

# Identification of TFEB gene genetic variants in acute myocardial infarction

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## Research article

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# Abstract

**Background:** Abnormal lipid metabolism and inflammation play critical roles in the initiation and progression of atherosclerosis and its associated complications, including coronary artery disease (CAD) and acute myocardial infarction (AMI). Autophagic-lysosomal system is involved in many physiological processes, such as lipid metabolism and inflammation. TFEB, a master regulator of the system, coordinates the expression of lysosomal hydrolases, lysosomal membrane proteins, and autophagic proteins. Altered level of TFEB gene expression and subsequent changes of autophagic-lysosomal system may be involved in the onset of CAD and AMI.

**Methods:** In this study, the promoter of the TFEB gene was genetically and functionally analyzed in AMI patients (n=352) and ethnic-matched healthy controls (n=337).

**Results:** A total of fifteen genetic variants, including eight single nucleotide polymorphisms (SNPs), were identified in the participants. Two novel genetic variants and four SNPs were only identified in six AMI patients, and significantly altered the transcriptional activity of the TFEB gene in cultured cells. Further electrophoretic mobility shift assay revealed that two genetic variants (g.41737144A>G and g.41736544C>T) and two SNPs [g.41737274T>C (rs533895008) and g.41736987C>T (rs760293138)] evidently affected the binding of transcription factors.

**Conclusions:** Our findings suggested that the genetic variants in TFEB gene promoter may change TFEB levels, contributing to AMI as a low-frequency risk factor.

## Introduction

Coronary artery disease (CAD) including acute myocardial infarction (AMI) is an inflammatory and metabolic disease, which is mainly caused by atherosclerosis. Abnormal lipid metabolism and inflammation play critical roles in the initiation and progression of atherosclerosis and its complications [1, 2]. Accumulating evidence indicate that genetic factors contribute to the onset of CAD and AMI. To date, many genome-wide association studies have identified a great number of genetic loci for CAD and AMI. However, the collective genetic loci could explain only < 10% of cases [3–5]. Therefore, genetic causes and underlying molecular mechanisms for CAD and AMI remain to be elucidated. Recent studies suggested that low-frequency and rare genetic variants may confer susceptibility to cardiovascular diseases [6–8].

There are three subtypes of autophagy, macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy (hereafter referred as to autophagy) degrades cytoplasmic macromolecules and organelles by delivering them to lysosomes. Autophagy has been involved in many physiological processes, including lipid metabolism and inflammation. Dysfunctional autophagy has been implicated in a wide range of human diseases, including cardiovascular diseases [9, 10]. The lysosome has long been viewed as the recycling center of the cell, and has recently been established to play a central role of nutrient-dependent signal transduction [11, 12]. Recent studies have demonstrated that transcription

factor EB (TFEB) has been involved in the co-regulation between lysosome, autophagy, and lipid metabolism [13].

TFEB belongs to the MiT-TFE family of basic helix-loop-helix leucine-zipper transcription factors, which include TFEB, transcription factor E3 (TFE3), transcription factor EC (TFEC) and microphthalmia-associated transcription factor (MITF). Activity of these transcription factors are regulated by their shuttling between the surface of lysosomes, the cytoplasm, and the nucleus. TFEB regulates several cellular processes, including lysosome biogenesis, cellular energy homeostasis, autophagy, mitochondrial turnover, innate immune response and inflammation [13–19]. At transcriptional level, TFEB coordinates the expression of lysosomal hydrolases, lysosomal membrane proteins and autophagy proteins in response to various stress and nutritional fluctuation of the cell. TFEB binds to CLEAR (coordinated lysosomal expression and regulation) motif within the promoters of the genes [12, 16].

Recent studies suggest that TFEB also controls vascular development by regulating the proliferation of endothelial cells [20]. Overexpression of TFEB gene in endothelial cells in mice increases angiogenesis and improves blood flow recovery after ischemic injury [21]. Animal experiments show that TFEB inhibits endothelial cell inflammation and reduces atherosclerosis [22, 23]. Therefore, we postulated that altered TFEB levels may contribute to the onset of CAD and AMI. In this case-control study, we genetically and functionally analyzed the promoter of TFEB gene in large cohorts of AMI patients and ethnic-matched healthy controls.

## Materials And Methods

### Study participants

AMI patients (n = 352; male 267 and female 85) were recruited from Division of Cardiology, Affiliated Hospital of Jining Medical University (Jining, Shandong, China) during the period from March, 2015 to June, 2017. AMI Patients were diagnosed according to clinical manifestations, electrocardiograms, elevated biochemical markers of myocardial necrosis or coronary angioplasty. Ethnically-matched healthy controls (n = 337; male 167 and female 170) were recruited from Physical Examination in the same hospital during the same time period. The controls with a familial history of CAD and other heart diseases were excluded. This study was conducted according to the principles of the Declaration of Helsinki. The study protocol was approved by the Human Ethics Committee of the Affiliated Hospital of Jining Medical University. Written informed consent was obtained from all participants.

