Original Article

Association between the *SIRT1* mRNA Expression and Acute Coronary Syndrome

Yongyan Hu^{1,2}, Laiyuan Wang¹, Shufeng Chen¹, Xuehui Liu^{1,2}, Hongfan Li^{1,2}, Xiangfeng Lu¹, Xueli Yang¹, Jianfeng Huang¹ and Dongfeng Gu¹

¹State Key Laboratory of Cardiovascular Disease, Division of Population Genetics, Fuwai Hospital & National Center of Cardiovascular Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

²National Human Genome Center at Beijing, Beijing, China

Aim: Silent mating type information regulator 2 homolog 1 (*SIRT1*) functions as an atheroprotective factor in vascular biology, and genetic variations in *SIRT1* are associated with coronary artery calcification and type 2 diabetes in several populations. In this study, we investigated the relationship between the mRNA expression levels of the *SIRT1* gene and single nucleotide polymorphisms (SNPs) in the context of acute coronary syndrome (ACS).

Methods: Whole-genome expression microarray and real-time PCR techniques were used to detect the gene expression levels, and Western blotting was performed to determine the protein expression level. The four selected SNPs were genotyped in a Taqman genotyping platform.

Results: Compared with that observed in the controls, the mRNA expression levels of the *SIRT1* gene in the microarray study were significantly lower in the acute myocardial infarction (AMI), unstable angina (UA) and overall ACS patients. These results were replicated in another independent cohort with respect to the mRNA (AMI, $p < 0.001$; UA, $p < 0.001$; ACS, $p < 0.001$) and protein ($p < 0.05$) levels. Furthermore, the relationship between the *SIRT1* mRNA expression and the genotypes of four possible functional SNPs (rs12778366, rs3758391, rs2273773 and rs4746720) was tested, the results of which showed significant differences in the *SIRT1* mRNA expression among the allelic genes of rs3758391 ($p < 0.01$) in the healthy participants.

Conclusions: The present results confirm that the *SIRT1* gene plays a protective role against ACS and that the rs3758391 SNP affects the mRNA expression in healthy participants, providing new insight into the processes regulating the genetic control of the *SIRT1* gene with respect to the pathogenesis of ACS.

J Atheroscler Thromb, 2015; 22:165-182.

Key words: *SIRT1*, ACS, Gene expression, SNP

Introduction

Acute coronary syndrome (ACS) is a leading cause of global morbidity and mortality, with a deleterious

impact on health and substantial economic burdens due to the need for hospitalization¹. ACS includes unstable angina (UA) and acute myocardial infarction (AMI). Studies of the pathogenesis of ACS have focused primarily on atherosclerosis. The physical rupture of the atherosclerotic plaque that accumulates in patients with this condition accounts for almost all cases of acute coronary thrombosis². A large amount of laboratory research conducted within the past decade has led to many achievements describing the biological and genetic foundations of atherosclerosis³.

Silent mating type information regulator 2

Address for correspondence: Laiyuan Wang, State Key Laboratory of Cardiovascular Disease, Division of Population Genetics, Fuwai Hospital & National Center of Cardiovascular Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College, No.167 Beilishi Road, Xicheng District, Beijing, 100037, China

E-mail: wanglaiyuandw@163.com

Received: March 10, 2014

Accepted for publication: July 23, 2014

homolog 1 (*SIRT1*) is the closest homolog of yeast sir2 and functions as an NAD⁺-dependent histone deacetylase⁴, contributing to regulation of the gene expression, metabolism and aging⁵. Stein S. *et al.*⁶ demonstrated that the SIRT1 protein expression in the aorta is lower in ApoE^{-/-} mice than in wild-type mice and that SIRT1 retards the progression of atherosclerosis by preventing macrophage foam cell formation. Breitenstein A. *et al.*⁷ also found that the *SIRT1* expression in monocytes to be decreased in 16 ACS patients. *SIRT1* exerts atheroprotective effects on the vascular biology by downregulating the expression of various pro-inflammatory cytokines, mediating vasodilatation via the actions of eNOS-derived nitric oxide (NO) and scavenging reactive oxygen species (ROS)⁸.

Polymorphisms related to the *SIRT1* gene have been reported to be associated with a higher incidence of type 2 diabetes⁹, Alzheimer's disease¹⁰, cholesterol metabolism, coronary artery calcification¹¹, greater body mass index (BMI) values, an increased risk of obesity¹², elevated amounts of body fat and blood pressure¹³, etc. In addition, the mRNA expression of the *SIRT1* gene is related to single nucleotide polymorphisms (SNPs) in subjects with severe obesity¹⁴. SNPs in the *SIRT1* gene may impact the mRNA expression by altering the binding ability of transcription factors or miRNAs.

In order to determine the role of *SIRT1* in ACS, we sought to compare the expression levels of *SIRT1* in peripheral blood mononuclear cells (PBMCs) between ACS patients and controls using a gene expression microarray and real-time PCR replication in a large sample size. In addition, four potential functional SNPs in *SIRT1* were assessed as to whether they affect the mRNA expression levels of the *SIRT1* gene.

Methods

Study Subjects

A total of 58 patients with ACS were enrolled from Fuwai Hospital at the National Center of Cardiovascular Diseases (Beijing, China). All patients underwent initial diagnostic coronary angiography and were found to have either a minimum of 70% stenosis in a major epicardial artery or 50% stenosis in the left main coronary artery. The subjects were divided into two groups: (1) those with UA ($n=30$, based on the following inclusion criteria: angina pectoris with a crescendo pattern, recurrent episodes at rest/low levels of activity and/or severe new onset with ischemic changes on electrocardiography); (2) and those with AMI ($n=28$, based on the following inclusion criteria: typical chest pain lasting more than 30

minutes within 24 hours before enrollment with ischemic changes on electrocardiography and significant elevation of the creatine kinase-MB and troponin I levels). Subjects with congenital heart disease, valvular disease, cardiomyopathy, stroke, secondary hypertension, diabetes and severe renal or hepatic disease were excluded. In patients with ACS, blood samples were obtained within 48 hours after the onset of the acute event.

A group of 229 participants from the rural district of Shandong Province were enrolled in the China Cardiovascular Health Study (CCHS) project, a population-based investigation of risk factors for cardiovascular disease conducted in China since 2006¹⁵. Individuals were excluded from the study if they had stage-two/three hypertension (systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg), a history of coronary heart disease, diabetes, stroke or renal or hepatic disease or took antihypertensive medications. One hundred and seventy-five age- and gender-matched participants were randomly selected from the above healthy participants as the control group in the *SIRT1* mRNA expression replication study. Sixty-seven participants were randomly selected from the above population for the assessment of the relationship between the gene expression of *SIRT1* and the inflammatory cytokine *IL-6*.

Informed consent was obtained from all participants prior to inclusion in the study. The investigation conformed to the principles outlined in the Declaration of Helsinki and was approved by the ethics review board of Fuwai Hospital (2011-341).

Cell Isolation and RNA Extraction

Peripheral blood was collected from all participants after an overnight fast, and PBMCs were isolated using Lymphocyte Separation Medium (TBD, Tianjin, China) according to the manufacturer's instructions. Total RNA was extracted from the PBMCs using TRIzol reagent (Ambion, Austin, USA) according to the manufacturer's protocol, and the RNA concentration was determined using a NanoDrop2000 spectrophotometer (Thermo Scientific, Wilmington, DE). All RNA samples were stored at -80°C prior to labeling, hybridization and cDNA synthesis.

