ORIGINAL ARTICLE

Analysis of *KLF7* and *KLF5* transcription factors gene variants in coronary artery disease

Vahid Akbari Kordkheyli[a], Arash Poursheikhani[b], Seyed Hasan Sharobandi[c], Sayed Mostafa Hosseini[d],*

[a] *Clinical Biochemistry, Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[b] *Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran*
[c] *Atherosclerosis Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*
[d] *Molecular Genetics, Human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

Abstract

Introduction and objective: Genetic susceptibility has a key role in the pathogenesis of coronary artery disease (CAD). *KLF5* and *KLF7* are transcriptional factors essential to cell development and differentiation. Their genetic variants have been associated with the risk of metabolic disorders. The present study aimed to evaluate the possible correlation of *KLF5* (rs3812852) and *KLF7* (rs2302870) single nucleotide polymorphisms (SNPs) with the risk of CAD for the first time in the world.

Methods: The clinical trial study comprised 150 patients with CAD and 150 control subjects without CAD from the Iranian population. After blood sampling, deoxyribonucleic acid was extracted and genotyped using the Tetra Primer ARMS-PCR method and confirmed by Sanger sequencing.

Results: The *KLF7* A/C genotypes and C allele frequency were meaningfully higher in the control group compared to the CAD+ group (p<0.05). No obvious association has been observed between *KLF5* variants and CAD risk. However, the distribution of the AG genotype of *KLF5* was statistically lower in CAD+ patients with diabetes than in CAD+ patients without diabetes (p<0.05).

Conclusion: This study identified *KLF7* SNP as a causative gene contributing to CAD, which presents novel insight into the molecular pathogenesis of the disease. It is, however, unlikely that *KLF5* SNP has an essential role in the risk of CAD in the studied population.

© 2023 Sociedade Portuguesa de Cardiologia. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* Corresponding author.
  E-mail address: smhosseini@bmsu.ac.ir (S.M. Hosseini).

PALAVRAS-CHAVE
Doença arterial
coronária;
KLF;
Fatores de
transcrição;
Polimorfismo;
Suscetibilidade
genética

Análise das variantes dos genes dos fatores de transcrição *KLF7* e *KLF*5 na doença arterial coronária

Resumo

Introdução e objetivo: A suscetibilidade genética tem um papel fundamental na patogénese da doença arterial coronária (DC). O *KLF5* e o *KLF7* são fatores de transcrição que desempenham papéis importantes no desenvolvimento e diferenciação celular e as suas variantes genéticas têm sido associadas ao risco de distúrbios metabólicos. O presente estudo teve como objetivo avaliar a possível correlação dos polimorfismos do nucleotídeo único (SNPs) *KLF5* (rs3812852) e *KLF7* (rs2302870) com o risco de DC pela primeira vez no mundo.

Métodos: O estudo de ensaio clínico compreende 150 pacientes com DC (CAD+) e 150 casos controlos sem DC na população iraniana. Após colheita de sangue, o DNA foi extraído e genotipado pelo método *Tetra Primer ARMS-PCR*, confirmado pela sequência de Sanger.

Resultados: Os genótipos *KLF7* A/C e a frequência do alelo C foram significativamente maiores no grupo controlo em comparação com o grupo CAD+ (p<0,05). Nenhuma associação óbvia foi observada entre as variantes do *KLF5* e o risco de CAD+. No entanto, a distribuição do genótipo AG de *KLF5* foi estatisticamente menor em doentes CAD+ com diabetes do que em doentes CAD+ sem diabetes (p<0,05).

Conclusão: Este estudo identificou o SNP *KLF7* como um gene causador da DC, que apresenta uma nova visão sobre a patogénese molecular da doença. Mas é improvável que o SNP *KLF5* tenha um papel essencial no risco de DC na população estudada.

© 2023 Sociedade Portuguesa de Cardiologia. Publicado por Elsevier España, S.L.U. Este é um artigo Open Access sob uma licença CC BY-NC-ND (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Coronary artery disease (CAD) is the most common cardiovascular abnormality and the main cause of human death around the globe. CAD is an atherosclerotic injury with an inflammatory basis, induced when an obstruction (more than 50%) is created in at least one of the coronary arteries.[1] Generally, CAD is common among the elderly. However, recent studies have demonstrated that 4–10% of patients who suffer from CAD are <45 years old. The World Health Organization report, presented in 2009, revealed that 17.3 million deaths are caused by cardiovascular diseases (CVD). In 2016, the American Heart Association reported that 15.5 million people >20 years old suffer from CAD complications, and approximately 500 000 deaths accrue annually due to CAD.[2] CAD-related mortality in Iran is approximately 175 000 annually, representing 50% of all deaths.[3]

Besides environmental factors and lifestyle, family history and several genetic polymorphisms are strongly associated with CAD. Therefore, the mutation in the genes linked to these diseases could be useful in distinguishing the disease vulnerability of a general population. Genome-wide association analysis of CAD determined that 396 single-nucleotide polymorphisms (SNPs) correlated with CAD. Of these, 30 SNPs were located in nine chromosomes.[4]