### Direct DNA sequencing

Fasting venous blood was collected and peripheral leukocytes were isolated with Human Leukocyte Isolation system (Haoyang Biological Products Technology Co., Ltd., Tianjin, China). Genomic DNAs were extracted with the QIAamp DNA Mini kit (Qiagen, Inc., Valencia, CA, USA). The promoter region of the human TFEB gene were generated with PCR and directly sequenced. Two overlapped DNA fragments, 705 bp (-1312 bp ~ -608 bp) and 801 bp (-657 bp ~ +144 bp), were overlapped, covering the TFEB gene

promoter region. The PCR primers were designed using the human TFEB genomic sequence (National Center for Biotechnology Information GenBank accession no. NC\_000006.12) and shown in Table 1. Bidirectional sequencing of PCR products was performed on a 3500XL genetic analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) by Sangon Biotech Co., Ltd. (Shanghai, China). DNA sequences were then compared with the wild type TFEB gene promoter using DNAMAN program (Version 5.2.2, Lynnon BioSoft, Quebec, Canada), and genetic variants including single-nucleotide polymorphisms (SNPs) were identified. Wild and variant TFEB gene promoters were analyzed using TRANSFAC program to predict the binding sites for transcription factor affected by genetic variants.

Table 1. PCR primers for the human TFEB gene promoter

PCR primers	Sequences	Location	Position	Products
Sequencing				
TFEB-F1	5'-GAACAAGGCTATGAAATAGAAG-3'	41737571	-1312bp	705bp
TFEB-R1	5'-AAAGCCTACTCTGTTGTTGC-3'	41736867	-608bp	
TFEB-F2	5'-TCAGGTCCTACTATTCCGTCTA-3'	41736916	-657bp	801bp
TFEB-R2	5'-TTATGGGTTCTACATTCACGC-3'	41736116	+144bp	
Functioning				
TFEB-F	5'-(KpnI)-CAAGGCTATGAAATAGAAGATTG-3'	41737568	-1309bp	1386bp
TFEB-R	5'-(HindIII)-GTCTGAGCTCCTTTCATGTCA-3'	41736183	+77bp	

PCR primers are designed based on the genomic DNA sequence of the TFEB gene (NC\_000006.12). The transcription start site is at the position of 41736259 (+1).

#### Functional analysis of the TFEB gene promoter by dual-luciferase reporter assay

Wild type and variant TFEB gene promoters (1386 bp, -1309 bp ~ + 77 bp) were generated by PCR, which were then inserted into the KpnI and HindIII sites of a luciferase reporter vector (pGL3-basic, Promega Corporation, Madison, WI, USA) to generate expression constructs. The PCR primers are presented in Table 1. The designated expression constructs were transiently transfected into cultured HEK-293 [CRL-1573; American Type Culture Collection, Manassas, VA, USA (ATCC), Manassas, VA, USA] and H9c2 cells (rat cardiomyocyte line; CRL-1446; ATCC) and dual-luciferase activity was examined using Dual-Luciferase® Reporter Assay on a Glomax 20/20 luminometer (Promega Corporation, Madison, WI, USA). TFEB gene promoter activity was expressed as the ratio of luciferase activity over Renilla luciferase activity. Activity of the wild type TFEB gene promoter was set as 100% and the activity of variant TFEB gene promoter was calculated.

#### Electrophoretic mobility shift assay (EMSA)

To examine the effects of genetic variants of the TFEB gene promoter on the binding sites for transcription factors, EMSA was conducted using the LightShift® Chemiluminescent EMSA kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Biotinylated double-stranded oligonucleotides (30 bp) containing genetic variants were used as probes. Nuclear extracts from HEK-293 and H9c2 cells were prepared using NE-PER® Nuclear and Cytoplasmic Extraction Reagent kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Protein concentrations were determined using the Bradford protein assay. DNA-protein binding reactions were conducted for 20 minutes at room temperature with equal amounts of probes (0.2pMol) and nuclear extracts (3.0µ g). The reaction mixtures were subsequently separated on a 6% polyacrylamide gel and transferred onto a nylon membrane (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The oligonucleotides were cross-linked to the membrane using the UV Stratalinker 1800 (Stratagene; Agilent Technologies, Inc., Santa Clara, CA, USA) and were detected by chemiluminescence using the LightShift® Chemiluminescent EMSA kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

## Statistical analysis

All transfection experiments were repeated three times independently, in triplicate. Transfection data are expressed as the means  $\pm$  standard error of the mean and were analyzed using two-way analysis of variance followed by Dunnett test. Frequency of genetic variants was compared between AMI patients and controls using SPSS v22.0 software (SPSS, Inc., Chicago, IL, USA).  $P < 0.05$  was considered as statistically significant.

## Results

### Clinical and biochemical characteristics of AMI patients and controls

This study included 352 AMI patients and 337 controls. Clinical data was collected, and related biochemical parameters were examined, including triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL). Clinical and biochemical characteristics were summarized in Table 2. Age, body mass index (BMI), TG, TC, HDL and LDL were expressed as mean  $\pm$  standard deviation ( $M \pm SD$ ). Mean age of AMI patients was 61.29 years, and mean age of controls was 51.25 years. A significant difference in age was observed between AMI patients and controls ( $P < 0.01$ ). The prevalence of traditional risk factors including male sex, hypertension, diabetes and smoking was significantly higher in AMI patients compared to controls ( $P < 0.01$ ). The levels of TG, TC, HDL and LDL in AMI patients were significantly lower compared to controls ( $P < 0.01$ ), probably due to application of lowering-lipid medicines in AMI patients. In addition, there was no significant difference of BMI between AMI patients and controls ( $P > 0.05$ ).