Whole Genome Expression

The quality of the RNA was determined using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Samples with an RNA integrity number greater than 9.5 were examined in the subsequent expression analyses. Amplification and labeling of the RNA was performed with the Illumina TotalPrep RNA Amplifi-

cation Kit from Life Technologies (Ambion, Austin, TX) using 150 ng of RNA per sample. Subsequently, 750 ng of labeled cRNA was hybridized to Human HT-12 v4 Expression BeadChips (Illumina, San Diego, CA) for 18 hours and processed according to the manufacturer's instructions. The slides were then scanned using a BeadArray Reader (Illumina, San Diego, CA).

Microarray Data Analysis

The GenomeStudio Gene Expression Module was used as a tool to analyze the gene expression data obtained from the scanned microarray results generated by the Illumina BeadArray Reader. The data processing technique included a normalization method, named Average, to reduce the obscuring variation between the microarrays, which may be introduced during the process of sample preparation, manufacturing, fluorescence labeling, hybridization and/or scanning. The statistical analysis of the differential gene expression data between the case and control groups was conducted using an Illumina custom software program. The moderated t statistic p -values derived from the Illumina custom analysis were further adjusted for multiple testing according to Benjamini and Hochberg's method¹⁶⁾ in order to control the false discovery rate (FDR). Differentially expressed genes were identified based on an FDR of <10% and a p -value of <0.01 between the case and control groups.

SNP Selection

According to the findings of previous studies and functional predictions (UCSC ENCODE¹⁷⁾: <http://genome.ucsc.edu/ENCODE/>; FastSNP¹⁸⁾: http://fast-snp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp), we selected four potential function SNPs (rs12778366, rs3758391, rs2273773 and rs4746720) in the *SIRT1* gene region. On the ENCODE website, we selected layered H3K27Ac, layered H3K4Me1, layered H3K4Me3, DNaseI Hypersensitivity Clusters and TS miRNA sites as functional criteria. Both rs3758391 and rs12778366 locate in the 5' flanking region of the *SIRT1* gene. Taking into consideration that rs4746720 is located in the 3' untranslated region (UTR) of the *SIRT1* gene, we used the computational prediction website miRNASNP2¹⁹⁾ (July 2013 Release; <http://www.bioguo.org/miRNASNP2/>) to explore the miRNA targets gained or lost by the existence of rs4746720.

DNA Extraction and Genotyping

Genomic DNA was isolated from blood leukocytes according to a standard procedure using a DNA

Fig. 1. Hierarchical clustering analysis of the gene expression in the peripheral blood mononuclear cells (PBMCs) of the acute myocardial infarction (AMI) patients, unstable angina (UA) patients, stable angina (SA) patients and control subjects. Ward's Minimum Method was used. The labels AMI, UA, SA and Control represent six PBMC samples each from the AMI patients, UA patients, SA patients and control subjects, respectively.

extraction kit (Tiangen Biotech, Beijing, China). The SNPs were genotyped using a Taqman genotyping platform (ABI 7900 HT Real-time PCR system, Applied Biosystems, Foster City, CA). The probes and primers are shown in supplementary material **Supplementary Table 1**. The thermocycler parameters were as follows: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds and 60°C for one minute. The rate of successful genotyping was over 98%, and 2% of the samples were randomly selected and genotyped in duplicate. The discrepancy between the samples was less than 1%.

cDNA Synthesis and Real-Time PCR Detection

cDNA was synthesized from 1 µg of RNA using reverse transcriptase with an oligo(dT) primer (Trans-Gen Biotech, Beijing, China).

Real-time PCR for *SIRT1* and *IL-6* was carried out in triplicate using SYBR Green on an Applied Biosystems 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, USA). The relative mRNA expression levels were normalized to that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and analyzed according to the $\Delta\Delta C_t$ method, after which the fold changes were calculated²⁰. Primers specific for the *SIRT1*, *IL-6* and *GAPDH* genes are provided in supplementary material **Supplementary Table 2**. The amplification program consisted of one cycle at 50°C for two minutes then another cycle at 95°C for two minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for one minute. A melting curve analysis was performed after amplification in order to verify the accuracy of the amplicon.

Western Blotting

Total protein extracted from the PBMCs was separated on a 10% polyacrylamide gel and then transferred onto a nitrocellulose membrane. The membrane was subsequently incubated overnight with one of the primary antibodies against SIRT1 (sc-15404, Santa Cruz Biotechnology, Santa Cruz, CA) and β -Actin (sc-47778, Santa Cruz Biotechnology, Santa Cruz, CA). Immunoblots were detected using a chemiluminescence kit (Thermo Scientific Pierce, Waltham, MA), and the autoradiographs were quantitated via densitometry (Science Imaging System, Bio-Rad, Hercules, CA).

Statistical Analysis

The genotype distributions, Hardy-Weinberg equilibrium and differences between categorical variables were tested using the Chi-square or Fisher's exact

Fig. 2. Venn diagram showing the number of differentially expressed genes common to the AMI and UA patients, but not expressed in the SA patients (all relative to that observed in the control subjects). There were 181 genes unique to the acute coronary syndrome (ACS) patients.

tests. Continuous variables, patients versus controls and the different genotype groups were compared using the independent Student's *t*-test or analysis of variance (ANOVA). The HaploView 4.2 software package was used to calculate and display the linkage disequilibrium (LD) values in the gene region. The statistical analyses of the real-time PCR results and descriptive statistics of the study population were performed using the SPSS software 17.0 (SPSS Inc., Chicago, Ill) and GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA) programs. All results are expressed as the mean $\pm$ standard error of the mean (SEM), and statistical significance was defined as a *p*-value less than 0.05.

Results

Whole-Genome Expression Analysis

A whole-genome expression analysis was performed among six AMI patients, six UA patients, six stable angina (SA) patients and six control individuals. The hierarchical clustering was performed based on the normalized signal data in order to show distinguishable gene expression profiling among the samples (**Fig. 1**). Genes differentially expressed in the patients relative to the controls were analyzed in an effort to distinguish genes uniquely expressed in ACS patients. A total of 181 genes common expressed in AMI and UA patients, but not in SA patients, were considered

Table 1. Differentially expressed genes with a potential role in AMI, UA and ACS compared with that observed in the controls