KLFs are zinc-finger-containing transcriptional controllers that mediate important cellular processes such as development, growth, proliferation, differentiation, and vascular remodeling and are also implicated in the pathogenesis of different diseases, including cardiovascular diseases (CVD), diabetes, inflammation, and cancer. 18 members of the *KLF* family have been distinguished in mammalian cells.[5] *KLF5*, also called *BTEB2*, *CKLF*, or *IKLF* in humans, are positioned on chromosome 13q22.1, and encodes a transcription factor protein with 457 amino acids. *KLF5* is highly expressed in human skin, esophagus, colon, small intestine, heart, and brain.[6] A recent study showed homozygous removal of *KLF5* in mice resulted in early embryo death.

In contrast, heterozygous *KLF5*-knockout led to diminished levels of angiogenesis, decreased neointimal formation, adventitia thickening, cardiac hypertrophy, and interstitial fibrosis in response to angiotensin II injection.[7] In this regard, *KLF5*-knockout in mice cardiomyocytes caused decreased expression of cardiac *PPAR1* and its downstream fatty acid metabolism-associated genes, which led to reduced cardiac fatty acid oxidation, increased triglyceride accumulation, abrogating ATP production, and cardiac invalidity. Moreover, *KLF5* overexpression notably increased cellular activity and diminished cellular apoptosis in response to myocardial damage.[8,9] Recent clinical trial studies revealed that *KLF5* is expressed in coronary atherosclerotic plaques, and its SNPs are associated with human hypertension, familial dilated cardiomyopathy (FDM), and basal and resting metabolic rate (BMR) in Japanese, Chinese and Korean populations, respectively.[7,10,11] These findings emphasize the critical function of *KLF5* in adaptive cardiovascular remodeling and make it causative to screen *KLF5* as a candidate gene for CAD vulnerability.

KLF7 (original name: ubiquitous klf) is located on chromosome 2q32 and encodes a protein with 269 amino acids and is generally expressed at a low scale in adult tissue. However, high-level expression is limited to neuronal tissue. It was reported that *KLF7* null mice died within two days of birth,

Table 1 Primers features.

Gene	Primers sequencing (5′–3′)
KLF5 outer F	TGAAAATGAGATCCATACCTTTGATAGA
KLF5 outer R	TATGTTGTTATAATGGGAGGGTGAATTT
KLF5 inner F (A allele)	TGATGAATAGGCTGTGGTATATGTCAA
KLF5 inner R (G allele)	TGTTGACACACAATATACCATTAGACATTC
Product size for A allele: 219	
Product size for G allele: 146	
Product size of two outer primers: 308	
KLF7 outer F	CACTCACATCCCTCCCATGAGCACTTAT
KLF7 outer R	TCAGAGGAAATGCTCTGACTCTGGGAAC
KLF7 inner F (A allele)	CTGGTCAGGCTTGGGAAAGGGCAGT
KLF7 inner R (C allele)	GACATCTAGTCTTTCAGGAATGAGTTGAC
Product size for A allele: 211	
Product size for C allele: 169	
Product size of two outer primers: 325	

as a result extensive neurological disorders.[12] Furthermore, it was demonstrated that *KLF7* overexpression suppressed adipogenesis and adiponectin gene expression in adipocytes. Also, *KLF7* inhibits glucose-induced insulin secretion in pancreatic β-cells and reduces hexokinase 2 expression in skeletal muscle of diabetes patients.[13] *KLF7* genetic polymorphism's role in obesity and diabetes has been proved in Danish and Japanese subjects, respectively.[14,15] Therefore, it can be hypothesized that *KLF7* may confer susceptibility to CAD via regulating adipocyte function and glucose metabolism.

The mediation of genetic factors in the pathogenesis of CAD is widely acknowledged; however, to date, just a few genes have been determined to increase susceptibility to CAD. Therefore, large-scale SNP genes are needed. Based on the role of *KLF5* and *KLF7* in adipose and glucose metabolism and the cardiovascular system and a robust correlation between *KLF5* and *KLF7* variants with metabolic disorders such as diabetes, CADs, body fat, and hypertension, these genes are considered to be candidate genes for conferring susceptibility to CAD.

Therefore, in this clinical trial we aimed to screen for the association between *KLF7* (rs2302870) and *KLF5* (rs3812852) SNPs with the risk of CAD for the first time in the globe and in the Iranian population.

Methods

Study patients

The case-control study comprised 150 patients with CAD (CAD+) and 150 control subjects without CAD (control) through convenient sampling from people undergoing coronary angiography and general checkup in the Tehran Heart Center Hospital, Tehran province, Iran, in 2019. The control group participants were enrolled at their annual health examination. They did not have any history of CAD, chest symptoms or electrocardiogram abnormalities suggesting CAD. The study did not include participants with renal failure, autoimmune diseases, pulmonary obstruction, liver diseases, malignancy, congenital heart disease, and concomitant cardiomyopathy.