Table 2  
Clinical and biochemical characteristics of AMI patients and controls

	Controls(n = 337)	AMI(n = 352)	P value
Age (years, mean ± SD)	51.25 ± 12.28	61.29 ± 12.01	< 0.01
Male (n, %)	167 (49.55%)	267 (75.85%)	< 0.01
Hypertension (n, %)	63 (18.69%)	122 (34.66%)	< 0.01
Diabetes (n, %)	12 (3.56%)	68 (19.32%)	< 0.01
Smoking (n, %)	24 (7.12%)	189 (53.69%)	< 0.01
BMI (Kg/M <sup>2</sup> )	25.12 ± 3.51	25.02 ± 3.73	0.783
TG(mmol/L)	1.74 ± 0.98	1.32 ± 1.00	< 0.01
TC(mmol/L)	4.88 ± 0.92	4.32 ± 1.13	< 0.01
HDL(mmol/L)	1.24 ± 0.25	1.10 ± 0.26	< 0.01
LDL(mmol/L)	2.95 ± 0.85	2.62 ± 0.84	< 0.01
BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol. *, Quantitative data including age, BMI, TG, TC, HDL and LDL was expressed as M ± SD.			

#### Identified genetic variants in the TFEB gene promoter

A total of fifteen genetic variants of the TFEB gene promoter were identified, including eight single nucleotide polymorphisms (SNPs). Frequency and locations of the genetic variants were presented in Fig. 1 and summarized in Table 3. Two novel heterozygous variants (g.41737144A > G and g.41736544C > T) and four SNPs [g.41737274T > C (rs533895008), g.41736987C > T (rs760293138), g.41736806C > T (rs748537297) and g.41736635T > C (rs975050638)] were only identified in six male AMI patients (Fig. 2). All the six cases were male, and the age was from 52 to 81 years old. Clinically, four AMI cases suffered from acute inferior myocardial infarction, and two acute anterior myocardial infarction. Three of the six AMI cases are accompanied with hypertension. Four of the six AMI cases had history of smoking. None of the six cases had diabetes. In addition, biochemical parameters of the six cases were within normal ranges.

Table 3. Genetic variants in the TFEB gene promoter in AMI patients and controls.

Genetic variants	Genotypes	Location <sup>1</sup>	Controls (n=337)	AMI (n=352)	P value
g.41737451T>G	TG	-1192bp	1	0	-
g.41737274T>C (rs533895008)	TC	-1015bp	0	1	-
g.41737144A>G	AG	-885bp	0	1	-
g.41737034G>C (rs73733015)	GG	-775bp	209	196	0.239
	GC		111	136	
	CC		17	20	
g.41737034G>A (rs73733015)	GA	-775bp	1	1	1.000
g.41737034G>C/G>A (rs73733015)	CA	-775bp	1	0	-
g.41737005G>A (rs149166358)	GA	-746bp	1	0	-
g.41736987C>T (rs760293138)	CT	-728bp	0	1	-
g.41736981A>G	AG	-722bp	1	0	-
g.41736806C>T (rs748537297)	CT	-547bp	0	1	-
g.41736740C>A	CA	-481bp	1	1	1.000
g.41736635T>C (rs975050638)	TC	-376bp	0	1	-
g.41736544C>T	CT	-285bp	0	1	-
g.41736407C>T	CT	-148bp	1	0	-
g.41736218T>G	TG	+42bp	1	0	-

<sup>1</sup>, Variants are located upstream (-) to the transcription start site of TFEB gene at 41736259 of NC\_000006.12.

Table 4  
The double-stranded biotinylated oligonucleotides for the EMSA

Genetic variants	Oligonucleotide sequences	Locations
g.41737274T > C (rs533895008)	5'- CTGATCTGCTCCAAA(T/C)TGGGAGCAGGAGGG- 3'	41737289 - 41737260
g.41737144A > G	5'- CTGCTCTGGTGGCTC(A/G)GTTGGCCTATGAGC- 3'	41737159 - 41737130
g.41736987C > T (rs760293138)	5'- CAACGGCTACTGCAC(C/T)GTGGGAGTCGAGCC- 3'	41737002 - 41736973
g.41736806C > T (rs748537297)	5'- TTCCTCTTGACCCTG(C/T)ACCCTCCTAGGGCA- 3'	41736821 - 41736792
g.41736635T > C (rs975050638)	5'- GTGGGTCACTGTGAT(T/C)CTAAGAGAAATGGG- 3'	41736650 - 41736621
g.41736544C > T	5'- CCTGGAAAGGGAAGA(C/T)AGTGGTAGCGCCAT- 3'	41736559 - 41736530

Four novel heterozygous variants (g.41737451T > G, g.41736981A > G, g.41736407C > T and g.41736218T > G) and two SNPs [g.41737005G > A (rs149166358) and g.41737034G > C/G > A (rs73733015)] were only found in healthy controls. One novel variant (g.41736740C > A) and one SNP with two variations (rs73733015: g.41737034G > C and g.41737034G > A) were found in both AMI patients and controls with similar frequencies ( $P > 0.05$ ).

