocol. PCR was performed by BioMark HD system (Fluidigm, South San Francisco, CA, USA) with TRP channel primers and, beta-actin (ACTB, housekeeping gene). We screened 26 TRP channel genes [*TRPA1, TRPC1-7, TRPM1-8, TRPV1-6, MCOLN1-3 (TRPML1-3),* and *PKD2 (TRPP2)*] for expression study. Data were analyzed using the $2^{-\Delta Ct}$ method, according to the formula: $\Delta C_t = C_{tTR} - C_{tACTB}$, where C_t = threshold cycle.

Statistical Analysis

Results are expressed as the means ± S.D. or percentage otherwise indicated. The Chi-square test for independence, Chi-square test with Yate's correction or Fisher's exact tests were used for calculation of the significance of differences in genotype and allele frequencies. The original significance level was set at a p value of 0.05. All probability values were based on two-tailed tests. To conclude the association, we used the Bonferroni method to correct the p values for multiple testing, using a stringent threshold. A p value of < 0.002 (0.05/25) was considered statistically significant after Bonferroni correction for multiple testing. For comparisons of the differences between mean values of two groups, the unpaired Student's t test was used. For calculation of the significance of differences in gene expressions, the Mann-Whitney U-test was used. Two-tailed significance tests were used. Statistical analysis was performed using GraphPad Instat version 3.05 (GraphPad Software Inc., San Diego, CA, USA).

Results

Demographic and clinical characteristics of the study population are presented in Table I. The prevalence of cardiovascular risk factors, including hypertension, fasting glucose levels, lipid profiles, smoking, body mass index (BMI), and waist circumference, are shown for control and MetS subjects. Compared with the control group, the average age, gender, percentage of smokers, alcohol intake, creatinine, and alanine aminotransferase levels in the MetS group were similar. BMI, waist circumference, blood pressure, fasting glucose, total cholesterol, LDL cholesterol, TG, and high-sensitive C-reactive levels protein were all greater among MetS subjects, and HDL cholesterol levels were lower among MetS subjects. All of the patients were obese with high BMI (40.19±6.32 kg/m^2). Of the MetS patients, 26.1% was diagnosed with type 2 diabetes (Table I).

Genotype and allele frequencies of *TRPM1, TRPM2, TRPM3, TRPM4,* and *TRPM5* gene polymorphisms in MetS and control groups are pre-

Table I. Baseline demographic and clinical characteristics of MetS patients and controls

	Patients (n = 142)	Controls (n = 166)	p value
Age (years)[a]	42.25 ± 12.22	41.89 ± 9.42	0.7708
Gender			
Male (n, %)	15 (10.6)	29 (17.5)	0.1025
Female (n, %)	127 (89.4)	137 (82.5)	
Smoking status			
Current (n, %)	19 (13.4)	20 (12.1)	0.9383
Never (n, %)	114 (80.3	135 (81.3)	
Past (n, %)	9 (6.3)	11 (6.6)	
Alcohol intake	6 (4.2)	9 (5.4)	0.7920
BMI (kg/m^2)[a]	40.19 ± 6.32	22.61 ± 2.00	< 0.0001
Waist circumference (cm)[a]	118.10 ± 12.99	82.12 ± 8.23	< 0.0001
Systolic BP (mm Hg)a	131.81 ± 15.69	117.95 ± 8.65	< 0.0001
Diastolic BP (mm Hg)[a]	86.39 ± 13.69	74.88 ± 5.36	< 0.0001
Diabetes mellitus (n, %)	37 (26.1)	–	
Fasting plasma glucose (mg/dl)[a]	116.62 ± 58.52	86.64 ± 6.89	< 0.0001
HbA1c (%)[a]	6.33 ± 1.81	–	
Creatinine (mg/dl)[a]	0.68 ± 0.14	0.71 ± 0.15	0.0722
Alanine aminotransferase (IU/l)[a]	24.83 ± 14.56	23.86 ± 13.38	0.5430
Total cholesterol (mg/dl)[a]	198.46 ± 45.46	152.64 ± 18.77	< 0.0001
Low density lipoprotein cholesterol (mg/dl)[a]	129.60 ± 32.63	96.12 ± 14.37	< 0.0001
High density lipoprotein cholesterol (mg/dl)[a]	40.68 ± 8.15	43.12 ± 5.75	0.0024
Triglyceride (mg/dl)[a]	174.04 ± 66.43	123.46 ± 26.45	< 0.0001
Uric acid (mg/dl)[a]	4.94 ± 1.30	–	
High-sensitive C-reactive protein	1.92 ± 3.62	0.27 ± 0.18	< 0.0001
Insulin (pmol/l)[a]	22.10 ± 16.43	–	
HOMA-IR[a]	6.00 ± 4.68	–	