The study protocol was approved by the university's local ethics committee and was planned in accordance with the concepts of the Helsinki Declaration (ethics code: 1400.1038). All the patients signed the informed consent forms before enrollment. The subjects underwent a physical examination and completed a question sheet of individual demographic and clinical information, including body weight, medical history, medication, smoking, body mass index (BMI), history of alcohol and substance abuse, hypertension, and other diseases exclusion criteria. Venous whole blood samples collected in EFTA tubes from the participants after overnight fasting and serum were separated by low-speed centrifugation. Blood cells were supplied at −20 °C for DNA extraction, and serum was used for the serology study. CAD was detected by observing intense coronary stenosis (≥50%) in at least one of the three main coronary arteries or their main branches via coronary angiography.

Biochemical analyses

Serum total cholesterol (TC), triglycerides (TGs), HDL, LDL, and fasting blood sugar (FBS) levels were evaluated using commercially available kits (Pars Azmoon Inc., Tehran, Iran) with an auto-analyzer (Prestige, Japan).

Genotyping

Genomic DNA was isolated with a salting-out approach and was checked for quality and quantity by nanodrop spectrophotometry and gel electrophoresis. The *KLF5* variant (rs3812852) is located in −1282 bp region within 2000 bp 5′ of the transcription initiation location. *KLF7* SNP (rs2302870) positioned at putative promoter at intron 2 +35092. Due to the lack of restriction enzymes for these genes, the Tetra Primer ARMS PCR technique was used to detect the single nucleotide variants of the study genes. For this purpose, a pair of outer primers and two allele-specific inner primers were designed for each gene
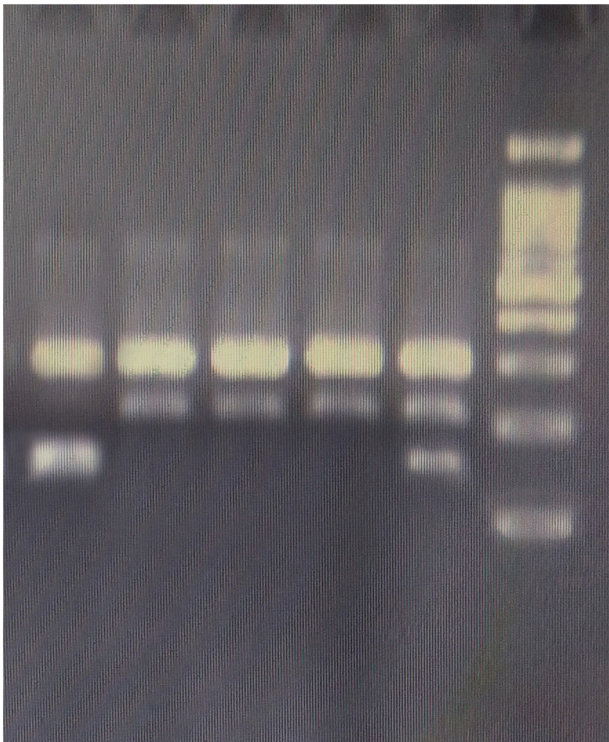
Figure 1 *KLF5* rs3812852 genotyping by Tetra Primer ARMS-PCR. The ladder is 100 bp. Pair band of 308 and 219 bp belongs to wild (AA) genotype, pair band of 308 and 146 bp homozygote mutant (GG), three band of 308, 219, and 146 bp heterozygote mutant (AG).

Figure 2 *KLF7* rs2302870 genotyping by Tetra Primer ARMS-PCR. The ladder is 100 bp. Pair band of 325 and 211 bp belongs to the wild (AA) genotype, pair band of 325 and 169 bp homozygote mutant (GG), three band of 325, 211 and 169 bp heterozygote mutant (AG).

(Table 1). To find the positive control for mutant genotypes, we randomly sequenced 15 samples with Sanger sequencing (ABI) according to the frequency of the mutation before studies. After identifying the mutant samples, we tested different concentrations of primers and DNAs until we reached the desired setup of tetra-ARMS PCR. Genomic DNA was amplified in 15 μl of reaction tube containing 100–200 ng genomic DNA. In this study, for a more accurate junction of primers, the samples were amplified with external primer pairs, then the PCR products were diluted at a ratio of 1–100 and used as a DNA sample for ARMS tetra primer. 0.5 μl of outer F and R primers (10 PM) (Gene fanavaran, Iran), 7 μl master mix containing 200 μmol/L of each dNTP, 1.4 mmol/L MgCl$_2$, and 1 unit of Taq polymerase (Amplicon, Denmark), 1 μl of extracted DNA and 6 μl distilled water were used for first step PCR. For the second step, 0.3 μl of outer F and R primers, 0.4 μl inner F and 0.8 μl inner R primers (10 PM), 7 μl master mix, 1 μl of dilated DNA, and 5.2 μl distilled water were used. PCR cycling plan was 95 °C for 5 min (initialization melting) followed by 35 cycles of 95 °C for 30 s (denaturing), 57 °C for *KLF5* and 60 for *KLF7* for 30 s (annealing), 72 °C for 30 s (extension), with a final extension phase of 5 min at 72 °C. The resultant amplicons were evaluated by 2.5% cyber-green gel electrophoresis in 90 V for 40 min. Approximately 30% of cases were randomly evaluated in duplicates for the genotyping quality control, and the concordance rate was 100% (Figures 1–4).