#### Altered activity of TFEB gene promoter by genetic variants

Expression constructs containing wild type and variant TFEB gene promoters: pGL3-WT (wild type), pGL3-41377451G, pGL3-41737274C, pGL3-41737144G, pGL3-41737034A, pGL3-41737034C, pGL3-41737005A, pGL3-41736987T, pGL3-41736981G, pGL3-41736806T, pGL3-41736635C, pGL3-41736544T, pGL3-41736407T and pGL3-41736218G, were transfected into HEK-293 and H9c2 cells. The dual-luciferase activities were measured and relative activity of wild type and variant TFEB gene promoters were examined.

In HEK-293 cells, two variants (g.41737144A > G and g.41736544C > T) and three SNPs [g.41737274T > C (rs533895008), g.41736806C > T (rs748537297) and g.41736635T > C (rs975050638)] identified in AMI patients significantly increased transcriptional activity of the TFEB gene promoter ( $P < 0.01$ ). The SNP [g.41736987C > T (rs760293138)], which was also identified in AMI patient, significantly decreased transcriptional activity of the TFEB gene promoter ( $P < 0.01$ ). These results indicated that the genetic

variants only identified in AMI patients altered transcriptional activity of the TFEB gene. In contrast, other genetic variants only identified in controls or both AMI patients and controls did not alter transcriptional activity of the TFEB gene ( $P > 0.05$ ) (Fig. 3).

As human cardiomyocyte cell lines are currently not available, the H9c2 rat cardiomyocyte cell line was used. Similar results to that in HEK-293 cells were observed in H9c2 cells. Two variants (g.41737144A > G and g.41736544C > T) and three SNPs [g.41737274T > C (rs533895008), g.41736806C > T (rs748537297) and g.41736635T > C (rs975050638)] significantly increased transcriptional activity of the TFEB gene promoter ( $P < 0.01$ ), and the SNP [g.41736987C > T (rs760293138)] significantly decreased transcriptional activity of the TFEB gene promoter ( $P < 0.01$ ) (Fig. 3).

#### Genetic variants-affected binding sites of transcription factors

The TFEB gene promoter was analyzed using TRANSFAC program to predict whether genetic variants identified in AMI patients alter the putative binding sites for transcription factors. As predicted, the SNP [g.41737274T > C (rs533895008)] may abolish the binding site for high mobility group A (HMGA) and nuclear transcription factor Y (NF-Y), and create the binding sites for nuclear receptor direct repeat (NR-DR), transcription factor 7 (TCF-7) and breast cancer type 1 factor (BRCA1). The variant (g.41737144A > G) may modify the binding sites for BRCA1 and sterol regulatory element-binding proteins (SREBP). The SNP [g.41736987C > T (rs760293138)] may modify the binding site for CBF-1/RBP-J, a Notch-regulated factor. The SNP [g.41736806C > T (rs748537297)] may create a binding site for peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ). The SNP [g.41736635T > C (rs975050638)] may disrupt the binding site for growth factor independent 1 (GFI1), create a binding site for glucocorticoid receptor-like receptors and modify the binding site for estrogen receptors. The variant (g.41736544C > T) may modify the binding sites for SREBP and glucocorticoid receptor-like receptors.

#### Transcription factor binding as determined by EMSA

To experimentally investigate whether the genetic variants affected the binding of transcription factors, EMSA was performed with wild type or variant oligonucleotides (30 bp). The genetic variants identified in AMI patients were examined. Biotinylated oligonucleotides for the EMSA were shown in Table 3. As shown in Fig. 4, the SNP [g.41737274T > C (rs533895008)] abolish the binding of a transcription factor and created a binding site for a new transcription factor. Two variants (g.41737144A > G and g.41736544C > T) and one SNP [g.41736987C > T (rs760293138)] markedly enhanced the binding of an unknown transcription factor in HEK-293 and H9c2 cells. The affected transcription factor, which acted as a transcriptional activator, requires further identification. The effects of the other two SNPs [g.41736806C > T (rs748537297) and g.41736635T > C (rs975050638)] on the binding of transcription factors were not detected, likely due to the sensitivity limit of EMSA experiments (data not shown).

## Discussion

Altered TFEB gene expression and subsequent dysfunctional autophagic-lysosomal system have been implicated in human diseases, including lysosomal storage diseases, neurodegenerative diseases and cancers [24–27]. However, mutations or genetic variants in TFEB gene have not been associated with human diseases. In this study, six genetic variants including four SNPs in the TFEB gene promoter were identified in AMI patients, but in none of controls. These genetic variants significantly altered the transcriptional activity of the TFEB gene. Further EMSA indicated that two variants (g.41737144A > G and g.41736544C > T) and two SNPs [g.41737274T > C (rs533895008) and g.41736987C > T (rs760293138)] evidently affected the binding of transcription factors. Collectively, 1.70% (6/352) of AMI patients were found to carry genetic variants of TFEB gene promoter.