[a]Data are mean ± S.D. BMI, body mass index. BP, blood pressure. Fasting glucose and insulin plasma levels are used to calculate homeostasis model assessment of insulin resistance (HOMA-IR).

sented in Table II. Although there was marked changes in allele frequency of the *TRPM5* gene rs4929982 (Arg578Gln) polymorphism (A, 49.5%; G, 50.5% in control vs. A, 64.6%; G, 35.4% in MetS $p < 0.0019$), no significant differences were noted in genotype distribution. Genotype and allele frequencies of *TRPM6, TRPM7,* and *TRPM8* gene polymorphisms in MetS and control groups are presented in Table III. We have observed maked associations of *TRPM8* rs12472151 polymorphism with MetS. CT genotype (41.6%) of rs12472151 polymorphism were more frequent, and CC genotype (56.9%) were less frequent among the patients than controls (CT, 16.8%, and CC, 82.0%, $p < 0.0001$). There was an increase in T allele (22.3% vs. 9.6%) and decrease in C allele frequencies (77.7% vs. 90.4%, $p < 0.0001$) in patients (Table III). However, no associations were found with the other 23 polymorphisms studied (Tables II and III).

Gene expression analysis showed that *TRPM2, TRPM5* mRNA contents in leukocytes were markedly depressed in MetS patients when compared to the control groups ($p < 0.05$, Figure 1A). There were also marked reductions in *TRPC1, TRPC3, TRPV4, TRPV5, TRPV6, MCOLN2 (TRPML2),* and *MCOLN3 (TRPML3)* gene expressions in MetS ($p < 0.05$, Figures 1B-D). Only significant increase was observed with *TRPC6* gene expression in patients (p=0.0035, Figure 1B). However, no significant changes in expressions were found with *TRPA1, TRPC4, TRPC5, TRPC7, TRPM1, TRPM3, TRPM4, TRPM6, TRPM7, TRPM8, TRPV1, TRPV2, TRPV3, MCOLN1 (TRPML1),* and *PKD2 (TRPP2)* genes in MetS patients ($p > 0.05$).

Discussion

In this case-control study, we have shown that *TRPM5* gene rs4929982 (Arg578Gln) and *TRPM8* gene rs12472151 polymorphisms were significantly associated with obesity-related MetS and could be the risk factor of developing MetS. Our results suggest A allele of the rs4929982 (Arg578Gln) polymorphism, and CT genotype and T allele of the rs12472151 polymorphism may increase the susceptibility to MetS. Additionally, suppressed *TRPC1, TRPC3,*

Table II. Distributions of genotypes and alleles for the *TRPM1*, *TRPM2*, *TRPM3*, *TRPM4*, and *TRPM5* gene polymorphisms between the case and control groups.

Gene SNP	Genotypes/alleles	Controls	n*	Cases with MetS	n*	*p* value
TRPM1	CC/CT/TT	109/43/14	166	91/31/16	138	0.5684
rs28441327	C/T	261/71		213/63		0.7426
TRPM1	GG/GA/AA	105/48/8	161	93/37/10	140	0.6337
rs11070811	G/A	258/64		223/57		0.9641
TRPM1	AA/AG/GG	86/52/20	158	87/35/17	139	0.3065
rs2241493 (Ser71Asn)	A/G	224/92		209/69		0.2791
TRPM1	CC/CT/TT	157/5/0	162	132/6/0	138	0.7596
rs111649153 (Arg1518His)	C/T	319/5		270/6		0.7619
TRPM2	TT/TG/GG	93/47/23	163	93/27/18	138	0.1376
rs1618355	T/G	233/93		213/63		0.1343
TRPM3	CC/CA/AA	130/22/9	161	109/20/6	135	0.8787
rs1328142	C/A	282/40		238/32		0.8998
TRPM4	CC/CT/TT	91/44/24	159	65/46/20	131	0.3575
rs3760663	C/T	226/92		176/86		0.3569
TRPM5	CC/CT/TT	157/0/4	161	134/0/5	139	0.7376
rs34364959 (Gly900Ser)	C/T	314/8		268/10		0.4774
TRPM5	AA/AG/GG	51/6/52	109	68/10/35	113	0.0354
rs4929982 (Arg578Gln)	A/G	108/110		146/80		0.0019
TRPM5	AA/AG/GG	68/57/34	129	63/44/25	132	0.6914
rs886277 (Asn235Ser)	A/G	193/125		170/94		0.4055
TRPM5	CC/CT/TT	52/2/6	60	70/2/5	77	0.7236
rs34551253 (Ala456Thr)	C/T	106/14		144/12		0.3016
TRPM5	GG/GA/AA	115/23/18	156	102/14/18	134	0.5203
rs3986599 (Val254Ala)	G/A	253/59		218/50		0.9379

