Review Article
Polymorphism of klotho G-395A and susceptibility of coronary artery disease in East-Asia population: a meta-analysis

Changlin Zhai[1*], Guanmin Tang[1*], Gang Qian[1], Huilin Hu[1], Aiming Gu[1], Ying Fang[1], Xiaohong Hu[2], Zhiyong Wang[1], Shijun Wang[1], Ming Yao[1], Jiayin Li[1]

[1]Department of Cardiology, First Affiliated Hospital of Jiaxing University, Jiaxing 314000, Zhejiang, China; [2]Department of ECG, Jiaxing Hospital of Traditional Chinese Medicine, Jiaxing 314000, Zhejiang, China. *Equal contributors.

Abstract: Objective: To investigate the association between polymorphism of Klotho G-395A and susceptibility of coronary artery disease (CAD) in East-Asia population. Methods: A total of 6 case-control studies involving 1560 patients and 1459 controls were analyzed in the study. PubMed, Embase, CBM disc, Wanfang database were searched for published case-control studies investigating the association between Klotho G-395A and CAD that were available before Dec. 2013. Fixed or random effect models were selected for odds ratio (OR) calculation. A Meta-analysis was performed to estimate heterogeneity and the pooled odds ratio (OR) to evaluate the relationship between Klotho G-395A polymorphism and CAD. The sensitivity analysis was also assessed. Results: There was no significant heterogeneity found (dominant genetic model: P = 0.2, I^2 = 30.8%). The pooled OR (95% CI) value of the frequencies of the Klotho G-395A genotype (GA + AA)/GG calculated by fixed effects mode was 1.24 (95% CI:1.06-1.45), P = 0.009. There was no significant heterogeneity among the remaining articles after using random effect model or excluding the article with the largest weight or the article with larger frequencies of the allele A, respectively. And the pooled OR (95% CI) value of the frequencies of the genotype (GA + AA)/GG were similar. Publication bias was not found by Begg's test. Conclusion: Klotho G-395A polymorphism may be a susceptible factor of CAD in East-Asia population.

Keywords: Coronary artery disease, polymorphism of klotho G-395A

Introduction

Coronary artery disease (CAD) is a common disease with a high incidence and mortality rate, which has serious effects on the health of human beings and brings heavy economic burden to our society. The pathogenesis of CAD is a little complicated including environmental, genetic and other kind of factors. Recently, some candidate genes had been identified that they might increase the susceptibility of CAD. Kuro-o et al. found a new gene firstly, in 1977, which was called Klotho gene and was considered associated with senescence [1]. In mouse experiments, when this gene was knocked out, senescence would be accelerated. On the contrary, once this gene expressed excessively, aging could be delayed. Therefore, this gene

was also called "anti-aginglin". Klotho gene mainly encodes membrane and secreted proteins [2]. Some previous studies showed that the changes of its gene polymorphism and expression products might be related with the pathogenesis of hypertension, atherosclerosis, chronic kidney disease, early onset of ischemic stroke and metabolism syndrome [3-9]. Moreover, all these diseases would accelerate the progression of CAD, which might indicate that Klotho gene played an important role in the control of the pathogenesis of cardiovascular disease [10]. It has been found that there were lots of SNPs in Klotho gene and the G-395A gene, which were located in Klotho gene's promoter region. Their polymorphisms were associated with the pathogenesis of CAD [11-16], which had been reported in many Asian coun-

tries. However, no consistent conclusions were obtained.

Therefore, we conducted a meta-analysis to evaluate the relation of the polymorphism of G-395A gene and the pathogenesis of CAD.

Methods

Literature search

To obtain an overall view of the relation between G-395A gene and CAD, we performed a comprehensive and systematic searching strategy. We searched for publications updated to October 2013 using PubMed, Embase, CBM and WanFang database. For English databases, we used the keyword or subject word search (coronary artery disease, coronary disease, myocardial, atherosclerosis, myocardial infarction, angina pectoris, Klotho, gene, polymorphism, variant, allele) to identify publications. For Chinese databases, we selected the keyword or subject word search (CHD, coronary atherosclerotic heart disease, angina pectoris, myocardial infarction, Klotho, polymorphism) to identify publications. Additional publications were assessed via cited references in the recruited articles.

