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Association of Rho/Rho-kinase gene polymorphisms and expressions with obesity-related metabolic syndrome

S. TABUR[1], S. OZTUZCU[2], E. OGUZ[3], H. KORKMAZ[1], S. EROGLU[2], M. OZKAYA[1], A.T. DEMIRYÜREK[4]

[1]Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[2]Department of Medical Biology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey
[3]Department of Medical Pharmacology, Faculty of Medicine, Harran University, Sanliurfa, Turkey
[4]Department of Medical Pharmacology, Faculty of Medicine, University of Gaziantep, Gaziantep, Turkey

Abstract. – OBJECTIVE: The metabolic syndrome (MetS) is a common multicomponent condition including abdominal obesity, dyslipidemia, hypertension and hyperglycemia. The aim of this study was to investigate the associations of Rho GTPase and Rho-kinase (ROCK) gene polymorphisms and expressions with MetS in a Turkish population.

PATIENTS AND METHODS: A total of 141 obese MetS patients and 163 healthy controls with similar age and sex were included to this study. Polymorphisms were analyzed in genomic DNA using a BioMark 96.96 dynamic array system. mRNA from blood samples was extracted, and real-time polymerase chain reaction was performed for gene expressions.

RESULTS: We observed that genotype (CC, 18.1%; CA, 13.4%, and AA, 68.5%) and allele (C, 24.8%; A, 75.2%) frequencies for the rs35996865 polymorphism of the ROCK1 gene in patients were markedly different from controls (CC, 84.2%; CA, 2.9%, and AA, 12.9%; C, 85.6%; A, 14.4%, *p* < 0.0001). In the rs2230774 (Thr431Asn) polymorphism of the ROCK2 gene, there were increases in the CC genotype (16.5%) and C allele frequencies (20.4%) in MetS patients when compared with the control group (CC, 6.0%, *p* = 0.0009, and C, 6.7%, *p* < 0.0001). However, no associations with the other 18 polymorphisms studied were found. Although there were an increase in peripheral blood mRNA RhoH expressions, marked decreases in RhoC, RhoBTB1, RhoV, Rnd1, and CDC42 gene expressions were noted in MetS patients.

CONCLUSIONS: This is the first study to provide evidence that ROCK gene polymorphisms and gene expressions of the Rho GTPase proteins may modify individual susceptibility to MetS in the Turkish population.

Key Words:
Diabetes mellitus, Metabolic syndrome, Obesity, Rho-kinase, Rho proteins.

Introduction

Obesity, hypertension, dyslipidemia, and type 2 diabetes (T2D) as a result of insulin resistance and impaired insulin secretion are characteristic parameters of the metabolic syndrome (MetS)[1,2]. MetS has evolved from a set of statistical associations believed to carry an excess of cardiovascular risk. In the various existing definitions, a mixture of physical, metabolic and clinical variables have been used on grounds of predictive value or practical ease. These variables belong to different phenotypes, which are upstream, intermediate and proximal, respectively, in their relation to clinical disease[3]. It appears that both insulin resistance per se and hyperinsulinaemia make an independent contribution to the MetS[4]. The prevalence of MetS is about 30% worldwide[5]. The incidence of the MetS has been increasing worldwide in parallel with an increase in overweight and obesity. The MetS is associated with an increased risk of cardiovascular (CV) disease, T2D, CV-specific mortality, and all-cause mortality. Yazici et al[6] showed that the prevalence of MetS is found to be 37.5% in Turkish patients with non-diabetic first acute ST elevation myocardial infarction.

The pathogenesis of MetS remains unknown. Insulin resistance plays an important role in the pathophysiology of MetS[7]. Insulin action is part-

Corresponding Author: Suzan Tabur, MD; e-mail: suzan2471@yahoo.com.tr

ly regulated by RhoA, a member of the Rho family of GTPases. RhoA activation reduces skeletal muscle glucose transport by the repression of signals of insulin receptor substrate (IRS)-1 and phosphatidylinositol 3-kinase/serine-threonine kinase[8]. Rho-kinase (ROCK) is the immediate downstream target of RhoA, and involved in diverse cellular functions, including smooth muscle contraction, actin cytoskeletal organization, gene expression, apoptosis, inflammation, and endothelial dysfunction[9]. ROCK consists of 2 isoforms; ROCK1 and ROCK2. ROCK also could regulate insulin signaling and glucose metabolism through direct phosphorylation of the IRS-1[10]. ROCK mediates the expression of plasminogen activator inhibitor-1 under hyperglycemic conditions[11]. Previous studies have shown that ROCK activity is increased in the serum from MetS patients, and in arteries from obese Zucker rats (a model of MetS)[12,13]. Furthermore, ROCK is upregulated under inflammatory conditions, and may be involved in adipocyte differentiation[14,15]. Thus, it is likely that Rho/ROCK pathway play an important role in the pathogenesis of MetS.