*Numbers do not always add up to total numbers because of missing values on the BioMark dynamic array system. SNP, single nucleotide polymorphism. MetS, metabolic syndrome.

TRPM2, TRPM5, TRPV4, TRPV5, TRPV6, MCOLN2 (TRPML2), and *MCOLN3 (TRPML3)* genes and elevated *TRPC6* gene expressions were observed in cases with MetS. To the best of our knowledge, this is the first study to examine the association of the *TRPM* gene polymorphisms with the risk of developing MetS. This is also first study to evaluate the TRP channels gene expressions in MetS.

The exact pathophysiological events leading to the development of MetS remain unknown. Almost all MetS patients suffer from abdominal obesity. The risk of MetS increases with age and increasing BMI. Our patients in the present study had high BMI. Obesity itself can precipitate an inflammatory response and lead to free radical generation[24]. Overnutrition will supply excess electrons to the mitochondrial respiratory chain, while lack of physical activity and the consequent low ATP demand will favor a high proton motive force, a low respiration rate, and mitochondrial superoxide formation[25-27]. Therefore, elevated mitochondrial oxidative stress may contribute to the metabolic syndrome[28,29]. Decrease mitochondrial reactive oxygen species levels prevent pathology in animal models of metabolic syndrome[30,31]. It is known that TRPM2 functions as a plasma membrane nonselective cation channel permeable to calcium, and it is activated by reactive oxygen and nitrogen species[32,33]. We have observed decrease in TRPM2 channel gene expression in MetS. The functional role of this gene reduction in the development of MetS remains to be established.

TRPM2, TRPM3 and TRPM5 are considered as the most important TRP channels in regulating insulin release[34]. TRPM2 is a non-selective Ca^{2+} permeable cation channel. This channel is shown to be expressed in insulin secreting cell lines and in human and mouse pancreatic islets[35-37]. The *Trpm2*$^{-/-}$ mice exhibit higher basal glucose levels and an impaired glucose tolerance caused by lower plasma insulin levels[38]. There is evidence that rosiglitazone, used in the treatment of type 2 diabetes as an peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, can inhibit TRPM2 and TRPM3 channels independently of PPAR-γ[39]. Troglitazone and pioglitazone also inhibit TRPM3[39]. Consequences of blocking TRPM3 would be reduced insulin secretion from the pan-

Table III. Distributions of genotypes and alleles for the *TRPM6*, *TRPM7*, and *TRPM8* gene polymorphisms between the case and control groups.

Gene SNP	Genotypes/alleles	Controls	n*	Cases with MetS	n*	*p* value
TRPM6	GG/GA/AA	120/35/7	162	98/32/8	138	0.7771
rs3750425 (Val1388Ile)	G/A	275/49		228/48		0.5217
TRPM6	AA/AG/GG	159/1/0	160	133/1/0	134	1.0000
rs62569677 (Asn877Asp)	A/G	319/1		267/1		1.0000
TRPM7	TT/TC/CC	159/3/0	162	135/1/0	136	0.6282
rs55924090 (Ile459Thr)	T/C	321/3		271/1		0.6295
TRPM8	CC/CT/TT	119/35/5	159	100/27/5	132	0.9153
rs1016062	C/T	273/45		227/37		0.9626
TRPM8	TT/TC/CC	61/42/56	159	44/40/58	142	0.3902
rs2362294	T/C	164/154		128/156		0.1306
TRPM8	AA/AG/GG	98/51/15	164	75/39/19	133	0.3841
rs2362295	A/G	247/81		189/77		0.2833
TRPM8	GG/GA/AA	97/52/14	163	81/44/12	137	0.9973
rs10490018	G/A	246/80		206/68		0.9374
TRPM8	GG/GA/AA	98/49/17	164	81/40/16	137	0.9352
rs2052029	G/A	245/83		202/72		0.8586
TRPM8	CC/CT/TT	93/55/12	160	91/34/14	139	0.1593
rs6431648	C/T	241/79		216/62		0.5560
TRPM8	GG/GC/CC	131/27/3	161	118/18/3	139	0.6472
rs10803666	G/C	289/33		254/24		0.5771
TRPM8	CC/CT/TT	132/27/2	161	78/57/2	137	< 0.0001
rs12472151	C/T	291/31		213/61		< 0.0001
TRPM8	CC/CT/TT	108/41/10	159	103/24/6	133	0.1944
rs2215173	C/T	257/61		230/36		0.0863
TRPM8	GG/GA/AA	108/42/11	161	101/25/7	133	0.2472
rs6740118	G/A	258/64		227/39		0.1220