Gene Symbol	Gene Entrez ID	Gene Name	AMI Fold	UA Fold	ACS Fold
G3BP1	10146	GTPase activating protein (SH3 domain) binding protein 1	16.06 ↓	35.41 ↓	22.10 ↓
SPON2	10417	spondin 2, extracellular matrix protein	69.71 ↓	4.65 ↓	8.73 ↓
MCTS1	28985	malignant T cell amplified sequence 1	5.07 ↓	2.89 ↓	3.68 ↓
SEPT7	989	septin 7	4.62 ↓	2.68 ↓	3.39 ↓
SFRS11	9295	splicing factor, arginine/serine-rich 11	3.29 ↓	2.60 ↓	2.90 ↓
USP8	9101	ubiquitin specific peptidase 8	3.03 ↓	2.62 ↓	2.81 ↓
MYBL1	4603	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	3.69 ↓	2.01 ↓	2.60 ↓
ZNF557	79230	zinc finger protein 557	2.35 ↓	2.90 ↓	2.60 ↓
NOC3L	64318	Homo sapiens nucleolar complex associated 3 homolog (S. cerevisiae)	2.62 ↓	2.46 ↓	2.54 ↓
CAPZA1	829	capping protein (actin filament) muscle Z-line, alpha 1	2.32 ↓	2.75 ↓	2.51 ↓
RPLP1	6176	ribosomal protein, large, P1	2.88 ↓	2.21 ↓	2.50 ↓
PTMA	5757	prothymosin, alpha	2.63 ↓	2.28 ↓	2.44 ↓
ERGIC1	57222	endoplasmic reticulum-Golgi intermediate compartment 1	2.40 ↓	2.25 ↓	2.32 ↓
ZNF146	7705	zinc finger protein 146	2.20 ↓	2.37 ↓	2.28 ↓
GNG10	2790	guanine nucleotide binding protein (G protein), gamma 10	2.55 ↑	1.66 ↑	2.11 ↑
LEP	3952	leptin (obesity homolog, mouse)	1.96 ↓	2.16 ↓	2.06 ↓
NNT	23530	nicotinamide nucleotide transhydrogenase (NNT), nuclear gene encoding mitochondrial protein	2.07 ↓	2.03 ↓	2.05 ↓
USP49	25862	ubiquitin specific peptidase 49	1.93 ↓	2.13 ↓	2.02 ↓
GNA13	10672	guanine nucleotide binding protein (G protein), alpha 13	2.11 ↓	1.92 ↓	2.01 ↓
TMEM106A	113277	transmembrane protein 106A	1.93 ↓	2.09 ↓	2.01 ↓
ETS1	2113	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	2.11 ↓	1.91 ↓	2.01 ↓
SIRT1	23411	silent mating type information regulator 2 homolog 1	2.12 ↓	1.90 ↓	2.00 ↓

There were 75 differentially decreased expressed genes and 23 differentially increased expressed genes in the AMI, UA and ACS patients versus the controls. The complete list of all 98 genes is available in **Supplementary Table 1**. The subset of genes with the greatest differential expression (fold change ≥ 2) is listed here. ↓ = decreased; ↑ = increased.

to be unique to ACS (shown in **Fig. 2**). Based on the above genes, 98 coding transcripts with well-established annotation, including 23 upregulated genes and 75 downregulated genes, were selected for the further analysis (shown in **Supplementary Table 3** and **Supplementary Table 4**). Twenty-three genes with the greatest differential expression (fold change > 2) are shown in **Table 1**.

We reviewed previous studies in order to examine whether the functions of the above 23 genes were related to ACS and found that the expression of *SIRT1* gene had the greatest association evidence^{7, 21, 22}. Our findings showed that the expression of the *SIRT1* gene was reduced considerably in the patients suffering from AMI, UA and ACS compared with that observed in the control group.

Replication of the *SIRT1* Expression in the Patients with ACS and the Controls

The expression levels of the *SIRT1* gene were replicated using real-time PCR. The replication cohort consisted of 58 ACS patients and 175 control subjects (**Table 2**). Compared with that observed in the controls (0.90 ± 0.72 fold, $n=175$), the *SIRT1* mRNA expression levels were reduced in the AMI (0.34 ± 0.41 fold, $p=5.37 \times 10^{-10}$; $n=28$; **Fig. 3A**), UA (0.43 ± 0.37 fold, $p=7.45 \times 10^{-7}$; $n=30$; **Fig. 3A**) and overall ACS patients (0.38 ± 0.34 fold, $n=58$; $p=6.59 \times 10^{-12}$; **Fig. 3A**).

In addition, protein in the PBMCs was isolated from a subgroup of age-matched ACS patients ($n=8$) and control subjects ($n=10$), and levels of SIRT1 proteins were detected using a Western blot analysis

Table 2. Demographic and clinical characteristics of the CAD and control samples

Characteristics	Control (<i>n</i> =175)	ACS (<i>n</i> =58)	<i>p</i> -value*
Gender (male, %)	70.3	69	0.85
Age	53.00 ± 4.95	56.03 ± 7.82	0.01
BMI (kg/m ²)	25.60 ± 3.23	25.53 ± 3.12	0.89
Smoker (%)	23.4	62.1	0.00
Drinker (%)	45.7	34.5	0.13

Continuous variables: mean ± SD. *Comparison of the ACS cases and controls. Independent Student's *t*-test for continuous variables, Chi-square test for categorical variables. ACS, acute coronary syndrome; BMI, body mass index.

(Fig. 3B). Consequently, the SIRT1 protein expression in the ACS patients (0.80 ± 0.04 fold, $p=0.01$) was lower than that observed in the control subjects (1.0 ± 0.06 fold). Taken together, the data in Fig. 3 suggest that patients with ACS have decreased levels of SIRT1 mRNA and proteins in their PBMCs.

Relationship between the Gene Expression of *SIRT1* and the Inflammatory Cytokine *IL-6*

In order to determine the relationship between the *SIRT1* and inflammatory cytokine *IL-6* gene expression, the transcript levels of *SIRT1* and *IL-6* in PBMCs obtained from healthy individuals were measured using real-time PCR. Consequently, the level of *SIRT1* negatively correlated with the inflammatory cytokine *IL-6* gene expression ($r = -0.45$, $p = 0.0002$, $n = 67$) (Fig. 4).

Functional SNP Prediction

In order to further explore the mechanisms underlying the regulation of the *SIRT1* gene in the setting of CAD with respect to genetic variants, we applied a bioinformatics tools to predict potential functional SNPs and subsequently evaluated the associations between the genotypes and the *SIRT1* mRNA expression. A search for potential functional features of the four SNPs on the ENCODE website showed that rs12778366 and rs3758391 were located in the enriched zone of H3K4Me1 markers, which are often found near regulatory elements (Fig. 5). In addition, rs4746720 was found to be located in an miRNA regulatory site on both the ENCODE and miRNASNP2 websites. The mutation of rs4746720 from T to C creates a binding site for hsa-miR-2115-3p (Supplementary Fig. 1). Rs2273773, which has been reported to be associated with body fat and blood pressure, is a synonymous variant in exon 5 of the *SIRT1* gene¹³. Hence, the above four SNPs were selected to examine whether their genotypes affect the mRNA expression of the *SIRT1* gene.

Association between the mRNA Expression Level of *SIRT1* and the Presence of the Four SNPs in the General Subjects

Genotyping of the four SNPs and real-time PCR detection of the *SIRT1* gene were implemented in the PBMC samples obtained from 58 ACS patients and 229 healthy participants. Among the healthy participants, the genotype frequencies of rs12778366, rs3758391, rs2273773 and rs4746720 were in compliance with the Hardy-Weinberg equilibrium ($p = 0.22$, $p = 0.84$, $p = 0.91$ and $p = 0.66$, respectively). We then analyzed the association between genotyping and the gene expression. However, there were no significant differences in the gene expression among the ACS patients with different genotypes (Supplementary Fig. 2).

We subsequently analyzed the allelic expression of rs12778366, rs3758391, rs2273773 and rs4746720 among the healthy participants and found notable differences for rs3758391. For example, the mRNA expression levels of *SIRT1* in rs3758391 were significantly higher in 175 individuals with the TT genotype (0.92 ± 0.08 fold) than in 54 individuals with the TC and CC genotypes (0.63 ± 0.07 fold, $p = 0.006$; Fig. 6A; only four CC homozygotes for rs3758391, thus TC and CC were combined). The characteristics and rs3758391 genotype distributions of the study participants are shown in Supplementary Table 5.