The etiology of MetS is complex. The progression of MetS is influenced by genetic susceptibility and environmental factors. MetS can be considered a polygenic, multifactorial, and genetically complex disorder. Several single nucleotide polymorphisms (SNPs) in the fat mass and obesity associated gene locus have been shown to be associated with obesity-related traits by genome-wide association study[16]. It is generally accepted that common forms of obesity are highly polygenic, with each variant contributing with very small effects[16]. Genetic variants in pathways related to inflammation, thrombosis, homeostasis, neurohormonal activation, and endothelial dysfunction may represent potential risk factors for the MetS[17]. Whether the Rho/ROCK gene polymorphisms and gene expressions participate in patients with the MetS are unknown. Therefore, the aim of the present study was to assess the associations of Rho/Rho-kinase gene polymorphisms and expressions with obesity-related MetS in a Turkish population.

Patients and Methods

Study Populations

A total of 304 unrelated Turkish subjects, 141 with obesity-related MetS and 163 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.

The diagnosis of the MetS was done by clinicians according to the the National Cholesterol Education Program Adult Treatment Panel III criteria which is an acceptable and well-recognized criterion for MetS diagnosis[1,2]. 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/diastolic blood pressure ≥130/85 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. Because statins could affect ROCK activity through modulation of Rho[8], patients taking statins before enrollment were also excluded. 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. All the control subjects were from the same geographical area with a similar socioeconomic and ethnic background. 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 the 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 Laboratory. Triglyc-

erides 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) as previously described[18]. 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, 20 SNPs [rs6784820, rs974495, rs2177268, rs11102522, rs2282502 (Asp88Glu), rs34270544 (Arg144Gln), rs61891303, rs816890, rs76447184, rs73963110, rs112108028 (Pro1164Leu), rs35996865, rs111312709 (Thr792Ala), rs2230774 (Thr431Ser), rs2230774 (Thr431Asn), rs726843, rs2290156, rs965665, rs10178332, rs6755196] were studied for *Rho* and *ROCK* gene polymorphisms.

cDNA Synthesis and Gene Expression

To confirm the expression of Rho Proteins and ROCK 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 Rho, ROCK primers and, beta-actin (ACTB, housekeeping gene). We screened 21 Rho GTPases and 2 ROCK genes for expression study. Data were analyzed using the $2^{-\Delta Ct}$ method, according to the formula: $\Delta C_t = C_{tROCK\ (or\ Rho)} - C_{tACTB}$, where C_t = threshold cycle.

Statistical Analysis

Results are expressed as the mean ± SD 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. For correcting *p* values in model-based analysis, including allele and full model, a *p* value of <0.0025 (0.05/20) was considered statistically significant. For comparisons of the differences between mean values of two groups, the unpaired Student's *t* test was used. The Mann-Whitney U test was performed to compare gene expression data. 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 in Table I for the 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 (40.01±6.34 kg/m^2). Of the MetS patients, 25.5% was diagnosed with type 2 diabetes (Table I).

Genotype and allele frequencies of *Rho* gene polymorphisms in MetS and the control groups are presented in Table II. There were no statistical significant differences in genotype and allele frequencies between the control and MetS groups (p>0.0025).

Genotype and allele frequencies of the *ROCK* gene polymorphisms in cases with MetS and the control groups are presented in Table III. rs35996865 and rs2230774 (Thr431Asn) polymorphisms of the *ROCK* gene were associated with the risk of developing MetS. There were