Statistical analysis

All statistical analyses were performed using SPSS (version 19.0) and R (version 3.0.1) software. The Kolmogorov-Smirnov test analyzed the normal distribution of the data. The t-test or analysis of variance was used to analyze the differences between the parametric variables, and the nonparametric variables were analyzed by Mann-Whitney. The Student's t-test or χ^2 test compared demographic and clinical data between cases and controls. The chi-square test was utilized to measure the deviation of allelic and genotype frequencies from the Hardy-Weinberg equilibrium expectations. Logistic regression analysis was carried out to estimate the possible effect of the study genotypes on CAD by odds ratios (ORs) and 95% confidence intervals (CI). p values <0.05 were considered to be significant. Data and materials analyzed during the current study are available from the corresponding author upon reasonable request.

Results

Clinical characteristics of the study subject

There was no statistical difference in the BMI, HDL, TC, and family history of metabolic diseases between the two studied groups. However, older age, being a man, smoking, diabetes, high blood pressure, FBS, LDL, and TG levels were remarkably associated with increased risk of CAD (Table 2).
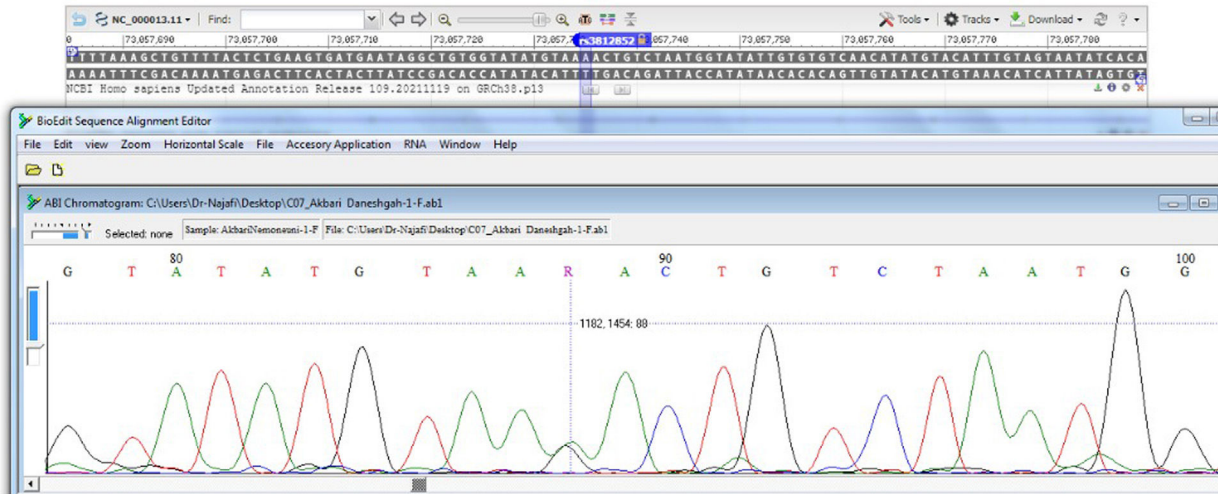
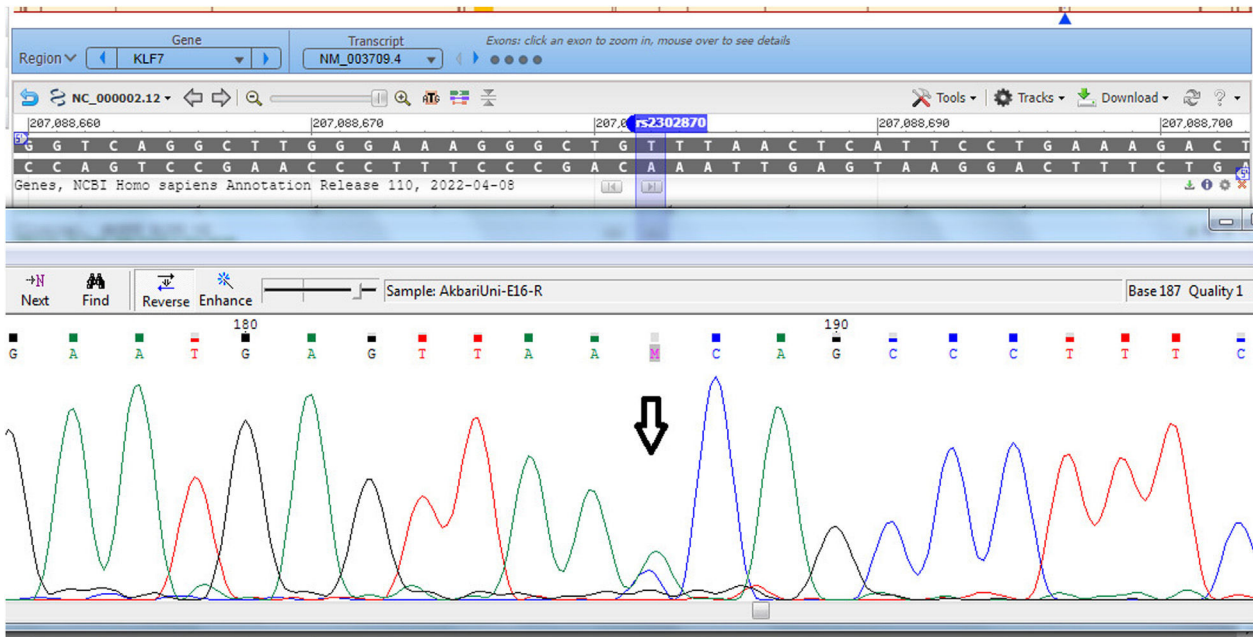**Figure 3** Sanger sequence curve of *KLF5* rs3812852 AG genotype.