Each retrieved publication was evaluated for the following criteria: 1) Independent case-control studies assessing the potential correlation between polymorphism of Klotho gene and CAD; 2) The distribution of gene frequency in control group meets the Hardy-Weinberg equilibrium; 3) Articles published before October 2013; 4) For repeated published literatures, we recruited the most significant one whose sample size and the data were the most complete; 5) Results must include the data of allelic or genotypic frequencies and can directly or indirectly supply odds ratios (ORs) or 95% confidence intervals (95% CIs); We tried to contact those authors whose studies with insufficient data (lacking of allelic or genotypic frequencies), if failed, those studies would be excluded.

Articles matching following criteria should be excluded: 1) Duplicated reports; 2) Articles with deficiencies in study design and qualities; 3) Articles with insufficient data and ambiguous outcomes; 4) Incorrect statistical analysis methods were used and cannot be modified,

directly or indirectly ORs and 95% CIs cannot be provided, and mean and standard deviation of measurement data were failed to be supplied.

Data extraction

Data extracted from the articles including: 1) the name of the first author; 2) year of publication; 3) study population; 4) diagnosis criterion of CAD; 5) Gene and genotype frequencies in both experiment and control group. All the data would be extracted by two reviewers independently. When outcomes from two reviewers were found inconsistent, another reviewer would get involved in discussions until the consensus was reached.

Statistical analysis

We used chi-square test to assess the H-W equilibrium of genotype in control group. We chose OR and 95% CI to evaluate the distribution of genotype between experiment and control group. A fixed-effect model was conducted to assess the correlation between experiment and control group when no heterogeneity was detected. Otherwise, the random-effect model was conducted for summarization. We used I^2 test to assess the extent of heterogeneity of pooled data. All the results would be shown in a forest graph, using the OR value as vertical coordinate and the standard error of natural logarithm of OR value as horizontal coordinate. Publication bias was evaluated by Begg's tests. The statistical significance level was set at 0.05.

Results

Literature's character

A total of 23 articles were found when the mentioned keywords were used. Nine articles were excluded for describing unrelated parts of Klotho gene's polymorphism and including repeated sample size data. One was excluded for lacking gene and allele frequencies, and we couldn't contact with its author. Another one was excluded for repeated sample size, and other six were excluded because they were reviews. Finally, we identified a total 6 articles [11-16]. All the study populations were from Southeast Asia, and genotype frequencies were in accord with H-W equilibrium. Among the included articles, 3 articles were written in

Table 1. Baseline characteristics of included studies

First author	Year of publish	Country	Age		Sex (male/female)		Diagnostic criteria of CAD	Examination of genetype
			Experiment	Control	Experiment	Control		
Akiko Imammnra [11]	2006	Japan	63.5 ± 9.8	53.6 ± 11.4	150/47	238/93	CAG ≥ 75%	TaqMan
Eun-Jung Rhee [12]	2006	Korea	NA	55.3 ± 11.5	NA	NA	CAG > 50%	TaqMan
Sang-Ho Jo [13]	2009	Korea	64.1 ± 11.5	59.3 ± 12.1	139/86	103/106	CAG ≥ 50%	TaqMan
Pingguo He [14]	2010	China	65.8 ± 11.0	62.0 ± 10.6	240/86	124/95	2002AHA/ACA ACS	Genechip
Ning You [15]	2012	China	68.9 ± 8.0	67.7 ± 10.0	191/126	184/117	2007 Chinese guidelines for CAD	Gene sequencing
Mengyun Cai [16]	2013	China	64.4 ± 12.0	63.1 ± 12.1	232/108	169/111	CAG > 50%	LDR

Note: NA = data of unknown; CAG = coronary angiography.