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

	Patients (n=141)	Controls (n=163)	*p* value
Age (years)[a]	42.12±12.16	41.93±9.49	0.8786
Gender			
Male (n, %)	17 (12.1)	28 (17.2)	0.2573
Female (n, %)	124 (87.9)	135 (82.8)	
Smoking status			0.8766
Current (n, %)	17 (12.1)	21 (12.9)	
Never (n, %)	117 (83.0)	132 (81.0)	
Past (n, %)	7 (4.9)	10 (6.1)	
Alcohol intake	4 (2.8)	7 (4.3)	0.5534
BMI (kg/m^2)[a]	40.01 ± 6.34	22.64 ± 1.96	<0.0001
Waist circumference (cm)[a]	117.62 ± 13.03	83.45 ± 7.37	<0.0001
Systolic BP (mm Hg)[a]	131.51 ± 15.71	117.91 ± 8.66	<0.0001
Diastolic BP (mm Hg)[a]	86.28 ± 13.55	74.85 ± 5.37	<0.0001
Diabetes mellitus (n, %)	36 (25.5)	-	
Fasting plasma glucose (mg/dl)[a]	116.15 ± 58.81	86.62 ± 6.87	<0.0001
HbA1c (%)[a]	6.32 ± 1.79	-	
Creatinine (mg/dl)[a]	0.67 ± 0.13	0.70 ± 0.14	0.0551
Alanine aminotransferase (IU/l)[a]	24.78 ± 14.09	24.02 ±13.50	0.6318
Total cholesterol (mg/dl)[a]	199.16 ± 45.05	157.22 ± 17.94	<0.0001
Low density lipoprotein cholesterol (mg/dl)[a]	129.30 ± 30.57	98.54 ± 12.88	<0.0001
High density lipoprotein cholesterol (mg/dl)[a]	40.61 ± 8.53	44.22 ± 5.83	<0.0001
Triglyceride (mg/dl)[a]	167.99 ± 74.49	123.15 ± 26.6	<0.0001
Uric acid (mg/dl)[a]	4.96 ± 1.31	-	
High-sensitive C-reactive protein	1.90 ± 3.58	0.26 ± 0.17	<0.0001
Insulin (pmol/l)[a]	21.86 ± 16.33	-	
HOMA-IR[a]	5.88 ± 4.57	-	

[a]Data are mean±SD. 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).

marked changes in both genotype (CC, 18.1%; CA, 13.4%, and AA, 68.5%) and allele (C, 24.8%; A, 75.2%) frequencies for the rs35996865 polymorphism of the *ROCK1* gene in patients compared with controls (CC, 84.2%; CA, 2.9%, and AA, 12.9%; C, 85.6%; A, 14.4%, $p<0.0001$). Although CC genotype (16.5%) of rs2230774 (Thr431Asn) polymorphism of the *ROCK2* gene were more frequent, GG genotype (75.7%) were less frequent among the patients than controls (CC, 6.0%, and GG, 92.5%, $p=0.0009$). There was an increase in C allele (20.4% vs. 6.7%) and decrease in G allele frequencies (79.6% vs. 93.3%, $p<0.0001$) in patients. However, no associations were found with rs73963110, rs112108028 (Pro1164Leu), rs111312709 (Thr792Ala), rs2230774 (Thr431Ser), rs726843, rs2290156, rs965665, rs10178332 and rs6755196 polymorphisms (Table III).

Gene expression analysis showed that *RhoC, RhoBTB1, RhoV, Rnd1,* and *CDC42* mRNA contents in leukocytes were markedly depressed in MetS patients when compared to the control groups (Figure 1A-C, $p<0.05$). However, there was a significant increase *RhoH* gene expression in patients ($p<0.0001$, Figure 1D). No marked changes in gene expressions were noted in other Rho GTPase proteins [*RhoA, RhoB, RhoD, RnD3 (RhoE), RhoF, RhoG, RhoJ, RhoQ, RhoU, RhoBTB2, RhoBTB3, Rnd2, RAC1, RAC2,* and *RAC3*] ($p>0.05$). Additionally, there were also no marked changes in *ROCK1* ($p=0.4457$) and *ROCK2* gene expresions ($p=0.4638$).

Discussion

In this case-control study, we have shown that rs35996865 (*ROCK1* gene) and rs2230774 (Thr431Asn) (*ROCK2* gene) polymorphisms were significantly associated with obesity-related MetS and could be the risk factor of developing MetS. Additionally, suppressed (*RhoC,*

RhoBTB1, RhoV, Rnd1, and *CDC42*) and elevated (*RhoH*) 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 Rho/ROCK gene polymorphisms with the risk of developing MetS. This is also first study to evaluate the Rho/ROCK gene expressions in MetS. Our results suggest CA and AA genotypes and A allele of the rs35996865 polymorphism, and CC genotype and C allele of the rs2230774 (Thr431Asn) polymorphism may increase the susceptibility to MetS.