The human TFEB gene has been localized to chromosome 6p21.1. TFEB recognizes the motif, CLEAR element, within its target genes [12, 28]. TFEB null mice die at embryonic stage due to defective placental vascularization [29]. Conditional disruption or transgenic mouse models reveal that TFEB has specialized functions in different tissues [11, 21, 30–32]. There are five transcript variant of TFEB, and the variant 2 encodes the longest isoform, promoter region of which was analyzed in this study. To date, the promoter of TFEB gene has not been characterized in details. There are numerous CLEAR sequences in the TFEB gene promoter, indicating that TFEB regulates its own expression in an autoregulatory loop [14]. In human endothelial cell, paternally expressed gene 3 (PEG3) is an upstream transcriptional regulator of TFEB gene [33]. In this study, the genetic variants identified in AMI patients altered transcriptional activity of the TFEB gene by affecting the binding of transcription factors. Taken transfection data together with EMSA results, the SNP [g.41737274T > C (rs533895008)] may create a binding site for a transcription activator. The variants (g.41737144A > G and g.41736544C > T) may enhance the binding of a transcription activator, and the SNP [g.41736987C > T (rs760293138)] may enhance the binding of a transcription repressor. These transcription factors need to be investigated and identified in further studies.

Under normal conditions, TFEB is located in the cytoplasm. The subcellular localization and activity of TFEB are regulated by its phosphorylation state. TFEB phosphorylation is mediated by several kinases, including mammalian target of rapamycin complex 1 (mTORC1), extracellular signal-regulated kinase 2 (ERK2), glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) and AKT (protein kinase B) [15, 34–39]. Protein phosphatase 2A stimulates activation of TFEB by dephosphorylation in response to oxidative stress [40]. Phosphorylated TFEB is retained in the cytoplasm, whereas dephosphorylated TFEB translocates to the nucleus to induce the transcription of its target genes. A great number of TFEB direct genes has been identified, which represent essential components of the CLEAR gene network [11, 41]. TFEB promotes the gene expression of the autophagic-lysosomal pathway, and regulates the lysosomal biogenesis, autophagy, lysosomal proteostasis, lysosomal exocytosis and lysosomal positioning [11, 15, 42–44]. Moreover, TFEB and TFE3 cooperate in regulating the expression of proinflammatory cytokine genes, controlling the adaptive response of whole body energy metabolism, and modulating the cellular response to endoplasmic reticulum stress [31, 45, 46]. Therefore, upregulation or downregulation of TFEB gene expression may contribute to AMI development through dysfunctional autophagic-lysosomal system and other pathways.

Accumulating studies have demonstrated that a window of optimal autophagic-lysosomal activity is critical to the maintenance of cardiovascular homeostasis and function. Excessive or insufficient levels of autophagic flux can each contribute to the pathogenesis of cardiovascular diseases, including AMI [47–49]. TFEB is differentially activated in patients with different diseases. In patients with Danon disease, TFEB and downstream targets are activated. Conversely, TFEB is inhibited and an autophagy is blocked in patients with glycogen storage disease type II [50]. In this study, the genetic variants in AMI patients exhibited upregulation or downregulation of TFEB gene promoter activity. Excessive or insufficient TFEB gene expression may similarly contribute to AMI development through diverse pathways.

## Conclusions

In the present study, the TFEB gene promoter was genetically and functionally analyzed in AMI patients and healthy controls. Two novel variants and four SNPs were only identified in AMI patients. These genetic variants significantly altered the transcriptional activity of the TFEB gene promoter. Furthermore, four of the six genetic variants evidently affected the binding of transcription factors. Therefore, these genetic variants may change TFEB level, contributing to AMI development through diverse pathways as a low-frequency risk factor.

## Abbreviations

AKT: Pprotein kinase B; AMI: acute myocardial infarction; BMI: Body mass index; BRCA1: Breast cancer type 1 factor; CAD: Coronary artery disease; CLEAR: Coordinated Lysosomal Expression and Regulation; ERK2: Extracellular signal–regulated kinase 2; GFI1: Growth factor independent 1; GSK-3 $\beta$ : Glycogen synthase kinase-3 $\beta$ ; H9c2: rat cardiomyocyte cells; HDL: High density lipoprotein cholesterol; HEK-293: Human Embryonic Kidney 293 cells; HMGA: High mobility group A; LDL: Low density lipoprotein cholesterol; mTORC1: Mammalian target of rapamycin complex 1; MITF: Microphthalmia-associated transcription factor; NF-Y: Nuclear transcription factor Y; NR-DR: Nuclear receptor direct repeat; PEG3: paternally expressed gene 3; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ; SNPs: Single nucleotide polymorphisms; SREBP: Sterol regulatory element-binding proteins; TC: Total cholesterol; TCF-7: Transcription factor 7; TFE3: Transcription factor E3; TFEB: Transcription factor EB; TFEC: Transcription factor EC; TG: Triglyceride.

## Declarations

### Ethics approval and consent to participate

The study was approved by the Human Ethics Committee of Jining Medical University, Jining, Shandong, China. Written informed consent was obtained from all participants.

### Consent for publication

Not applicable.

## Availability of data and materials

All data generated or analyzed in this study are included in article.