Figure 4 Sanger sequence curve of *KLF7* rs2302870 AC genotype.

KLF5 and *KLF7* genotypes distribution

Univariate logistic regression analysis of *KLF7* variants revealed that the distribution of C allele and AC genotypes were statistically higher in the control group compared to CAD+. However, we did not observe any significant association between the *KLF5* variants and the risk of CAD, a clinical characteristic of study subjects, according to different genotypes of *KLF5* and *KLF7* (Tables 3 and 4). Moreover, we analyzed whether the *KLF5* and *KLF7* SNPs impact diabetes and hypertension risk among CAD+ patients or not, and we found that the distribution of AG genotypes of *KLF5* is meaningfully lower in CAD+ with diabetes (D+) patients compared to CAD+ and D− patients (Table 5). However, no significant correlation was found in *KLF5* and *KLF7* SNPs with the risk of hypertension among CAD+ patients (Table 6).

Discussion

KLF5 was detected in vascular smooth muscle cells of the rabbit aorta for the first time. *KLF5* has been determined to be downregulated during development but stimulated in the neointima after damage in the adult aorta to induce cell proliferation and angiogenesis, perhaps via elevating *PDGFA* expression. Previous investigations have reported that *KLF5* can regulate the expression of several target genes such as *TGFβ*, *PPAR1*, *PDGF-A*, and *IGF1* in the heart, which have an essential function in cardiac structural remodeling functional adaptation processes.[8,16−18] A recent study indicated that *KLF5* knockout rats were eliminated in utero before E8.5 and *KLF5* +/− heterozygote rats triggered a decrease in neointimal formation, angiogenesis, adventitia thickening, and fibrosis.[19]

Table 2 Clinical characteristics of the study groups.

	CAD+	Control	p value
Age	59.4±9.6	53.3±9.8	<0.05
Gender	Male: 106 (70.6%)	Male: 60 (40%)	<0.01
	Female: 44 (29.3%)	Female: 90 (60%)	
Smoking	31 (20.6%)	18 (12%)	<0.01
BMI (kg/m^2)	27.5±3.7	28.13±4.2	>0.05
Familial history	31 (20.6%)	24 (16%)	>0.05
Hypertension	95 (63.3%)	69 (46%)	<0.01
Diabetes	65 (43.3%)	17 (11.3%)	<0.001
TG	179.4±111.7	155±86.4	<0.01
TC	188.2±50.3	179.3±37.4	>0.05
HDL	42.6±10.7	45.3±10.5	>0.05
LDL	126.2±45.12	101.2±31.6	<0.05
FBS	126.2±52.3	101.2±30.3	<0.01

Data are shown as mean ± standard deviation. BMI: body mass index; FBS: fasting blood sugar; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TC: total cholesterol; TG: triglycerides.

Table 3 Genotypes and allelic frequencies in controls and CAD+ patients.

	CAD+	Control	OR	95% CI	p value
KLF5	AA 129 (86%)	125 (83.3%)	0.54[a]	0.218–1.1	>0.05
	AG 20 (13.3%)	23 (15.3%)	1.18[b]	0.7–2.63	>0.05
	GG 1 (0.7%)	2 (1.5%)	1.41[c]	0.23–9.2	>0.05
	A allele: 92.5%	91%			>0.05
	G allele: 7.5%	9%	1.38[d]	0.6–1.9	>0.05
KLF7	AA 131 (87%)	122 (81%)	0.77	0.414–1.3	>0.05
	AC 18 (12%)	27 (18%)	1.76	0.9–3.1	<0.05
	CC 1 (0.7%)	1 (0.7%)	1.81	0.27–9.8	>0.05
	A allele: 93.5%	90%			>0.05
	C allele: 6.5%	10%	1.89	0.8–2.7	<0.05

[a] NN vs. NM+MM, M: mutant allele; N: normal allele.
[b] NM vs. NN+MM.
[c] MM vs. NN+NM.
[d] M vs. N.