We have observed that there were no changes in *ROCK1* and *ROCK2* gene expressions in leukocytes of the MetS patients when compared to controls. Liu et al[12] studied the leukocyte ROCK protein expression, and found that ROCK1 and ROCK2 protein expression were similar in control and MetS subjects. However, *ROCK1* and *ROCK2* gene expressions or the gene expressions of the Rho GTPase proteins have not been examined in that study. Liu et al[12] found an increase in leukocyte ROCK activity in MetS subjects. On the other hand, we have observed an increase in *RhoH* gene and decrease in 5 Rho GTPase gene expressions (*RhoC, RhoBTB1, RhoV, Rnd1,* and *CDC42*) in MetS patients. Our findings suggest that alterations of the Rho proteins gene expressions rather than induction of *ROCK* gene expression are the underlying mechanisms that are associated with components of the MetS. RhoH, Rnd1, and RhoBTB1 are among the atypical members of the Rho family. These Rho GTPases do not possess the ability to hydrolyze GTP and lack a GDP binding site, and are thus constitutively bound to GTP. In these cases, regulation is achieved through modulation of gene expression, phosphorylation, and protein degradation. The physiologic function of RhoH is largely unknown. RhoH is produced specifically in hematopoietic cells and aberrant expression has been linked to various forms of leukemia[19]. It is indicated that RhoH functions as an antagonist for the classical Rho GTPases, since it was found to inhibit CDC42/RAC1/RhoA-dependent activation of NF-κB and p38 MAPK[20]. RhoH also an-

Table II. Genotype and allele frequencies of the *RHO* gene polymorphisms in cases with MetS and controls

Gene SNP	Genotypes/ Alleles	Controls	n*	Cases with MetS	n*	*p* value
RhoA	AA/AG/GG	65/63/30	158	39/54/34	127	0.1275
rs6784820	A/G	193/123		132/122		0.0359
RhoA	CC/CT/TT	87/54/20	161	92/32/14	138	0.0786
rs974495	C/T	228/94		216/60		0.0472
RhoA	TT/TA/AA	89/33/41		89/15/27	131	0.0445
rs2177268	T/A	211/115	163	193/69		0.0255
RhoC	AA/AG/GG	89/43/23	155	79/24/15		0.2589
rs11102522	A/G	221/89		182/54	118	0.1509
RhoD	GG/GA/AA	66/58/37	161	54/47/36	137	0.8041
rs2282502 (Asp88Glu)	G/A	190/132		155/119		0.6050
RhoD	CC/CT/TT	157/4/0	161	134/1/0	135	0.3805
rs34270544 (Arg144Gln)	C/T	318/4		269/1		0.3826
RhoD	GG/GA/AA	158/2/0	160	135/0/0	135	0.5019
rs61891303	G/A	318/4		270/0		0.1295
Rnd3 (RhoE)	CC/CT/TT	153/9/0	162	133/2/0	135	0.0725
rs816890	C/T	315/9		268/2		0.0750
Rnd3 (RhoE)	CC/CT/TT	160/2/0	162	131/4/0	135	0.4162
rs76447184	C/T	322/2		266/4		0.4185

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

Table III. Genotype and allele frequencies of the *ROCK* gene polymorphisms in cases with MetS and controls

Gene SNP	Genotypes/ Alleles	Controls	n*	Cases with MetS	n*	*p* value
ROCK1 rs73963110	TT/TC/CC T/C	159/3/0 321/3	162	135/1/0 271/1	136	0.6282 0.6295
ROCK1 rs112108028 (Pro1164Leu)	GG/GA/AA G/A	160/1/0 321/1	160	135/6/0 276/6	141	0.0532 0.0545
ROCK1 rs35996865	CC/CA/AA C/A	117/4/18 238/40	139	23/17/87 63/191	127	<0.0001 <0.0001
ROCK1 rs111312709 (Thr792Ala)	AA/AG/GG A/G	162/0/0 324/0	162	133/3/0 269/3	136	0.0939 0.0945
ROCK2 rs2230774 (Thr431Ser)	GG/GT/TT G/T	45/81/37 171/155	163	39/64/36 142/136	139	0.7667 0.7985
ROCK2 rs2230774 (Thr431Asn)	GG/GC/CC G/C	124/2/8 250/18	134	87/9/19 183/47	115	0.0009 <0.0001
ROCK2 rs726843	CC/CT/TT C/T	53/74/36 180/146	163	43/50/41 136/132	134	0.2008 0.3156
ROCK2 rs2290156	GG/GC/CC G/C	70/72/18 212/108	160	77/39/13 193/65	129	0.0211 0.0322
ROCK2 rs965665	CC/CG/GG C/G	120/32/8 272/48	160	109/27/2 245/31	138	0.2295 0.1848
ROCK2 rs10178332	TT/TG/GG T/G	119/36/8 274/52	163	108/24/3 240/30	135	0.2728 0.0952
ROCK2 rs6755196	CC/CT/TT C/T	130/26/7 286/40	163	107/19/4 233/27	130	0.8074 0.5152