*Numbers do not always add up to total numbers because of missing values on the BioMark dynamic array system. SNP, single nucleotide polymorphism. MetS, metabolic syndrome.

creas[40]. It has been demonstrated that disruption of *Trpm2* or *Trpm5* genes in mice was found to impair insulin secretion[38,41], and stimulation of insulin secretion by pregnenolone sulfate was transduced by TRPM3 in isolated mouse pancreatic β-cells[40].

TRPM5 is predominately expressed in the proximal small intestine where it plays a key role in nutrient sensing and underlies a dynamic metabolic control. Interestingly, *Trpm5* expression is negatively correlated with blood glucose concentrations in the small intestine from diabetic patients[42]. Moreover, a recent study reports an association of *TRPM5* variants with prediabetic phenotypes in subjects at risk for type 2 diabetes, including insulin secretion, insulin sensitivity and plasma glucose and glucagon-like peptide-1 (GLP-1) levels[43]. Indeed, TRPM5 SNP rs2301699 was significantly associated with insulin secretion and associated with lower GLP-1 levels during an oral glucose tolerance test. Moreover, three TRPM5 SNPs (rs800344, rs800345 and rs2301699) were significantly associated with glucose levels during oral glucose tolerance test (OGTT). These SNPs were also associated with OGTT-derived insulin sensitivity[43]. How these *TRPM5* variants might affect insulin sensitivity remains elusive, since *Trpm5*$^{-/-}$ mice showed a normal insulin tolerance test[41]. Furthermore, the functional impact of these mutations on the TRPM5 channel has not been clarified yet. However, these data indicate a possible link between TRPM5 and type 2 diabetes mellitus. We have studied 5 SNPs, rs34364959 (Gly900Ser), rs4929982 (Arg578Gln), rs886277 (Asn235Ser), rs34551253 (Ala456Thr), and rs3986599 (Val254Ala) of the TRPM5. We found that the presence of A allele of the rs4929982 (Arg578Gln) polymorphism was associated with MetS, but no other marked associations with MetS were observed. We have also shown that TRPM5 gene experession was significantly depressed in MetS patients suggesting that TRPM5 may contribute to the development of MetS.