Table 4 Clinical characteristics of study subjects according to different genotypes of *KLF5* and *KLF7*.

	KLF5 (AA)	*KLF5* (AG and GG)	p value	*KLF7* (AA)	*KLF7* (AC and CC)	p value
Age	55.3±8.1	57.5±7.2	>0.05	57.8±9.2	55.4±6.4	>0.05
BMI (kg/m^2)	26.1±3.7	26.9±3.2	>0.05	28.1±3.9	27.4±3.4	>0.05
Hypertension	135 (53.1%)	25 (55.9%)	>0.05	133 (53.3%)	27 (56.1%)	>0.05
Diabetes	69 (27.1%)	13 (27.7%)	>0.05	67 (26.5%)	14 (28.1%)	>0.05
TG	163.2±73.2	171±59.9	>0.05	170.5±80.8	164±63.2	>0.05
TC	180.2±37.2	186.5±35.1	>0.05	179.4±24.8	185.1±23.8	>0.05
HDL	45.1±8.3	43.3±9.1	>0.05	44.3±10.5	43.7±9.3	>0.05
LDL	110.5±45.2	116.4±40.1	>0.05	109.9±30.3	115.1±32.4	>0.05
FBS	114.5±43.7	110.2±37.3	>0.05	115.6±34.34	109.4±30.9	>0.05

Data are shown as mean ± standard deviation. BMI: body mass index; FBS: fasting blood sugar; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TC: total cholesterol; TG: triglycerides.

Oishi et al. reported that the *KLF5* rs3812852 A/A and A/G genotypes are associated with an increased risk of hypertension in the Japanese population. They determined that people with the G allele have a weaker response to angiotensinogen II (AII) compared to the A allele. They proved that *KLF5* expression and activation are increased in response to AII, and its overexpression is associated with an increment of cardiovascular lesions. Therefore, *KLF5* A/A and A/G, change the responsiveness of *KLF5* to AII, which may lead to hypertension. Also, they revealed a

Table 5 *KLF5* and *KLF7* genotype distribution in CAD+ patients with (D+) and without (D−) diabetes.

		CAD+ and D+	CAD+ and D−	OR	95% CI	p value
KLF5	AA	58 (89.4%)	73 (85.5%)	0.59	0.216−1.1	>0.05
	AG	6 (10.1%)	12 (14.5%)	1.39	0.6−2.53	<0.05
	GG	1 (0.5%)	0 (0%)	1.49	0.22−9.1	>0.05
	A allele: 94.4%	92.8%				>0.05
	G allele: 5.6%	7.2%		1.19	0.5−1.8	>0.05
KLF7	AA	57 (87.6%)	74 (87%)	0.87	0.419−1.4	>0.05
	AC	8 (12.3%)	10 (11.7%)	1.86	0.1−3.1	>0.05
	CC	0 (0%)	1 (1.1%)	1.91	0.29−9.9	>0.05
	A allele: 93.7%	93%				>0.05
	C allele: 6.3%	7%		1.99	0.9−2.8	>0.05

Table 6 *KLF5* and *KLF7* genotypes distribution in CAD+ patients with (H+) and without (H−) hypertension.

		CAD+ and H+	CAD+ and H−	OR	95% CI	p value
KLF5	AA	82 (86.3%)	46 (83.6%)	0.65	0.22−1.5	>0.05
	AG	12 (12.6%)	9 (16.4%)	1.19	0.8−2.77	>0.05
	GG	1 (1%)	0 (0%)	1.36	0.38−9.1	>0.05
	A allele: 92.6%	91.8%				>0.05
	G allele: 7.3%	8.2%		1.49	0.7−2	>0.05
KLF7	AA	84 (88.4%)	47 (87%)	0.77	0.314−1.2	>0.05
	AC	11 (11.5%)	7 (13%)	1.26	0.7−3.19	>0.05
	CC	0 (0%)	1 (1.8%)	1.21	0.27−9.8	>0.05
	A allele: 94.5%	91.7%				>0.05
	C allele: 5.6%	8.3%		1.49	0.7−2.3	>0.05

novel signaling and transcription network, including *MEF2A* and *KLF5* as an important regulator of the pathogenesis of cardiovascular diseases such as hypertension. *KLF5* and *MEF2* are coexpressed in coronary sclerotic lesions. It was identified that ROS are essential for AII-associated activation of *KLF5* and *KLF5*area novel functional molecules in the ROS-p38 MAPK signaling pathway.[7]