In contrast, the rs12778366 genotype was not associated with the *SIRT1* mRNA expression (TT, 0.85 ± 0.07 fold, $n = 178$; TC + CC, 0.88 ± 0.14 fold, $n = 48$; $p = 0.80$; Fig. 6B; only five CC homozygotes for rs3758391, thus TC and CC were combined). The characteristics and rs12778366 genotype distributions of the study participants are shown in Supplementary Table 6. The *SIRT1* expression levels did not differ among the genotypes of rs2273773 (TT, 0.81 ± 0.07 fold, $n = 121$; TC, 0.96 ± 0.12 fold, $n = 89$; CC, 0.62 ± 0.10 fold, $n = 17$; $p = 0.31$; Fig. 6C). The characteristics and rs2273773 genotype distributions of the study participants are shown in Supplementary Table

Fig. 3. Decreased levels of SIRT1 mRNA and proteins in the peripheral blood mononuclear cells (PBMCs) obtained from the patients with acute coronary syndrome (ACS). **A.** Decreased levels of SIRT1 mRNA in the PBMCs derived from the patients with AMI, UA and ACS (control group, 0.90 ± 0.72 fold, $n = 175$; AMI group, 0.34 ± 0.41 fold, $n = 28$; UA group, 0.43 ± 0.37 fold, $n = 30$; ACS group, $n = 58$). **B.** The levels of SIRT1 proteins were determined using a Western blot analysis. Top, representative Western blots; bottom, the results were quantified from the pixel values in grey scale (control group, 1.0 ± 0.06 fold, $n = 10$; ACS group, 0.80 ± 0.04 fold, $n = 8$). The data are presented as the mean $\pm$ SEM. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, compared with the control. Independent Student's *t*-test

7. Meanwhile, the genotypes of rs4746720 were not related to the *SIRT1* mRNA expression (TT, 0.64 ± 0.07 fold, $n = 64$; TC, 1.00 ± 0.11 fold, $n = 111$; CC, 0.79 ± 0.10 fold, $n = 54$; $p = 0.06$; **Fig. 6D**). The char-

Fig. 4. Negative correlation between the SIRT1 mRNA level and the inflammatory cytokine IL-6 gene expression. $r = -0.45$, $p = 0.0002$.

acteristics and rs4746720 genotype distributions of the study participants are shown in **Supplementary Table 8**.

Discussion

In the present study, the *SIRT1* mRNA expression was found to be reduced in the PBMCs obtained from the ACS patients. Furthermore, we found that rs3758391 was significantly associated with the expression of the *SIRT1* gene in the healthy participants.

Our case-control study using gene expression microarrays and real-time PCR replication demonstrated that the *SIRT1* mRNA expression level is associated with ACS, with a decreased expression in the PBMCs of the ACS patients, while earlier studies of the *SIRT1* gene expression reported a reduced expression of the *SIRT1* gene in monocytes derived from ACS patients⁷. PBMCs and monocytes are both cells related to the pathogenesis of ACS. Furthermore, it has been reported that *p66Shc* is negatively regulated by *SIRT1*²¹, while another study²² showed that the expression of *p66Shc* is increased in PBMCs in ACS patients. It has also been reported that the blood levels of SIRT1 proteins are increased in patients suffering from cardiovascular disease (CVD) compared to that noted in healthy subjects²³. These observations conflict with the results of our studies of ACS and another study of coronary artery disease⁷. In addition, we compared the mRNA expression levels of the *SIRT1* gene in the AMI and UA patients and found that the *SIRT1* expression levels were lower in the AMI patients than in the UA patients. This finding suggests that the *SIRT1* expression is more significantly related

Fig. 5. ENCODE functional annotation of the *SIRT1* gene with rs12778366, rs3758391, rs2273773 and rs4746720. H3K27Ac, acetylation of lysine 27 of the H3 histone protein; H3K4me1, mono-methylation of lysine 4 of the H3 histone protein; H3K4Me3, tri-methylation of lysine 4 of the H3 histone protein.

to AMI than to UA, although no studies have been conducted to explain this result.

ACS is the result of the physical rupture of atherosclerotic plaque, and inflammation plays a major role in all stages of atherogenesis²⁾. In addition, *SIRT1* exerts anti-inflammatory effects in the setting of atherosclerosis²⁴⁾, and the level of *SIRT1* negatively correlated with the gene expression of the inflammatory cytokine *IL-6* in our study. Moreover, it has been reported that ACS patients have significantly elevated levels of the inflammatory cytokine *IL-6*²⁵⁾ and that the knockdown of *SIRT1* results in an increased *IL-6* expression²⁶⁾. These findings indicate that inflammation is a contributing factor affecting the downregulation of the *SIRT1* gene expression.

Another contributing factor may be the effects of the non-coding genetic variant of the *SIRT1* gene. Increasing evidence suggests that non-coding genetic variants impact the gene expression by binding to different transcription factors²⁷⁾. In the present study, we observed a strong relationship between rs3758391 and the *SIRT1* mRNA expression in healthy individuals, and rs3758391 was not in LD with the other three SNPs (**Supplementary Table 9**). It has also been reported that rs3758391 is associated with the calorie restriction-stimulated *SIRT1* expression and may affect the *SIRT1* mRNA expression via the loss of binding sites of the transcription factor p53. The C allele of rs3758391 disrupts the mirror-image symme-

try of the p53 consensus binding sequence²⁸⁾. Therefore, the change from T to C in a conserved region of the p53-binding element decreases binding to p53 *in vitro*. Furthermore, p53 has been found to induce myocyte apoptosis via activation of the renin-angiotensin system²⁹⁾. The high *SIRT1* expression of the T allele of rs3758391 may be the result of a protective negative feedback loop with respect to the detrimental effects of p53. To our knowledge, the present study is the first to confirm this finding in the general population. Finally, *SIRT1* has been found to deacetylate p53 and regulate the functions of p53 in cells³⁰⁾. That is to say, rs3758391 regulates the *SIRT1* expression by changing the p53 binding site. Therefore, inflammation and *SIRT1*-related genetic variants may be contributing factors affecting the downregulation of the *SIRT1* gene expression.

We also analyzed the association between the presence of the four functional SNPs and the *SIRT1* mRNA expression in the ACS patients; however, we did not find any significant differences among the different genotypes. The primary explanation for this finding is the use of medications prior to blood sampling in the ACS patients, which may have affected the *SIRT1* gene expression.

Concerning rs2273773, we found no significant associations with the *SIRT1* mRNA expression, although the discrepant data for the alleles with respect to fasting glucose, BMI and diastolic blood pressure

Fig. 6. Relationship between the SIRT1 mRNA expression levels in the healthy subjects and the genotypes of rs3758391, rs4746720, rs12778366 and rs2273773.

A. Expression levels of SIRT1 mRNA according to the different genotypes of rs3758391 (TT, 0.92 ± 0.08 fold, $n = 175$; TC + CC, 0.63 ± 0.07 fold, $n = 54$; only four CC homozygotes for rs3758391, thus TC and CC were combined. Independent Student's t -test).

B. Expression levels of SIRT1 mRNA according to the different genotypes of rs12778366 (TT, 0.85 ± 0.07 fold, $n = 178$; TC + CC, 0.88 ± 0.14 fold, $n = 48$; only five CC homozygotes for rs12778366, thus TC and CC were combined. Independent Student's t -test).