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

tagonize RAC activation, RAC-mediated actin reorganization and cell migration[21]. It has been shown that neutrophils are RhoH positive under inflammatory conditions, and RhoH negatively regulates LTB4 production in these cells[22]. RhoC associates with ROCK and activates it. RhoC may be able to regulate cell motility[23]. RhoBTBs are believed to act as tumor suppressors through regulating ubiquitinylation[21]. RhoV is localized to focal adhesions in endothelial cells[24]. Rnd1 interacts with and activates p190RhoGAP, a protein which recruits and downregulates RhoA, thus acting as a RhoA antagonist[25]. Rnd1 expression was found to be induced during inflammation in endothelial cells[26]. CDC42 involves in the apolipoprotein A-I-induced cholesterol efflux, an important mechanism by which high density lipoproteins protect against atherosclerosis[27]. CDC42 activity is essential for endothelial barrier repair, adherens junction stability, and restoration of permeability. Therefore, dysregulated CDC42 activity might contribute to the failure to restore endothelial barrier function associated with atheroslerosis. CDC42 activity is probably involved in chemottractant-induced monocyte recruitment to sites of vascular inflammation during the formation and the progression of atherosclerosis. CDC42 is involved in cholesterol movement in macrophages. CDC42 plays an important role in cellular cholesterol efflux, and its dysregulation might lead to the development of atherosclerotic cardiovascular disease[28]. Activated CDC42 can mediate insulin-stimulated GLUT4 translocation and glucose transport in a phosphatidylinositol 3-kinase-dependent manner[29]. Insulin can also stimulate CDC42 activity[29]. Collectively, little is known about the function of the RhoH, RhoC, RhoBTB1, RhoV,