Ruo-Min and coworkers reported *KLF5* as a novel disease gene responsible for familial dilated cardiomyopathy (FDC) in Chinese people. They identified heterozygous mutant *KLF5* c.1100T>A; p.(Leu367) increased the risk of FDC; moreover has no transcriptional activity. Furthermore, the variants eliminate the synergistic transactivation between *KLF5* and *NFK-β1*another important transcription factor implicated in FDC.[10] Jung Ran and a coworker determined mutant homozygote of *KLF5* (rs3782933) is a pivotal factor in increasing basal metabolic rate (BMR) in the Korean population. They revealed that people with the TT genotype had markedly higher BMR than the CC genotype. Also, heavier muscle mass, insulin and C-peptide were higher in TT subjects than in CC.[11] Yanagi et al. illustrated that *KLF5* is expressed in neurons in the human brain's prefrontal cortex and positioned in granular and pyramidal cells in the hippocampus as the main site of glutamatergic neurotransmission. Furthermore, they determined mutation in *KLF5* rs3812852 is accompanied by a higher risk of schizophrenia in the Japanese population.[20]

Based on this research, *KLF5* polymorphism contributed to FDC and BMR, and the rs3812852 polymorphism is associated with hypertension (an important risk factor of CAD) and schizophrenia. Therefore, we considered that investigating the possible relationship between rs3812852 polymorphism and CAD for the first time in the world, might be useful to clarify the molecular pathogenesis and genetic susceptibility of CAD. Our results showed that the frequency of the rs3812852 A allele is greater (92.5% vs. 84% and 90%), and the G allele is less (7.5% vs. 16% and 10%) compared to the Japanese population. We failed to find any meaningful difference in *KLF5* SNP distribution among CAD+ and the control population. But more analysis revealed that CAD+ patients with AG genotypes have less risk of developing to diabetes in comparison with AA genotypes.

KLF7 has been indicated to block the expression of multiple genes implicated in adipogenesis, including adiponectin, *leptin*, *PPARg*, *CCAAT*, and *CEBPa*. Previous studies have shown that the expression of *Klf7* is suppressed in response to the differentiation of 3T3-L1 adipocytes. Moreover, it has been demonstrated that green tea polyphenol (K)-catechin is able to increase adiponectin expression and glucose uptake into 3T3-L1 adipocytes via downregulation of *KLF7*. Furthermore, it was determined that *KLF7* inhibits glucose-induced insulin secretion in pancreatic β-cells and reduces hexokinase 2 expression in skeletal muscle of T2D patients.[13,21] These findings induce the hypothesis that *KLF7*

can impact cardiovascular function through regulating the expression of the genes involved in glucose metabolism and adipocyte function. Zobl et al. reported no statistical difference in the frequency of the *KLF7* rs2302870 and rs7568369 SNPs among T2D and control subjects in Danish people, however the minor A allele of *KLF7* rs7568369 protected against obesity, and the frequency of the minor C allele of rs2302870 was remarkably higher in overweight/abdominally obese subjects and abdominally obese individuals compared with lean individuals.[15] Kanazawa and a coworker reported that the frequency of heterozygote mutants in *KLF7* rs2302870 is statistically lower in T2D patients than in control participants in the Japanese population.[14] Based on the research, *KLF7* rs2302870 polymorphism contributed to obesity and diabetes, which is an important risk factors for CAD. In this regard, we hypothesize that investigating the possible relationship between rs2302870 polymorphism and CAD for the first time in the world, might be useful to clarify the molecular pathogenesis and genetic susceptibility of CAD. Our research has shown that the frequency of the minor C allele of *KLF5* rs2302870 is lower than in Danish and Japanese people (8.5% vs. 10.5%) and meaningfully lower in CAD+ patients than in control participants.

We had some limitations when conducting this study, including sampling during the COVID-19 pandemic, the absence of restriction enzymes for the SNPs to perform the RFLP method and low frequency of some genotypes.

Conclusion

In conclusion, the current study identifies *KLF7* gene as a novel candidate for conferring genetic susceptibility to CAD, which provides innovative insights into the molecular pathogenesis of the disease. The exact mechanism is unknown, but it could be speculated that a single nucleotide mutation in the gene caused an alteration its transcriptional activities leading to an increased risk of CAD. We have determined that the *KLF5* SNP is unlikely to be implicated in CAD risk. However, in the study population, a mutation in the SNP may have a protective role against the development of diabetes among CAD+ patients. This was the first study to make an association between *KLF5* and *KLF7* SNP and CAD risk. However, more functional studies are required to clarify the exact correlation of other polymorphisms in *KLF5* and *KLF7* with the risk of CAD and other metabolic diseases in different recesses.