C. Expression levels of SIRT1 mRNA according to the different genotypes of rs2273773 (TT, 0.81 ± 0.07 fold, $n = 121$; TC, 0.96 ± 0.12 fold, $n = 89$; CC, 0.62 ± 0.10 fold, $n = 17$. Analysis of variance).

D. Expression levels of SIRT1 mRNA according to the different genotypes of rs4746720 (TT, 0.64 ± 0.07 fold, $n = 64$; TC, 1.00 ± 0.11 fold, $n = 111$; CC, 0.79 ± 0.10 fold, $n = 54$. Analysis of variance).

The data are presented as the mean $\pm$ SEM. ** $p < 0.01$; n.s., non significant.

(Supplementary Table 7) were in accordance with the findings of a Japanese health checkup examinee study¹³). Although rs2273773 is a synonymous mutation, it may act via noncoding functions pre-transcriptionally at the DNA level or post-transcriptionally at the RNA level²⁷). *SIRT1* participates in fat mobilization via the actions of peroxisome proliferator-acti-

vated receptor- γ (PPAR γ)³¹), which inhibits the *SIRT1* expression at the transcriptional level. Rs2273773 may therefore affect BMI and blood pressure via PPAR γ . Our results are the first to indicate this phenomenon in a Chinese Han population, although further confirmation is required in other populations with larger sample sizes.

The limitation of our study is that the actions of PBMCs only partly reflect the biological events occurring in the vessel wall during the inflammatory response. However, endothelial and/or smooth muscle cell samples, which may better represent the condition of the coronary vessels, were inaccessible in this study due to the large sample size. In addition, the complexity of our population study means that the results must be verified in a larger sample.

Conclusion

The present findings further confirm that the *SIRT1* mRNA expression is reduced in ACS patients and that the gene *SIRT1* therefore confers a positive effect against the development of ACS. In addition, the detection of rs3758391 may be used in *SIRT1*-related disease prediction as a biomarker. The fact that the change in *SIRT1* mRNA is associated with the presence of nearby SNPs provides insight into the mechanisms by which common noncoding genetic variants affect the target gene expression via the actions of transcription factors. Hence, a more thorough functional analysis is warranted to verify the role of these variants in the pathogenesis of ACS.

Acknowledgements

This work was supported by grants from the National Basic Research Program of China (973 Plan) (2011CB503901) and the National Science Foundation of China (81273153 and 81270334).

Conflicts of Interest

None declared.

References

- 1) Delgado GA, Abbott JD: Acute Coronary Syndromes. In: Evidence-Based Cardiology Consult. Springer, 2014; 15-36
- 2) Libby P, Theroux P: Pathophysiology of coronary artery disease. *Circulation*, 2005; 111: 3481-3488
- 3) Libby P, Ridker PM, Hansson GK: Progress and challenges in translating the biology of atherosclerosis. *Nature*, 2011; 473: 317-325
- 4) Gasser SM, Cockell MM: The molecular biology of the SIR proteins. *Gene*, 2001; 279: 1-16
- 5) Pillarisetti S: A review of Sirt1 and Sirt1 modulators in cardiovascular and metabolic diseases. *Recent Pat Cardiovasc Drug Discov*, 2008; 3: 156-164
- 6) Stein S, Lohmann C, Schäfer N, Hofmann J, Rohrer L, Besler C, Rothgiesser KM, Becher B, Hottiger MO, Borén J: SIRT1 decreases Lox-1-mediated foam cell formation in atherogenesis. *Eur Heart J*, 2010; 31: 2301-2309
- 7) Breitenstein A, Wyss CA, Spescha RD, Franzeck FC, Hof D, Riwanoto M, Hasun M, Akhmedov A, von Eckardstein A, Maier W: Peripheral Blood Monocyte Sirt1 Expression Is Reduced in Patients with Coronary Artery Disease. *PLoS One*, 2013; 8: e53106
- 8) Stein S, Matter CM: Protective roles of SIRT1 in atherosclerosis. *Cell Cycle*, 2011; 10: 640-647
- 9) Zillikens MC, van Meurs JB, Sijbrands EJ, Rivadeneira F, Dehghan A, van Leeuwen JP, Hofman A, van Duijn CM, Witteman J, Uitterlinden AG: SIRT1 genetic variation and mortality in type 2 diabetes: interaction with smoking and dietary niacin. *Free Radical Biol Med*, 2009; 46: 836-841
- 10) Kuningas M, Putters M, Westendorp RG, Slagboom PE, van Heemst D: SIRT1 gene, age-related diseases, and mortality: the Leiden 85-plus study. *J Gerontol A Biol Sci Med Sci*, 2007; 62: 960-965
- 11) Shimoyama Y, Mitsuda Y, Tsuruta Y, Suzuki K, Hamajima N, Niwa T: SIRTUIN 1 Gene Polymorphisms are Associated With Cholesterol Metabolism and Coronary Artery Calcification in Japanese Hemodialysis Patients. *J Ren Nutr*, 2012; 22: 114-119
- 12) Zillikens MC, van Meurs JB, Rivadeneira F, Amin N, Hofman A, Oostra BA, Sijbrands EJ, Witteman JC, Pols HA, van Duijn CM: SIRT1 genetic variation is related to BMI and risk of obesity. *Diabetes*, 2009; 58: 2828-2834
- 13) Shimoyama Y, Suzuki K, Hamajima N, Niwa T: Sirtuin 1 gene polymorphisms are associated with body fat and blood pressure in Japanese. *Transl Res*, 2011; 157: 339-347
- 14) Clark SJ, Falchi M, Olsson B, Jacobson P, Cauchi S, Balkau B, Marre M, Lantieri O, Andersson JC, Jernäs M: Association of sirtuin 1 (SIRT1) gene SNPs and transcript expression levels with severe obesity. *Obesity*, 2012; 20: 178-185
- 15) Lu X, Wang L, Chen S, He L, Yang X, Shi Y, Cheng J, Zhang L, Gu CC, Huang J, Wu T, Ma Y, Li J, Cao J, Chen J, Ge D, Fan Z, Li Y, Zhao L, Li H, Zhou X, Chen L, Liu D, Duan X, Hao Y, Lu F, Liu Z, Yao C, Shen C, Pu X, Yu L, Fang X, Xu L, Mu J, Wu X, Zheng R, Wu N, Zhao Q, Liu X, Wang M, Yu D, Hu D, Ji X, Guo D, Sun D, Wang Q, Yang Y, Liu F, Mao Q, Liang X, Ji J, Chen P, Mo X, Li D, Chai G, Tang Y, Li X, Du Z, Dou C, Yang Z, Meng Q, Wang D, Wang R, Yang J, Schunkert H, Samani NJ, Kathiresan S, Reilly MP, Erdmann J, Peng X, Chen R, Qiang B, Gu D: Genome-wide association study in Han Chinese identifies four new susceptibility loci for coronary artery disease. *Nat Genet*, 2012; 44: 890-894
- 16) Benjamini Y, Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*, 1995; 289-300
- 17) Feingold E, Good P, Guyer M, Kamholz S, Liefer L, Wetterstrand K, Collins F, Gingeras T, Kampa D, Sekinger E: The ENCODE (ENCyclopedia of DNA elements) project. *Science*, 2004; 306: 636-640
- 18) Yuan H-Y, Chiou J-J, Tseng W-H, Liu C-H, Liu C-K, Lin Y-J, Wang H-H, Yao A, Chen Y-T, Hsu C-N: FAST-SNP: an always up-to-date and extendable service for

