

Absence of kl-vs variant of klotho gene in Iranian cardiac patients (comparison to the world populations)

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Abstract. *Objective:* Klotho has an important role in development of coronary artery (CAD) disease. A functional variant of klotho gene (kl-vs) has been found as an independent risk factor for early-onset occult coronary artery disease (CAD) in previous studies. The Frequency of this variant was not known in Iranian population. We have examined the allele frequency of the kl-vs variant in a case-control study in an Iranian population.

Methods and results: Genotyping for kl-vs variant was carried out in $N = 107$ individuals including $N = 54$ cases and $N = 53$ control who all underwent coronary angiogram for CAD evaluation. Patients with $>50\%$ stenosis in vessels considered as case groups (or CAD⁺) and patients with normal vessels (or CAD⁻) as controls. The frequency of kl-vs variant was determined in these patients using PCR-RFLP technique. None of the individual was carrying the kl-vs mutation in our samples. The frequency of kl-vs mutation was significantly different from previous studies in different populations.

Conclusion: The kl-vs variant seems to be scarce found in the Iranian population in comparison to other populations reported previously. Klotho gene might be a candidate gene of atherosclerosis in some populations but not in Iranian population. Further studies are required to examine the frequency of kl-vs variant in other populations from the Middle East.

Keywords: Klotho gene, atherosclerosis, coronary artery disease

1. Introduction

Cardiovascular diseases are the main cause of mortality in the world and atherosclerosis plays a pivotal role in the pathogenesis of CVD. Therefore prevention of atherosclerosis is the main goal for clinicians [1]. Atherosclerosis is a complex, polygenic disease and there are more than 400 genes regulating its processes [2]. Klotho is a novel β -glucuronidase [3] which is a circulating factor detectable in serum and declines with aging. It reaches to highest level in 30-40 yrs, and reaches the peak several years earlier in females compared to males [4]. A novel mouse mutant, the klotho

knockout mouse, was discovered by Kuro-o et al. [5]. It was observed that a defect in klotho gene expression in homozygous phenotypes leads to a premature aging syndrome [2]. This syndrome includes arteriosclerosis, osteoporosis, pulmonary emphysema, etc. The most notable histological changes in klotho knockout mouse is the expression of atherosclerosis that is associated with impairments of angiogenesis, vasculogenesis [5, 6] and decreased nitric oxide metabolites in the urine (No2 and No3) [7,8]. All together these data suggest that klotho protein may have a protective role in the cardiovascular system [8].

Although more than 10 mutations or single nucleotide polymorphisms (SNPs) have been reported in the klotho gene in human [9], subsequent studies have identified a functional variant of klotho (kl-vs) that were proven to be an independent risk factor for early-onset occult coronary artery disease (CAD) [8].

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The associations between the homozygote kl-vs variant with some cardiovascular risk factors such as high-density lipoprotein cholesterol (HDL-C), high systolic blood pressure, smoking status and stroke have also been reported [8,10]. The kl-vs frequency is reported to be common in general population with frequency of 0.157 [8]. Two of the mutations within gene including F325V and C370S can alter klotho metabolism and subsequently increase the risk of CAD [8].

There was no report of kl-vs variant frequency in Iranian population. Thus, we conducted a case-control study in subjects undergoing coronary angiograms, to examine kl-vs frequency in an Iranian population with and without CAD.

2. Methods

This case-control study was performed in 107 ($N = 54$ cases and $N = 53$ controls) patients whose chief complaint was chest pain and who underwent coronary artery angiography at Cath Lab Center of Dr. Shariati Hospital, Tehran, Iran, from February 2008 to March 2010. All of them were Iranian. After obtaining informed consent, personal and demographic questionnaire were completed for all patients.

Coronary artery angiography was performed in all patients. The procedures were done and interpreted by trained cardiologists. Significant stenosis was defined as a decrease of the internal diameter of more than 50% in all 3 main coronary arteries. Then patients were grouped according to the results of angiography, including patients with normal vessels grouped as control (or CAD⁻) and patients with >50% stenosis in vessels considered as case groups (or CAD⁺).

Subjects with a history of taking medication for hypertension or those with an average blood pressure of $\geq 140/90$ mmHg were labeled as having hypertension. Diabetes mellitus and hyperlipidemia were diagnosed according to American Diabetes Association (ADA) criteria [11] and National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) [12], respectively.

Also, the smoking status and past history of myocardial infarction (MI) or premature coronary heart diseases (premature CHD) were recorded. Myocardial infarction was confirmed by a review of medical records based on World Health Organization (WHO) criteria including characteristic symptoms besides typical electrocardiographic changes or elevations of cardiac enzymes [12] or by past history of hospitalization. Pre-

Table 1
Baseline characteristics in Iranian subjects with and without CAD

Variable	With coronary artery stenosis	Without coronary artery stenosis
sex (male) (n%)	60.4 (32)	68.5 (37)
age (yr)	63 \pm 8.5	55 \pm 11
Current smokers	25.9 (14)	13.2 (7)
Hypertension	75.9 (41)	50.9 (27)
Diabetes mellitus	53.7 (29)	22.6 (12)
Hyperlipidemia	50 (27)	24.5 (13)
TChol	188 \pm 47	175 \pm 38
TG	188 \pm 84	165 \pm 80
LDL	116 \pm 41	118 \pm 37
HDL	44 \pm 17	43 \pm 12
Past MI	51.9 (28)	5.7 (3)
Premature CHD	40.7 (22)	62.3 (33)

TChol: Total Cholesterol, TG: Triglyceride, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, Past MI: past history of myocardial infarction, Premature CHD: premature coronary heart disease in first degree relatives. Variables are described based mean \pm standard deviation.

mature CHD was defined based on NCEP ATP III [13] criteria.