## Competing interests

Authors declare that they have no competing interests.

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## Authors' contributions

JZ was responsible for conducting experiments, collecting data, , interpretation of data and drafting the manuscript. BY was responsible for conception and design, project administration, supervision, reviewing and editing the manuscript. JZ, YZ, XH and SW were responsible conducting experiments, formal analysis, and interpretation of data. SP was responsible for project administration, formal analysis and data curation. All authors approved the final manuscript version.

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Not applicable.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

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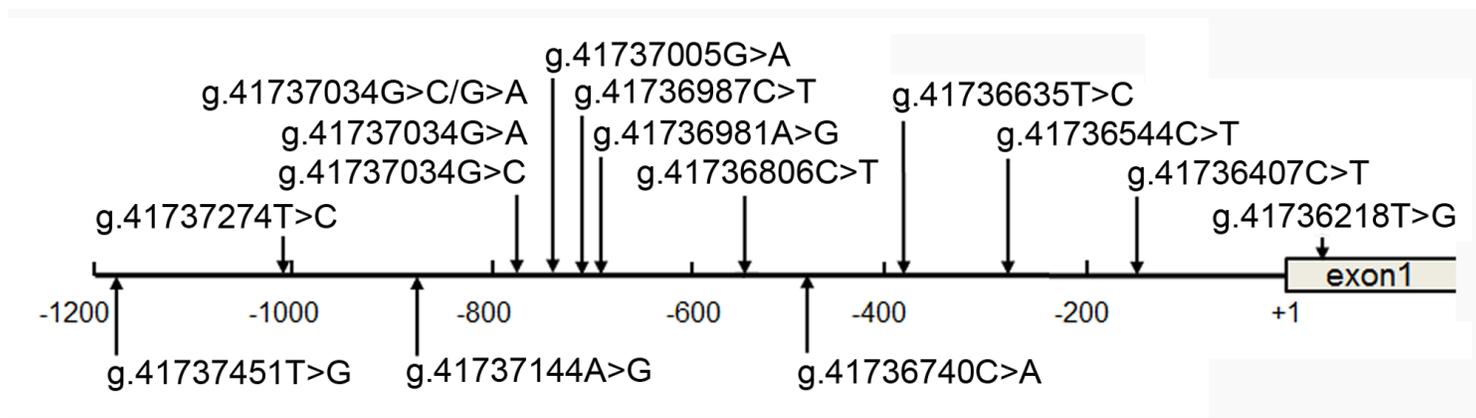
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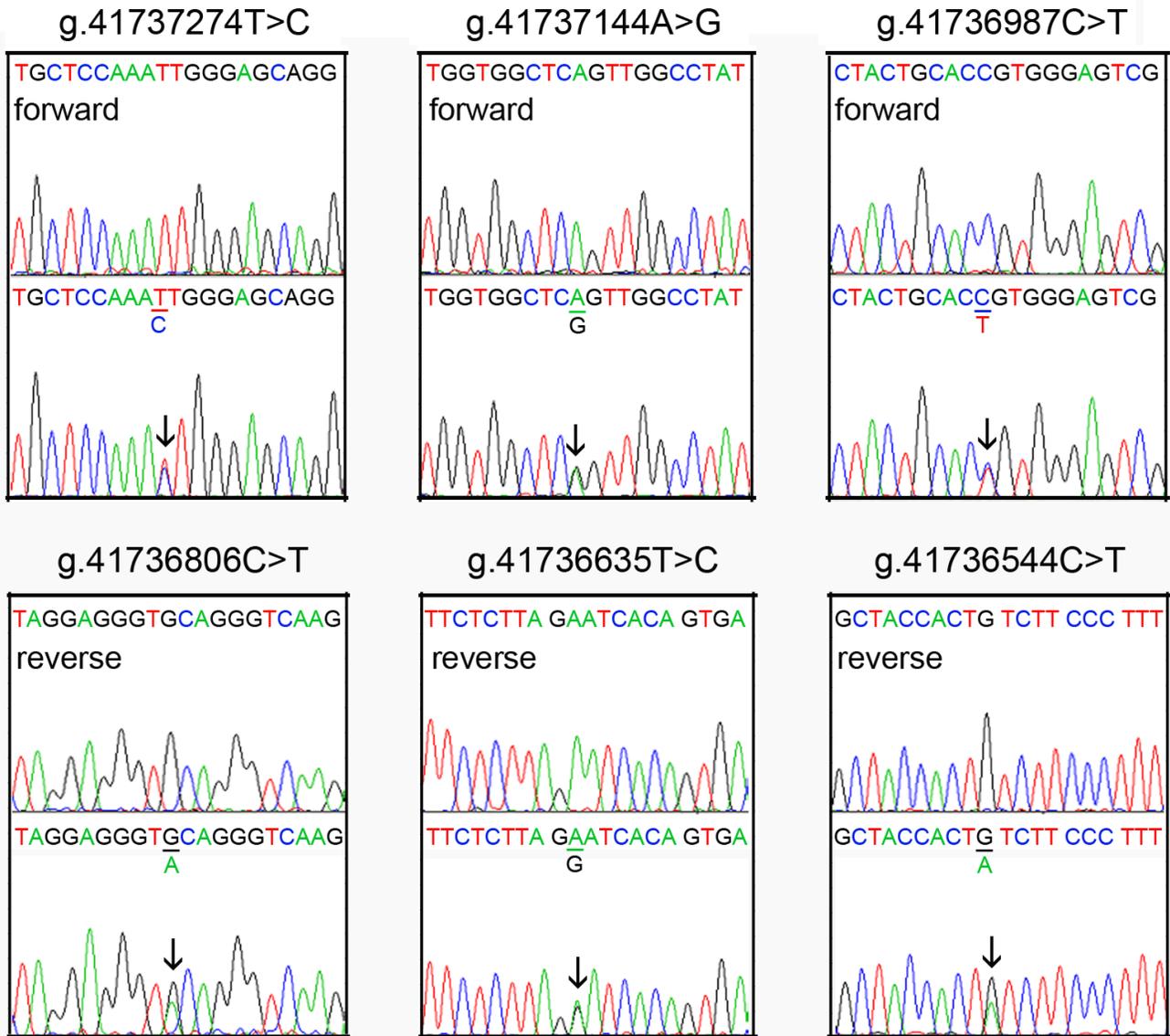
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## Figures