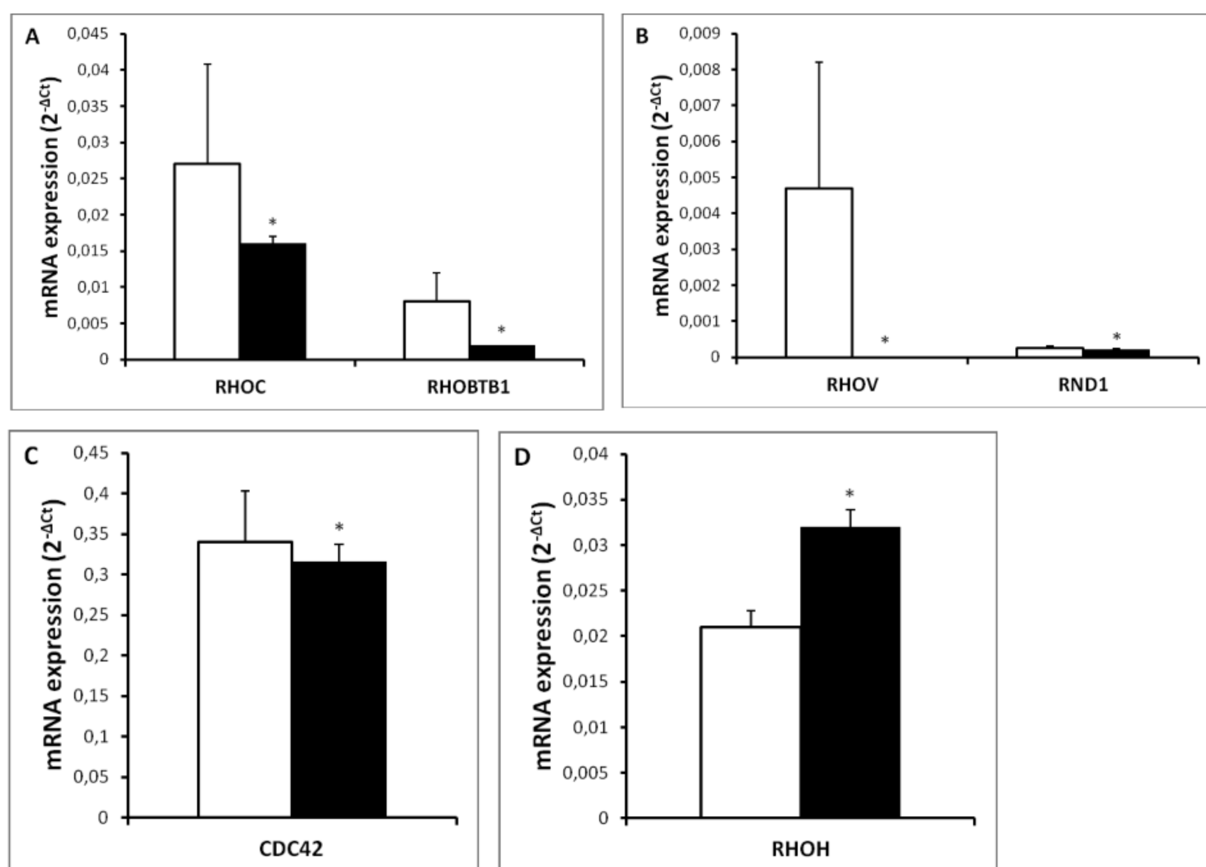

Figure 1. Comparison of the peripheral blood mRNA RHOC, RHOBTB1, (A), RHOV, RND1 (B), CDC42 (C), and RHOH (D) expressions in healthy controls (n=42, open bars) and in patients with metabolic syndrome (n=54, solid bars). Values are given as mean ± SEM, $^*p = 0.0344$, $p = 0.0130$, $p = 0.0033$, $p = 0.0389$, $p = 0.0222$, and $p < 0.0001$ values were obtained for RHOC, RHOBTB1, RHOV, RND1, CDC42 and RHOH, respectively.

Rnd1, and CDC42 members of the Rho GTPase family. Moreover, their roles in the MetS are currently unknown.

The exact pathophysiological events leading to the development of MetS remain unknown. Almost all MetS patients suffer from abdominal obesity. Obesity itself can precipitate an inflammatory response and lead to free radical generation[30], thus further augmenting ROCK signaling. Inhibition of ROCK ameliorates insulin sensitivity in obese, but not lean, rats suggesting that ROCK activity is increased by obesity[31]. ROCKs are involved in adipocyte differentiation[15], which also may affect the pathogenesis of MetS. Increased ROCK activity could lead to the phosphorylation of IRS-1 and subsequently impaired the glycogen synthase or glucose transportation in peripheral tissues, thereby increasing insulin resistance[10,15]. High-sensitivity C-reactive protein (hs-CRP), the best characterized biomarker of inflammation, was high in our patients group. Higher hs-CRP levels were associated with greater ROCK activity[12]. Taken together, these finding suggest that inhibition of the Rho/ROCK pathway play a role in the treatment of the MetS.

MetS is associated with impaired endothelial function. The NO production was lower in obese subjects with MetS, and blunted endothelial-dependent vasodilation along with augmented vasoconstriction has been demonstrated in obese humans[7]. In the forearm circulation of patients with the MetS, ROCK inhibition by fasudil improves endothelium-dependent and -independent vasodilator responsiveness during hyperinsulinemia; increased oxidative stress plays a role in this mechanism[32]. High oxidative stress is strongly associated with visceral fat accumulation and MetS[33]. Oxidative stress exacerbates MetS factors such as insulin resistance, hypertriglyceridemia, decrement of HDL cholesterol, and hyperten-

sion[34]. Enhanced oxidative stress triggers the activation of ROCK[35]. Upregulated ROCK activity was involved in the pathogenesis of MetS, suggesting that ROCK is an important molecular target for the prevention of the disorder.

Our results showed strong association of rs35996865 and rs2230774 (Thr431Asn) polymorphisms with MetS. There are only limited numbers of published studies related to ROCK polymorphisms in humans. Seasholtz et al[36] reported that rs2230774 (Thr431Asn) polymorphism predicts increased blood pressure, systemic vascular resistance, and resistance in response to the endogenous renin-angiotensin system in twins. However, it has been shown that this polymorphism does not exhibit any significant allele or genotype association with hypertension in the Chinese Han population[37]. rs2230774 (Thr431Asn) polymorphism did not show a significant association with diabetes or diabetic retinopathy[38]. It has been demonstrated that rs2230774 (Thr431Asn) polymorphism is significantly associated with chronic kidney disease in individuals with a low serum concentration of triglycerides[39]. Collectively, these findings suggest that *ROCK* polymorphisms may influence the risk of MetS. Since MetS is the combination of the effects of more than one risk factor, the genetic component of each part of the MetS is complex.