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) of Iran.

2.1. DNA extraction and genotyping

DNA from cases and controls was extracted from anticoagulated blood collected in EDTA using salting out method. Genotyping was performed using PCR-RFLP technique as previously described [8]. After DNA was extracted from whole blood buffy coat preparations or Guthrie cards, according to standard protocols, sample DNA was amplified using PCR (sense primer 5'-AGGCTCATGCCAAAGTCTGG-3'; antisense primer 5'-GTTTCCATGATGAACCTTTTGGAGG-3') with AmpliTaq Gold (Perkin Elmer) and supplied buffer under the following conditions: 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, followed by a 10-min 72°C final extension. PCR products were then digested with MaeIII (Roche) at 55°C for 16 h and were electrophoretically separated on a 1.6% agarose gel. The KL-VS allele is characterized by diagnostic MaeIII restriction fragments of 265 and 185 bp. The genotyping has been validated by direct sequencing.

2.2. Statistical analysis

The qualitative or quantitative results were expressed as the frequency or mean \pm standard deviation, respec-

Table 2
Frequency distribution of kl-vs variant of klotho gene in Iranian subjects compared with other populations

Population	Genotype (%)		
	FF	FV	VV
Iranian population (this study)			
With CAD	54 (100)	0 (0)	0 (0)
Without CAD	53 (100)	0 (0)	0 (0)
Czech (Arking et al., 2002)			
Elderly Bohemian Czech	308 (74.2)	103 (24.8)	4 (1.0)
Elderly Baltimore Caucasian	530 (73.3)	185 (25.6)	8 (1.1)
Elderly Baltimore African- American	169 (69.8)	68 (28.1)	5 (2.1)
Baltimore with occult CAD (Arking et al., 2003)	373 (71.7)	135 (26)	12 (2.3)
Male Ashkenazi Jews (Arking et al., 2005)	357 (33.3)	149 (29.5)	19 (26.3)
Italian population (Invidia et al., 2010)			
Age Class 1 (male < 66, female < 73 yr)	348 (75.2)	103 (22.2)	12 (2.6)
Age Class 2 (male 66–88, female 73–91 yr)	203 (67.7)	94 (31.3)	3 (1.0)
Age Class 3 (male > 88, female > 91 yr)	236 (72.4)	80 (24.4)	10 (3.1)

Legend: FF: homozygous wild-type, FV: heterozygous, VV: homozygous kl-vs.

tively. Analysis were performed by SPSS version 15 and $P \leq 0.05$ was considered as significant statistical analysis.

3. Results

None of the individuals was carrying the kl-vs polymorphism. The frequency of kl-vs allele distribution in various populations reported previously is shown in Table 2.

4. Discussion

In this study, we did not detect kl-vs polymorphism in patients who underwent coronary angiogram in Iranian population which requires further investigations to elucidate the reveloutinary origin of this variant or any other mechanism involved. Various frequencies of kl-vs mutation have been reported in previous studies [2,8–10,14,15] in different populations including, African American, Caucasians, Italian and Japanese, with lower frequency in Korean. Therefore it seems that the prevalence of kl-vs polymorphism is considerable in some populations, but it is rarely observed in others. On the other hand, prevalence of kl-vs is strongly affected by the ethnic background. In a study by Imamura et al. [16] it was shown that the human klotho gene polymorphism (–395A) may be a genetic risk factor for CAD but not for vasospastic angina in Japanese patients without significant fixed stenosis of the coronary arteries. Kim et al. [17] reported the

klotho gene polymorphism as a risk factor for ischemic stroke. However there were discrepancies for the results in different populations. Rhee et al. [18] observed an association between mean systolic blood pressure and –395 polymorphism of klotho in healthy Korean women, while Shimoyama et al. [19] did not found similar associations in healthy Japanese subjects. These differences might be related to different genetic backgrounds in various populations. However these data are not supported by our finding as none of the patients in our study population were carrier for KL-VS mutation.

It is well established that the family history of premature CAD in the first degree relatives is a major predictor of CAD [20,21] indicating the role of both genetics and environmental factors. Kl-vs variant was absent in our patients who had major cardiovascular risks. Our finding hilights the necessity of future studies to further clarify the role of klotho variants in various clinical conditions in different populations. The frequency of klotho kl-vs variant should be examined in more studies in defined populations. These studies must be carried out on subjects recruited from different ethnic backgrounds in areas which population admixture is rare and their characteristics have been described. At least one ethnic group with known presence of kl-vs variant must serve as positive control. Also detection of serum level of klotho protein or its gene expression in tissues might be helpful in identification of klotho role in CAD in various populations.

Conflict of interest

None declared.

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