- SNP function analysis and prioritization. *Nucleic Acids Res*, 2006; 34: W635-W641
- 19) Gong J, Tong Y, Zhang HM, Wang K, Hu T, Shan G, Sun J, Guo AY: Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Hum Mutat*, 2012; 33: 254-263
 - 20) Livak KJ, Schmittgen TD: Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 2001; 25: 402-408
 - 21) Chen H-Z, Wan Y-Z, Liu D-P: Cross-talk between SIRT1 and p66Shc in vascular diseases. *Trends Cardiovasc Med*, 2013; 23: 237-241
 - 22) Franzeck FC, Hof D, Spescha RD, Hasun M, Akhmedov A, Steffel J, Shi Y, Cosentino F, Tanner FC, von Eckardstein A: Expression of the aging gene p66Shc is increased in peripheral blood monocytes of patients with acute coronary syndrome but not with stable coronary artery disease. *Atherosclerosis*, 2012; 220: 282-286
 - 23) Kilic U, Gok O, Bacaksiz A, Izmirli M, Elibol-Can B, Uysal O: SIRT1 gene polymorphisms affect the protein expression in cardiovascular diseases. *PLoS One*, 2014; 9: e90428
 - 24) Winnik S, Stein S, Matter CM: SIRT1 - an anti-inflammatory pathway at the crossroads between metabolic disease and atherosclerosis. *Curr Vasc Pharmacol*, 2012; 10: 693-696
 - 25) Oudi ME, Aouni Z, Mazigh C, Khochkar R, Gazoueni E, Haouela H, Machghoul S: Homocysteine and markers of inflammation in acute coronary syndrome. *Exp Clin Cardiol*, 2010; 15: e25-28
 - 26) Zhu X, Liu Q, Wang M, Liang M, Yang X, Xu X, Zou H, Qiu J: Activation of Sirt1 by resveratrol inhibits TNF-alpha induced inflammation in fibroblasts. *PLoS One*, 2011; 6: e27081
 - 27) Ward LD, Kellis M: Interpreting noncoding genetic variation in complex traits and human disease. *Nat Biotechnol*, 2012; 30: 1095-1106
 - 28) Naqvi A, Hoffman TA, DeRicco J, Kumar A, Kim C-S, Jung S-B, Yamamori T, Kim Y-R, Mehdi F, Kumar S: A single-nucleotide variation in a p53-binding site affects nutrient-sensitive human SIRT1 expression. *Hum Mol Genet*, 2010; 19: 4123-4133
 - 29) Pierzchalski P, Reiss K, Cheng W, Cirielli C, Kajstura J, Nitahara JA, Rizk M, Capogrossi MC, Anversa P: p53 induces myocyte apoptosis via the activation of the renin-angiotensin system. *Exp Cell Res*, 1997; 234: 57-65
 - 30) Luo J, Nikolaev AY, Imai S, Chen D, Su F, Shiloh A, Guarente L, Gu W: Negative control of p53 by Sir2alpha promotes cell survival under stress. *Cell*, 2001; 107: 137-148
 - 31) Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, De Oliveira RM, Leid M, McBurney MW, Guarente L: Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature*, 2004; 429: 771-776

Supplementary Table 1.

Primer and probe sequences of variants at the SIRT1 locus

SNP probes	Sequences
rs3758391-F	5'- TGCCATAACAAACACTGGCTCTA -3'
rs3758391-R	5'- GCACACTGTGACTCCATATCTAATCTTA -3'
rs3758391-P-T	5'- FAM-ATCTACCA <i>T</i> GGGTATAT-MGB -3'
rs3758391-P-C	5'- HEX-ATCTACCA <i>C</i> GGGTAT-MGB -3'
rs12778366-F	5'- GCTTCTAGGACTGGAGATGATTACTTTC -3'
rs12778366-R	5'- TCCTATCTACATCCAAAAGTCTTATTTCA -3'
rs12778366-P-T	5'- FAM-AAATGAA <i>T</i> AGTGGTGACCAG-MGB -3'
rs12778366-P-C	5'- HEX-AAATGAA <i>C</i> AGTGGTGACC-MGB -3'
rs4746720-F	5'- CACTTTTCTTTGTAACATTGAATGGTTT -3'
rs4746720-R	5'- TGCCAGTGTTTAAAAATAATTGTGTT -3'
rs4746720-P-C	5'- FAM-CGCTAAACTT <i>C</i> TGATTC-MGB -3'
rs4746720-P-T	5'- HEX-CGCTAAACTT <i>T</i> TGATTC-MGB -3'
rs2273773-F	5'- TCCAGCCATCTCTCTGTCACAA -3'
rs2273773-R	5'- GCGTGTCTATGTTCTGGGTATAGTTG -3'
rs2273773-P-T	5'- FAM-TTCATAGCC <i>T</i> TGTCAGAT-MGB -3'
rs2273773-P-C	5'- HEX-TTCATAGCC <i>C</i> TGTCAGA-MGB -3'

F, forward; R, reverse; P, probe; Bold and italic letters denote SNP alleles.

Supplementary Table 2.

Primers used to evaluate the expression levels of SIRT1 and IL-6 on real-time PCR

Gene	Sequences	Amplicon size (bp)
SIRT1-F	5'- ATGCAAGCTCTAGTGACTGGACT -3'	134
SIRT1-R	5'- CTCAGGTGGAGGTATTGTTTCC -3'	
IL-6-F	5'- GATGGCTGAAAAAGATGGATGC -3'	230
IL-6-R	5'- TGGTTGGGTCAGGGGTGGTT -3'	
GAPDH-F	5'- AGCCACATCGCTCAGACAC -3'	66
GAPDH-R	5'- GCCCAATACGACCAATCC -3'	

F, forward; R, reverse.

Supplementary Table 3.

Differentially upregulated genes with a potential role in AMI, UA and ACS compared with that observed in the controls

Gene Symbol	Gene Entrez ID	Gene Name	AMI Fold	UA Fold	ACS Fold
GNG10	2790	guanine nucleotide binding protein (G protein), gamma 10	2.55	1.66	2.11
GNAQ	2776	guanine nucleotide binding protein (G protein), q polypeptide	1.83	1.91	1.87
LOC401357	401357	hypothetical LOC401357	1.89	1.73	1.81
RRAGD	58528	Ras-related GTP binding D	1.86	1.70	1.78
ZNF394	84124	zinc finger protein 394	1.63	1.76	1.70
IMPDH1	3614	IMP (inosine monophosphate) dehydrogenase 1	1.76	1.63	1.69
MAP3K7IP1	10454	mitogen-activated protein kinase kinase kinase 7 interacting protein 1	1.74	1.60	1.67
EVI2B	2124	ecotropic viral integration site 2B	1.71	1.61	1.66
C10orf32	119032	chromosome 10 open reading frame 32	1.57	1.69	1.63
PGAM4	441531	phosphoglycerate mutase family member 4	1.67	1.58	1.63
GRB2	2885	growth factor receptor-bound protein 2	1.72	1.49	1.60
MOBKL3	25843	MOB1, Mps One Binder kinase activator-like 3 (yeast)	1.62	1.55	1.58
CMTM7	112616	CKLF-like MARVEL transmembrane domain containing 7	1.54	1.57	1.55
PTPRC	5788	protein tyrosine phosphatase, receptor type, C	1.60	1.46	1.53
EIF3I	8668	eukaryotic translation initiation factor 3, subunit I	1.53	1.52	1.52
SPG21	51324	spastic paraplegia 21 (autosomal recessive, Mast syndrome)	1.56	1.47	1.52
TIMP1	7076	TIMP metalloproteinase inhibitor 1	1.67	1.35	1.51
TRAK1	22906	trafficking protein, kinesin binding 1	1.45	1.50	1.48
CARM1	10498	coactivator-associated arginine methyltransferase 1	1.40	1.37	1.39
LOC200030	200030	neuroblastoma breakpoint family, member 11-like	1.39	1.38	1.38
UBE2D3	7323	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	1.40	1.35	1.38
H3F3A	3020	H3 histone, family 3A	1.33	1.32	1.32
PABPC1	26986	Homo sapiens poly(A) binding protein, cytoplasmic 1	1.32	1.32	1.32

AMI, acute myocardial infarction; UA, unstable angina; ACS, acute coronary syndrome.