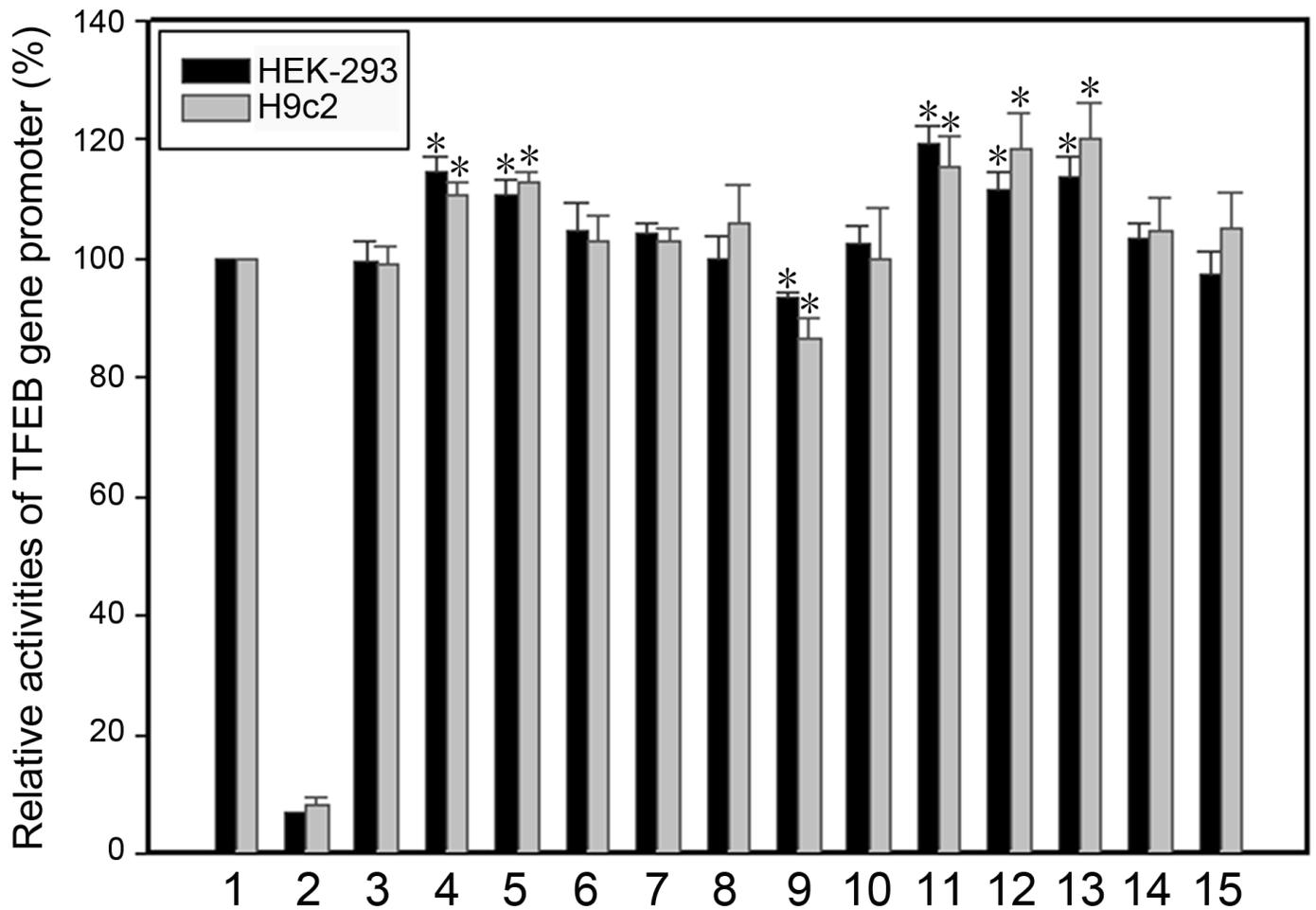
**Figure 1**

Locations of the genetic variants in the TFEB gene promoter. The numbers represents the genomic DNA sequences of the human TFEB gene (Genebank accession number NC\_000006.12). The transcription start site is at the position of 41736259 (+1) in the first exon.