Conclusions

Our data indicate that rs35996865 (*ROCK1* gene) and rs2230774 (Thr431Asn) (*ROCK2* gene) polymorphisms may contribute to individual susceptibility to MetS. Our data also imply that gene expressions of the Rho GTPase proteins play a role in the pathogenesis of MetS. ROCK polymorphisms and gene expressions of the Rho GTPase proteins are likely novel risk markers for MetS and suggest that genetics of the Rho/ROCK are involved in the development or progression of MetS. Our data may suggest that Rho/ROCK pathway may 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.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-2497.
2) Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004; 109: 433-438.
3) Ferrannini E. Is insulin resistance the cause of the metabolic syndrome? Ann Med 2006; 38: 42-51.
4) Ferrannini E, Balkau B. Insulin: in search of a syndrome. Diabet Med 2002; 19: 724-729.
5) Grundy SM. Metabolic syndrome pandemic. Arterioscler Thromb Vasc Biol 2008; 28: 629-636.
6) Yazici HU, Poyraz F, Turfan M, Sen N, Tavil Y, Tulmac M, Vatankulu MA, Aygül N, Ozdo ru I, Abaci A. The prevalence of the metabolic syndrome and its impact on the left ventricular systolic function in the patients with non-diabetic first ST elevation myocardial infarction. Eur Rev Med Pharmacol Sci 2012; 16: 90-95.
7) Prieto D, Contreras C, Sánchez A. Endothelial dysfunction, obesity and insulin resistance. Curr Vasc Pharmacol 2014; 12: 412-426.
8) Begum N, Sandu OA, Ito M, Lohmann SM, Smolenski A. Active Rho kinase (ROK-alpha) associates with insulin receptor substrate-1 and inhibits insulin signaling in vascular smooth muscle cells. J Biol Chem 2002; 277: 6214-6222.
9) Zhou Q, Gensch C, Liao JK. Rho-associated coiled-coil-forming kinases (ROCKs): potential targets for the treatment of atherosclerosis and vascular disease. Trends Pharmacol Sci 2011; 32: 167-173.
10) Furukawa N, Ongusaha P, Jahng WJ, Araki K, Choi CS, Kim HJ, Lee YH, Kaibuchi K, Kahn BB, Masuzaki H, Kim JK, Lee SW, Kim YB. Role of Rho-kinase in regulation of insulin action and glucose homeostasis. Cell Metab 2005; 2: 119-129.
11) Rikitake Y, Liao JK. Rho-kinase mediates hyperglycemia-induced plasminogen activator inhibitor-1 expression in vascular endothelial cells. Circulation 2005; 111: 3261-3268.
12) Liu PY, Chen JH, Lin LJ, Liao JK. Increased Rho kinase activity in a Taiwanese population with metabolic syndrome. J Am Coll Cardiol 2007; 49: 1619-1624.
13) Naik JS, Xiang L, Hester RL. Enhanced role for RhoA-associated kinase in adrenergic-mediated vasoconstriction in gracilis arteries from obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 2006; 290: R154-R161.

14) Hiroki J, Shimokawa H, Higashi M, Morikawa K, Kandabashi T, Kawamura N, Kubota T, Ichiki T, Amano M, Kaibuchi K, Takeshita A. Inflammatory stimuli upregulate Rho-kinase in human coronary vascular smooth muscle cells. J Mol Cell Cardiol 2004; 37: 537-546.

15) Sordella R, Jiang W, Chen GC, Curto M, Settleman J. Modulation of Rho GTPase signaling regulates a switch between adipogenesis and myogenesis. Cell 2003; 113: 147-158.

16) Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. Mol Cell Endocrinol 2014; 382: 740-757.

17) Guo X, Cheng S, Taylor KD, Cui J, Hughes R, Quiñones MJ, Bulnes-Enriquez I, De la Rosa R, Aurea G, Yang H, Hsueh W, Rotter JI. Hypertension genes are genetic markers for insulin sensitivity and resistance. Hypertension 2005; 45: 799-803.

18) Tabur S, Oztuzcu S, Duzen IV, Eraydin A, Eroglu S, Ozkaya M, Demiryürek AT. Role of the transient receptor potential (TRP) channel gene expressions and TRP melastatin (TRPM) channel gene polymorphisms in obesity-related metabolic syndrome. Eur Rev Med Pharmacol Sci 2015 (in press).

19) Gündogdu MS, Liu H, Metzdorf D, Hildebrand D, Aigner M, Aktories K, Heeg K, Kubatzky KF. The haematopoietic GTPase RhoH modulates IL3 signalling through regulation of STAT activity and IL3 receptor expression. Mol Cancer 2010; 9: 225.

20) Li X, Bu X, Lu B, Avraham H, Flavell RA, Lim B. The hematopoiesis-specific GTP-binding protein RhoH is GTPase deficient and modulates activities of other Rho GTPases by an inhibitory function. Mol Cell Biol 2002; 22: 1158-1171.