Supplementary Table 4.

Differentially downregulated genes with a potential role in AMI, UA and ACS compared with that observed in the controls

Gene Symbol	Gene Entrez ID	Gene Name	AMI Fold	UA Fold	ACS Fold
G3BP1	10146	GTPase activating protein (SH3 domain) binding protein 1	16.06	35.41	22.10
SPON2	10417	spondin 2, extracellular matrix protein	69.71	4.65	8.73
MCTS1	28985	malignant T cell amplified sequence 1	5.07	2.89	3.68
SEPT7	989	septin 7	4.62	2.68	3.39
SFRS11	9295	splicing factor, arginine/serine-rich 11	3.29	2.60	2.90
USP8	9101	ubiquitin specific peptidase 8	3.03	2.62	2.81
MYBL1	4603	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	3.69	2.01	2.60
ZNF557	79230	zinc finger protein 557	2.35	2.90	2.60
NOC3L	64318	Homo sapiens nucleolar complex associated 3 homolog (S. cerevisiae)	2.62	2.46	2.54
CAPZA1	829	capping protein (actin filament) muscle Z-line, alpha 1	2.32	2.75	2.51
RPLP1	6176	ribosomal protein, large, P1	2.88	2.21	2.50
PTMA	5757	prothymosin, alpha	2.63	2.28	2.44
ERGIC1	57222	endoplasmic reticulum-Golgi intermediate compartment 1	2.40	2.25	2.32
ZNF146	7705	zinc finger protein 146	2.20	2.37	2.28
LEP	3952	leptin (obesity homolog, mouse)	1.96	2.16	2.06
NNT	23530	nicotinamide nucleotide transhydrogenase (NNT), nuclear gene encoding mitochondrial protein	2.07	2.03	2.05
USP49	25862	ubiquitin specific peptidase 49	1.93	2.13	2.02
GNA13	10672	guanine nucleotide binding protein (G protein), alpha 13	2.11	1.92	2.01
TMEM106A	113277	transmembrane protein 106A	1.93	2.09	2.01
ETS1	2113	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)	2.11	1.91	2.01
SIRT1	23411	silent information regulator 1	2.12	1.86	1.98
FYTTD1	84248	forty-two-three domain containing 1	1.98	1.98	1.98
VPS41	27072	vacuolar protein sorting 41 homolog	1.97	1.91	1.94
DTWD2	285605	DTW domain containing 2	1.85	2.02	1.93
PLA2G2D	26279	phospholipase A2	1.80	2.06	1.92
ACTR3	10096	ARP3 actin-related protein 3 homolog (yeast)	2.00	1.84	1.92
RPS28	6234	ribosomal protein S28	2.05	1.78	1.90
PTPLAD2	401494	protein tyrosine phosphatase-like A domain containing 2	1.79	2.03	1.90
HLA-G	3135	HLA-G histocompatibility antigen, class I, G	2.08	1.73	1.88
NLRP8	126205	NLR family, pyrin domain containing 8	1.76	1.99	1.87
PTGES3	10728	prostaglandin E synthase 3 (cytosolic)	1.97	1.78	1.87
ATP11B	23200	ATPase, class VI, type 11B	1.93	1.80	1.86
C14orf169	79697	chromosome 14 open reading frame 169	2.06	1.70	1.86
GDPD1	284161	glycerophosphodiester phosphodiesterase domain containing 1	1.69	2.06	1.86
SNAPC1	6617	small nuclear RNA activating complex, polypeptide 1	1.76	1.96	1.85
UBE4A	9354	ubiquitination factor E4A (UFD2 homolog, yeast)	1.85	1.82	1.84
RASGRP1	10125	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	1.92	1.68	1.79
CLK1	1195	CDC-like kinase 1	1.95	1.63	1.78
GPR65	8477	G protein-coupled receptor 65	1.87	1.67	1.76
SF3B14	51639	splicing factor 3B, 14 kDa subunit	1.73	1.78	1.76
LEPROTL1	23484	leptin receptor overlapping transcript-like 1	1.75	1.72	1.73

(Cont Supplementary Table 4)

Gene Symbol	Gene Entrez ID	Gene Name	AMI Fold	UA Fold	ACS Fold
RPL23	9349	ribosomal protein L23	1.85	1.62	1.73
IPP	3652	intracisternal A particle-promoted polypeptide	1.64	1.83	1.73
FASTK	10922	Fas-activated serine/threonine kinase	1.69	1.78	1.73
DENR	8562	density-regulated protein	1.71	1.74	1.73
MTMR6	9107	myotubularin related protein 6	1.83	1.61	1.71
UBLCP1	134510	ubiquitin-like domain containing CTD phosphatase 1	1.72	1.68	1.70
ZNF223	7766	zinc finger protein 223	1.61	1.77	1.68
SPTLC1	10558	serine palmitoyltransferase, long chain base subunit 1	1.58	1.79	1.68
KIAA1370	56204	KIAA1370	1.80	1.56	1.67
RPL9	6133	ribosomal protein L9	1.67	1.65	1.66
AFTPH	54812	aftiphilin	1.61	1.70	1.66
EXOSC10	5394	exosome component 10	1.74	1.57	1.65
WSB1	26118	WD repeat and SOCS box-containing 1	1.50	1.82	1.64
MST4	51765	serine/threonine protein kinase MST4	1.79	1.52	1.64
YIPF3	25844	Yip1 domain family, member 3	1.55	1.74	1.64
BIRC3	330	baculoviral IAP repeat-containing 3	1.53	1.74	1.62
EIF2AK4	440275	eukaryotic translation initiation factor 2 alpha kinase 4	1.49	1.79	1.62
SKA2	348235	spindle and kinetochore associated complex subunit 2	1.69	1.56	1.62
CUL4A	8451	cullin 4A	1.72	1.53	1.62
ZNF669	79862	zinc finger protein 669	1.51	1.72	1.61
IBTK	25998	inhibitor of Bruton agammaglobulinemia tyrosine kinase	1.54	1.67	1.61
DDX17	10521	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	1.59	1.56	1.57
EIF3M	10480	eukaryotic translation initiation factor 3	1.57	1.58	1.57
CD247	919	CD247 molecule	1.76	1.40	1.57
CEP27	55142	centrosomal protein 27kDa	1.51	1.63	1.57
RBM34	23029	RNA binding motif protein 34	1.56	1.55	1.56
AKAP11	11215	A kinase (PRKA) anchor protein 11	1.62	1.49	1.55
EXOSC9	5393	exosome component 9	1.67	1.44	1.55
PGRMC2	10424	progesterone receptor membrane component 2	1.62	1.47	1.54
CNBP	7555	CCHC-type zinc finger, nucleic acid binding protein	1.54	1.51	1.53
SLC4A5	57835	solute carrier family 4, sodium bicarbonate cotransporter, member 5	1.50	1.53	1.52
PTPLB	201562	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member b	1.45	1.53	1.49
TRMT112	51504	tRNA methyltransferase 11-2 homolog (<i>S. cerevisiae</i>)	1.58	1.36	1.46
NARS	4677	asparaginyl-tRNA synthetase	1.43	1.39	1.41

AMI, acute myocardial infarction; UA, unstable angina; ACS, acute coronary syndrome.