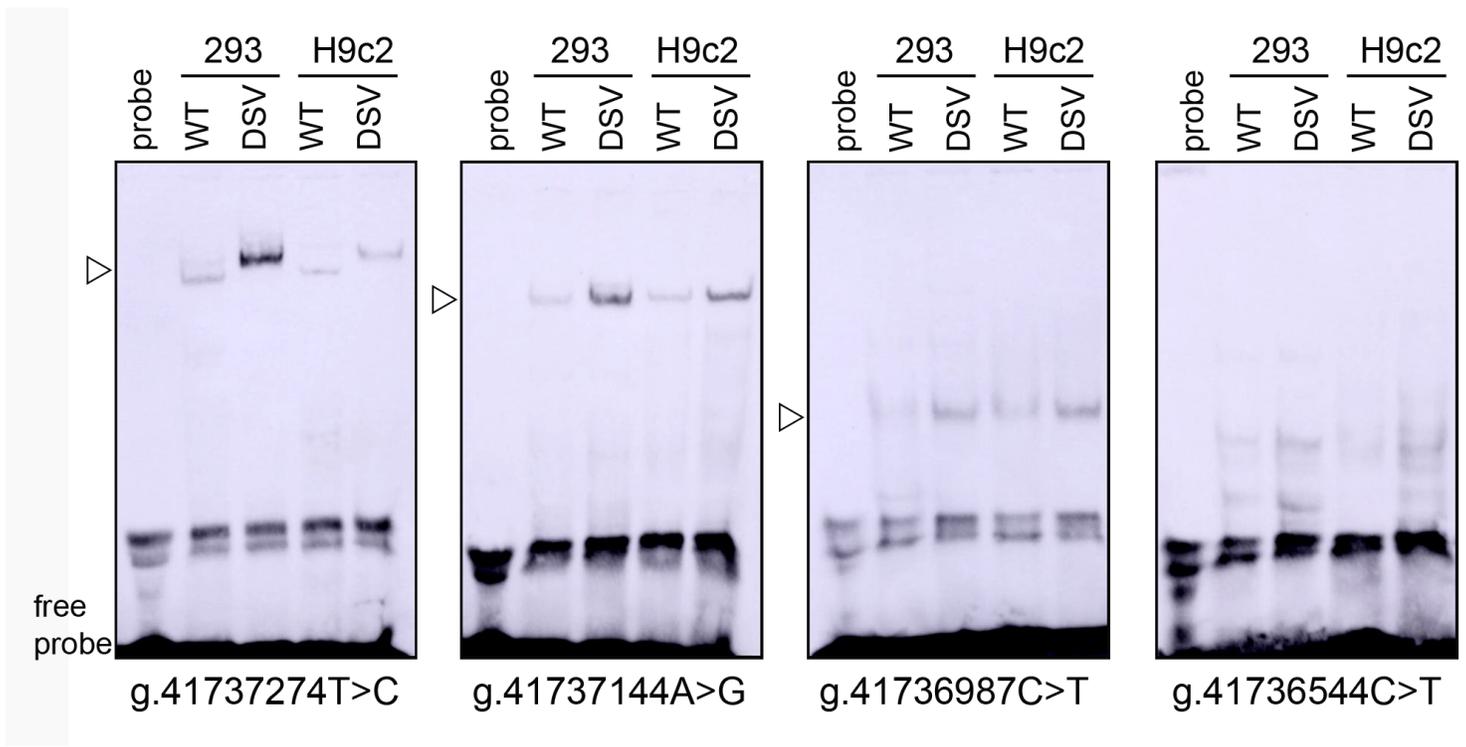
**Figure 2**

Sequencing chromatograms of the genetic variants of the TFEB gene promoter identified in AMI patients. Sequence orientations of the genetic variants are marked. Top panels show wild type and bottom heterozygous DNA sequences. Arrows indicate the heterozygous variant.



**Figure 3**

Relative transcriptional activity of wild type and variant TFEB gene promoters in HEK-293 cells and H9c2 cells. Black bars indicate HEK-293 cells and grey bars H9c2 cells. Empty vector pGL3-basic was used as a negative control. Transcriptional activity of the wild type TFEB gene promoter was designed as 100%. Relative activity of variant TFEB gene promoters was calculated. Lanes 1, pGL3-WT; 2, pGL3-basic; 3, pGL3-41377451G; 4, pGL3-41737274C; 5, pGL3-41737144G; 6, pGL3-41737034A; 7, pGL3-41737034C; 8, pGL3-41737005A; 9, pGL3-41736987T; 10, pGL3-41736981G; 11, pGL3-41736806T; 12, pGL3-41736635C; 13, pGL3-41736544T; 14, pGL3-41736407T; 15, pGL3-41736218G. WT, wild type. \*,  $P < 0.01$ .



**Figure 4**

EMSA of biotin-labeled oligonucleotide containing genetic variants with nuclear extracts of HEK-293 (293) and H9c2 cells. Free probe was marked at the bottom. The affected binding for an unknown transcription factor was marked with an open arrow.