21) Sadok A, Marshall CJ. Rho GTPases: masters of cell migration. Small GTPases 2014; 5: e29710.

22) Daryadel A, Yousefi S, Troi D, Schmid I, Schmidt-Mende J, Mordasini C, Dahinden CA, Ziemiecki A, Simon HU. RhoH/TTF negatively regulates leukotriene production in neutrophils. J Immunol 2009; 182: 6527-6532.

23) Wheeler AP, Ridley AJ. Why three Rho proteins? RhoA, RhoB, RhoC, and cell motility. Exp Cell Res 2004; 301: 43-49.

24) Aspenström P, Fransson A, Saras J. Rho GTPases have diverse effects on the organization of the actin filament system. Biochem J 2004; 377: 327-337.

25) Wennerberg K, Forget MA, Ellerbroek SM, Arthur WT, Burridge K, Settleman J, Der CJ, Hansen SH. Rnd proteins function as RhoA antagonists by activating p190 RhoGAP. Curr Biol 2003; 13: 1106-1115.

26 Warton K, Foster NC, Gold WA, Stanley KK. A novel gene family induced by acute inflammation in endothelial cells. Gene 2004; 342: 85-95.

27) Nofer JR, Feuerborn R, Levkau B, Sokoll A, Seedorf U, Assmann G. Involvement of Cdc42 signaling in apoA-I-induced cholesterol efflux. J Biol Chem 2003; 278: 53055-53062.

28) Loirand G, Sauzeau V, Pacaud P. Small G proteins in the cardiovascular system: physiological and pathological aspects. Physiol Rev 2013; 93: 1659-1720.

29) Usui I, Imamura T, Huang J, Satoh H, Olefsky JM. Cdc42 is a Rho GTPase family member that can mediate insulin signaling to glucose transport in 3T3-L1 adipocytes. J Biol Chem 2003; 278: 13765-13774.

30) Milagro FI, Campión J, Martínez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. Obesity (Silver Spring) 2006; 14: 1118-1123.

31) Kanda T, Wakino S, Homma K, Yoshioka K, Tatematsu S, Hasegawa K, Takamatsu I, Sugano N, Hayashi K, Saruta T. Rho-kinase as a molecular target for insulin resistance and hypertension. FASEB J 2006; 20: 169-171.

32) Schinzari F, Tesauro M, Rovella V, Di Daniele N, Gentileschi P, Mores N, Campia U, Cardillo C. Rho-kinase inhibition improves vasodilator responsiveness during hyperinsulinemia in the metabolic syndrome. Am J Physiol Endocrinol Metab 2012; 303: E806-E811.

33) Fujita K, Nishizawa H, Funahashi T, Shimomura I, Shimabukuro M. Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. Circ J 2006; 70: 1437-1442.

34) Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int J Obes (Lond) 2006; 30: 400-418.

35) Jin L, Ying Z, Webb RC. Activation of Rho/Rho kinase signaling pathway by reactive oxygen species in rat aorta. Am J Physiol Heart Circ Physiol 2004; 287: H1495-H1500.

36) Seasholtz TM, Wessel J, Rao F, Rana BK, Khandrika S, Kennedy BP, Lillie EO, Ziegler MG, Smith DW, Schork NJ, Brown JH, O'Connor DT. Rho kinase polymorphism influences blood pressure and systemic vascular resistance in human twins: role of heredity. Hypertension 2006; 47: 937-947.

37) Zhao Q, Wang L, Yang W, Chen S, Huang J, Fan Z, Li H, Lu X, Gu D. Interactions among genetic variants from contractile pathway of vascular smooth muscle cell in essential hypertension susceptibility of Chinese Han population. Pharmacogenet Genomics 2008; 18: 459-466.

38) Demiryurek AT, Erbagci I, Oztuzcu S, Alasehirli B, Ozkara E, Seker M, Sönmez A, Ozsan M, Camci C. Lack of association between the Thr431Asn and Arg83Lys polymorphisms of the ROCK2 gene and diabetic retinopathy. Curr Eye Res 2010; 35: 1128-1134.

39) Yoshida T, Kato K, Yokoi K, Oguri M, Watanabe S, Metoki N, Yoshida H, Satoh K, Aoyagi Y, Nishigaki Y, Nozawa Y, Yamada Y. Association of genetic variants with chronic kidney disease in individuals with different lipid profiles. Int J Mol Med 2009; 24: 233-246.