SNP in gene 3'UTR	miRNA	SNP location	Energy change (kcal/mol)	miRNA/SNP-target duplexes	Effect by SNP on 3'UTR
SIRT1(NM_001142498); rs4746720 (U/C)	hsa-miR-2115-3p	chr10:69676830	Wild Type: -10.50 SNP Type: -13.10	miRNA: 3'gauCGGAGGUACUU-AAGACUAc 5' UTR: 5'tctGTTACGCTAAACTTCTGATt 3' rs4746720: U → C	gain
SIRT1(NM_012238); rs4746720 (U/C)	hsa-miR-2115-3p	chr10:69676830	Wild Type: -10.50 SNP Type: -13.10	miRNA: 3'gauCGGAGGUACUU-AAGACUAc 5' UTR: 5'tctGTTACGCTAAACTTCTGATt 3' rs4746720: U → C	gain

Supplementary Fig. 1.
miRNASNP2 prediction results for rs4746720

Supplementary Fig. 2.
Relationships between the SIRT1 mRNA expression levels in the ACS patients and the genotypes of rs12778366 rs3758391, rs2273773 and rs4746720

Supplementary Table 5.

Characteristics and rs3758391 genotype distributions of the study participants

Characteristics	rs3758391		<i>p</i> -value*
	TT (<i>n</i> = 175)	TC + CC (<i>n</i> = 54)	
Age	50.65 ± 5.67	50.76 ± 5.73	0.91
BMI (kg/m ²)	25.05 ± 2.71	24.96 ± 2.85	0.87
GLU (mg/dL)	102.34 ± 21.90	103.71 ± 23.99	0.92
CHOL (mg/dL)	177.92 ± 47.77	175.35 ± 45.80	0.76
TG (mg/dL)	123.60 ± 74.84	103.83 ± 57.09	0.12
SBP (mmHg)	130.14 ± 12.85	130.77 ± 12.74	0.83
DBP (mmHg)	83.20 ± 8.22	82.41 ± 7.86	0.47
Smoker (%)	13.7	11.1	0.62
Drinker (%)	28	20.4	0.27

*Comparison of the TT genotype with the TC and CC genotypes. Independent Student's *t*-test for continuous variables, Chi-square test for categorical variables. Continuous variables, mean ± SD. BMI, body mass index; GLU, glucose; CHOL, cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplementary Table 6.

Characteristics and rs12778366 genotype distributions of the study participants

Characteristics	rs12778366		<i>p</i> -value*
	TT (<i>n</i> = 178)	TC + CC (<i>n</i> = 48)	
Age	50.88 ± 12.41	49.9 ± 5.34	0.29
BMI (kg/m ²)	24.83 ± 2.78	25.75 ± 2.43	0.04
GLU (mg/dL)	102.93 ± 21.56	101.66 ± 25.87	0.73
CHOL (mg/dL)	180.11 ± 46.68	168.49 ± 44.61	0.13
TG (mg/dL)	121.14 ± 76.16	110.50 ± 49.58	0.37
SBP (mmHg)	130.39 ± 12.41	129.99 ± 14.02	0.85
DBP (mmHg)	83.03 ± 7.71	82.70 ± 9.32	0.80
Smoker (%)	12.4	14.6	0.69
Drinker (%)	27.5	20.8	0.35

*Comparison of the TT genotype with the TC and CC genotypes. Independent Student's *t*-test for continuous variables, Chi-square test for categorical variables. Continuous variables, mean ± SD. BMI, body mass index; GLU, glucose; CHOL, cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplementary Table 7.

Characteristics and rs2273773 genotype distributions of the study participants

Characteristics	rs2273773			<i>p</i> -value*
	TT (<i>n</i> = 121)	TC (<i>n</i> = 89)	CC (<i>n</i> = 17)	
Age	50.86 ± 5.79	50.15 ± 5.38	52.06 ± 6.49	0.39
BMI (kg/m ²)	25.45 ± 2.71	24.51 ± 2.63	24.30 ± 2.94	0.03
GLU (mg/dL)	104.54 ± 23.72	102.82 ± 20.62	89.80 ± 17.94	0.04
CHOL (mg/dL)	176.79 ± 46.73	177.36 ± 46.96	180.52 ± 54.63	0.96
TG (mg/dL)	113.46 ± 65.80	121.39 ± 70.76	147.30 ± 105.03	0.18
SBP (mmHg)	130.56 ± 13.54	129.00 ± 12.02	132.84 ± 10.08	0.45
DBP (mmHg)	83.54 ± 8.24	81.48 ± 8.06	86.55 ± 10.08	0.03
Smoker (%)	11.6	14.6	17.6	0.70
Drinker (%)	29.8	22.5	23.5	0.48

*Comparison among the TT, TC and CC genotypes. Analysis of variance (ANOVA) for continuous variables, Chi-square test for categorical variables. Continuous variables, mean ± SD. BMI, body mass index; GLU, glucose; CHOL, cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplementary Table 8.

Characteristics and rs4746720 genotype distributions of the study participants

Characteristics	rs4746720			<i>p</i> -value*
	TT (<i>n</i> = 64)	TC (<i>n</i> = 111)	CC (<i>n</i> = 54)	
Age	50.78 ± 5.87	50.45 ± 5.36	51.00 ± 6.11	0.83
BMI (kg/m ²)	24.40 ± 2.52	25.43 ± 2.76	24.95 ± 2.86	0.06
GLU (mg/dL)	98.42 ± 22.42	103.55 ± 22.71	105.79 ± 21.26	0.18
CHOL (mg/dL)	167.99 ± 44.74	181.15 ± 50.84	180.18 ± 41.29	0.19
TG (mg/dL)	109.11 ± 67.35	125.17 ± 72.83	117.53 ± 72.98	0.36
SBP (mmHg)	129.33 ± 12.17	131.52 ± 12.54	128.88 ± 14.01	0.36
DBP (mmHg)	82.17 ± 8.23	83.48 ± 8.29	83.06 ± 7.07	0.59
Smoker (%)	15.6	11.7	13	0.76
Drinker (%)	18.8	25.2	37	0.08

*Comparison among the TT, TC and CC genotypes. Analysis of variance (ANOVA) for continuous variables, Chi-square test for categorical variables. Continuous variables, mean ± SD. BMI, body mass index; GLU, glucose; CHOL, cholesterol; TG, triglycerides; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Supplementary Table 9.Linkage disequilibrium plot of the SNPs with *r*² among the 229 healthy subjects

<i>r</i> ²	rs12778366	rs3758391	rs2273773	rs4746720
rs12778366		0.02	0.13	0.05
rs3758391			0.12	0.05
rs2273773				0.33
rs4746720				