Learning points

The current study identifies *KLF7* gene as a novel candidate for conferring genetic susceptibility to CAD, which provides novel insight into the molecular pathogenesis of the disease. A single nucleotide mutation in the gene caused altering its transcriptional activities may lead to an increased risk of CAD. We have determined that the *KLF5* SNP is unlikely to be implicated in CAD risk. However, in the study population, a mutation in the SNP may have a protective role against the development of diabetes among CAD+ patients. This may occur as angiotensinogen and NF-KB signal transduction is affected. This was the first study to make an association between *KLF5* and *KLF7* SNP and CAD risk.

Conflict of interests

Acknowledgments

References

1. Shahjehan RD, Bhutta BS. Coronary artery disease. StatPearls [Internet]. StatPearls Publishing; 2021.
2. Ralapanawa U, Sivakanesan R. Epidemiology and the magnitude of coronary artery disease and acute coronary syndrome: a narrative review. J Epidemiol Glob Health. 2021;11:169.
3. Ebrahimi M, Kazemi-Bajestani S, Ghayour-Mobarhan M, et al. Coronary artery disease and its risk factors status in Iran: a review. Iran Red Crescent Med J. 2011;13:610.
4. Kessler T, Schunkert H. Coronary artery disease genetics enlightened by genome-wide association studies. JACC Basic Transl Sci. 2021;6:610–23.
5. Pearson R, Fleetwood J, Eaton S, et al. Krüppel-like transcription factors: a functional family. Int J Biochem Cell Biol. 2008;40:1996–2001.
6. Gao Y, Ding Y, Chen H, et al. Targeting Kruppel-like factor 5 (KLF5) for cancer therapy. Curr Top Med Chem. 2015;15:699–713.
7. Oishi Y, Manabe I, Imai Y, et al. Regulatory polymorphism in transcription factor KLF5 at the MEF2 element alters the response to angiotensin II and is associated with human hypertension. FASEB J. 2010;24:1780–8.
8. Drosatos K, Pollak NM, Pol CJ, et al. Cardiac myocyte KLF5 regulates Ppara expression and cardiac function. Circ Res. 2016;118:241–53.
9. Li Y, Li J, Hou Z, et al. KLF5 overexpression attenuates cardiomyocyte inflammation induced by oxygen-glucose deprivation/reperfusion through the PPARγ/PGC-1α/TNF-α signaling pathway. Biomed Pharmacother. 2016;84:940–6.
10. Di R-M, Yang C-X, Zhao C-M, et al. Identification and functional characterization of KLF5 as a novel disease gene responsible for familial dilated cardiomyopathy. Eur J Med Genet. 2020;63:103827.
11. Choi JR, Kwon I-S, Kwon DY, et al. TT mutant homozygote of Kruppel-like factor 5 is a key factor for increasing basal metabolic rate and resting metabolic rate in Korean elementary school children. Genomics Inform. 2013;11:263.
12. Chen Y-C, Wei H, Zhang Z-W. Research progress of Krüppel-like factor 7. Sheng Li Xue Bao. 2016;68:809–15.
13. Kawamura Y, Tanaka Y, Kawamori R, et al. Overexpression of Kruppel-like factor 7 regulates adipocytokine gene expressions in human adipocytes and inhibits glucose-induced insulin secretion in pancreatic β-cell line. Mol Endocrinol. 2006;20:844–56.
14. Kanazawa A, Kawamura Y, Sekine A, et al. Single nucleotide polymorphisms in the gene encoding Krüppel-like factor 7 are associated with type 2 diabetes. Diabetologia. 2005;48:1315–22.
15. Zobel DP, Andreasen CH, Burgdorf KS, et al. Variation in the gene encoding Kruppel-like factor 7 influences body fat: studies of 14818 Danes. Eur J Endocrinol. 2009;160:603.
16. Aizawa K, Suzuki T, Kada N, et al. Regulation of platelet-derived growth factor-A chain by Krüppel-like factor 5: new pathway

of cooperative activation with nuclear factor-κB. J Biol Chem. 2004;279:70–6.

17. Nagai R, Suzuki T, Aizawa K, et al. Significance of the transcription factor KLF5 in cardiovascular remodeling. J Thromb Haemost. 2005;3:1569–76.

18. Takeda N, Manabe I, Uchino Y, et al. Cardiac fibroblasts are essential for the adaptive response of the murine heart to pressure overload. J Clin Invest. 2010;120:254–65.

19. Shindo T, Manabe I, Fukushima Y, et al. Krüppel-like zinc-finger transcription factor KLF5/BTEB2 is a target for angiotensin II signaling and an essential regulator of cardiovascular remodeling. Nat Med. 2002;8:856–63 [Epub 08.07.02].

20. Yanagi M, Hashimoto T, Kitamura NF. Expression of Kruppel-like factor 5 gene in human brain and association of the gene with the susceptibility to schizophrenia. Schizophr Res. 2008;100:291–301.

21. Cho SY, Park PJ, Shin HJ, et al. (−)-Catechin suppresses expression of Kruppel-like factor 7 and increases expression and secretion of adiponectin protein in 3T3-L1 cells. Am J Physiol Endocrinol Metab. 2007;294:1166–72.