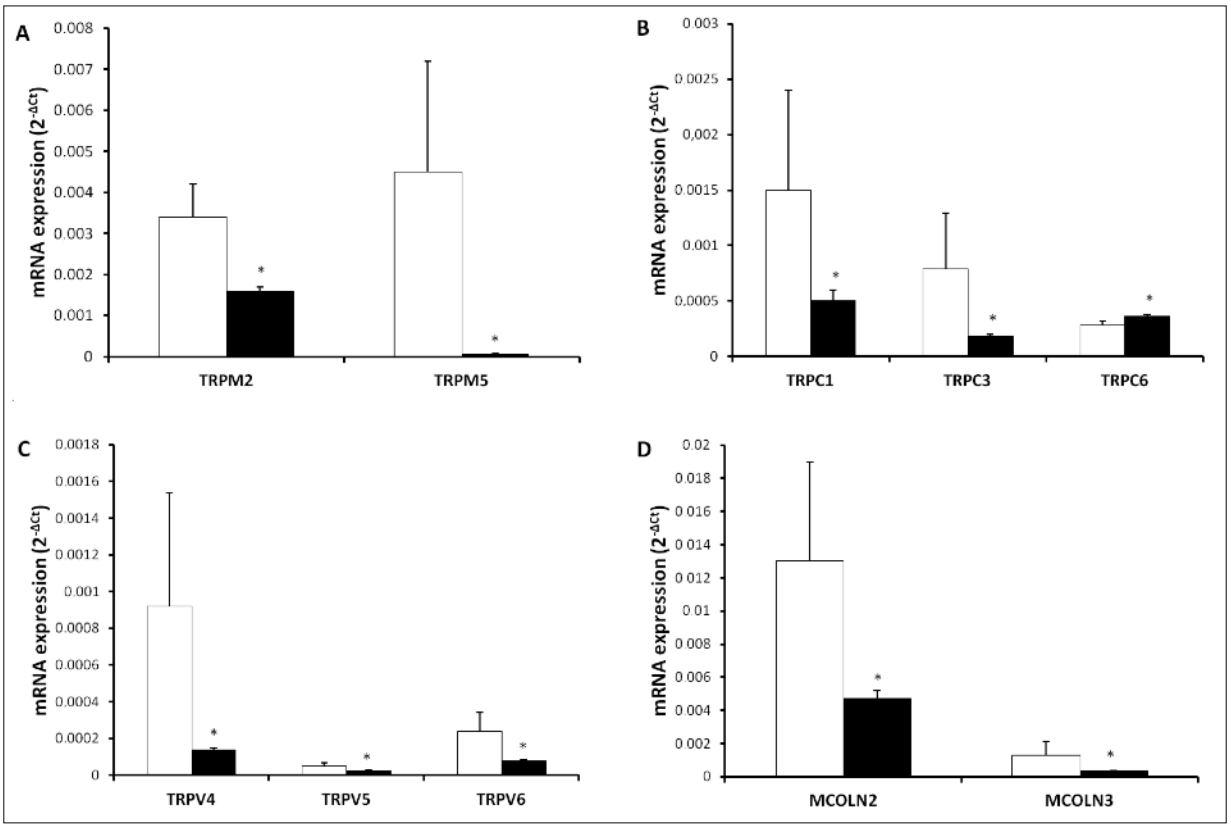

Figure 1. Comparison of the peripheral blood mRNA TRPM2, TRPM5 ***(A)***, TRPC1, TRPC3, TRPC6 ***(B)***, TRPV4, TRPV5, TRPV6 ***(C)***, MCOLN2 (TRPML2), and MCOLN3 (TRPML3) ***(D)*** expressions in healthy controls (n = 41, open bars) and in patients with metabolic syndrome (n = 54, solid bars). Values are given as mean ± SEM, *$p = 0.0004$, $p < 0.0001$, $p = 0.0082$, $p = 0.0025$, $p = 0.0035$, $p = 0.0326$, $p = 0.0079$, $p = 0.0003$, $p = 0.0029$, and $p < 0.0001$ values were obtained for TRPM2, TRPM5 ***(A)***, TRPC1, TRPC3, TRPC6 ***(B)***, TRPV4, TRPV5, TRPV6 ***(C)***, MCOLN2 (TRPML2), and MCOLN3 (TRPML3), respectively.

TRPM channels have also been associated with hypertension. TRP channels are expressed in every cell type present in the heart, including cardiomyocytes, fibroblasts, endothelial cells and vascular smooth muscle cells[10,44]. TRPM4 is a Ca^{2+} activated non-selective, Ca^{2+} impermeable cation channel that is expressed in atrial and ventricular tissue, in pacemaker cells, and in Purkinje fibres[45,46]. Several TRPs have been implicated already in blood pressure regulation[47,48]. It has been shown that *Trpm4* deficient mice display increased blood pressure through elevated catecholamine release[48]. Because TRPM7 preferentially mediates agonist-induced transplasma membrane magnesium influx, reduced TRPM7 expression is associated with reduced cytosolic magnesium concentrations, which may facilitate vasoconstriction[49]. Hu et al[50] demonstrated that TRPC1, TRPC5, and TRPC6 expression was higher in the adrenal medulla from pigs manifesting the MetS. However, we have observed decreased TRPC1 gene expression from leukocytes of the MetS patients. On the other hand, our TRPC6 results support the data presented by Hu et al[50]. Collectively, these findings suggest that TRP channels play a role in increased blood pressure observed in MetS.

Conclusions

Our data indicate that rs4929982 (Arg578Gln) (*TRPM5* gene) and rs12472151 (*TRPM8* gene) polymorphisms may contribute to individual susceptibility to MetS. Our data also imply that gene expressions of the TRP proteins play a role in the pathogenesis of MetS. TRPM, TRPC, TRPV and TRPML channels are likely novel risk markers for MetS and suggest that genetics of the TRP

channels are involved in the development or progression of MetS. Our data may suggest that TRP channels be a promising future therapeutic target in the treatment of MetS. However, further studies with a larger sample size are required to validate these results.

Acknowledgements

This study was funded by a project (TF.13.20) from the University of Gaziantep.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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