Table 2. Klotho G-395A gene Polymorphism's characteristics

First author	Sample size		Genetype of case group		Genetype of control group		Allele A frequency		H-W equilibrium[*]		
	Exp.	Con.	GG/GA/AA	GA + AA	GG/GA/AA	GA + AA	Exp.	Con.	Total	χ^2	P
Akiko Imammnra	197	331	138/59/0	59	268/62/1	63	0.149	0.096	0.116	1.74	0.19
Eun-Jung Rhee	155	119	116/NA/NA	39	87/NA/NA	32	NA	NA	0.148	3.71	0.16
Sang-Ho Jo	225	209	157/60/8	68	164/39/6	45	0.168	0.122	0.146	3.48	0.06
Guoping He	326	219	240/79/7	86	162/54/3	57	0.142	0.137	0.140	0.40	0.53
Ning You	317	301	150/124/43	167	150/129/22	151	0.331	0.287	0.309	0.65	0.42
Mengyun Cai	340	280	235/99/6	105	205/67/8	75	0.163	0.148	0.156	0.77	0.38

Note: Exp. = experiment; Con. = control; NA = data of unknown; [*]the total sample of the study by Eun-Jung Rhee et al. is in accord with H-W equilibrium, and the other studies' control group data is in accord with H-W equilibrium.
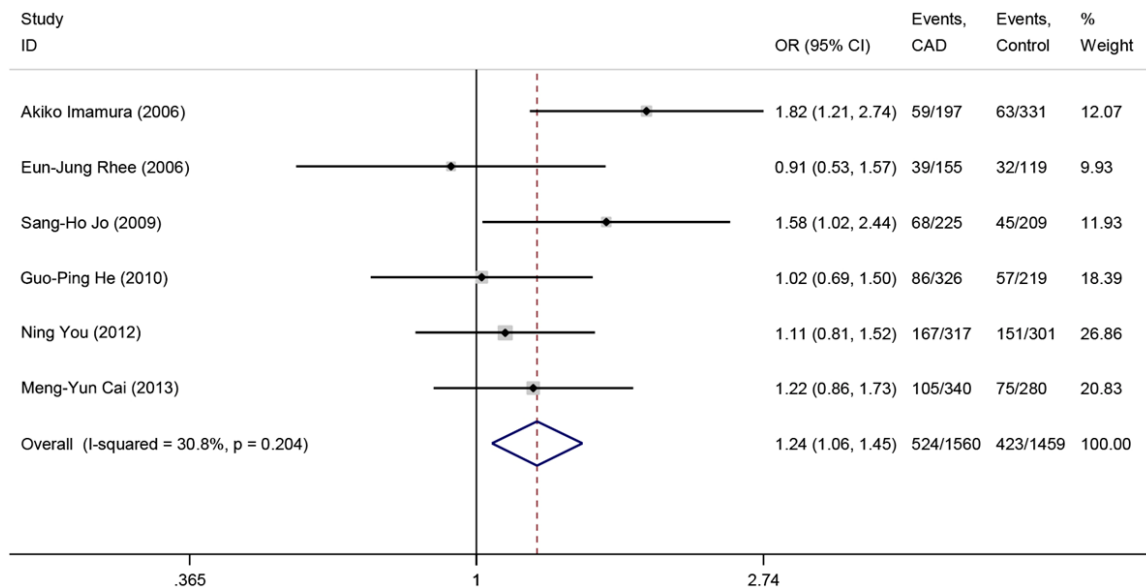

Figure 1. The relation between Polymorphism of Klotho G-395A gene and susceptibility of CAD (GA + AA) vs. GG.

English and 6 articles were written in Chinese. Moreover, the diagnosis of CAD in 4 articles was completely dependent on coronary angiography and the other 2 articles were not incompletely dependent on coronary angiography. The total study population in our study were 3019, including 1560 CAD patients and 1459 healthy people. Detailed characteristics of the eligible studies are listed in **Tables 1** and **2**.

Results of meta-analysis

The Meta-analysis showed that there weren't statistical heterogeneity. Therefore, fixed-effect model was performed (P = 0.2, I^2 = 30.8%), OR = 1.24 (95% CI: 1.06-1.45), P = 0.009, which was thought to be statistical significant (**Figure 1**). In the subgroup analysis, we detected statistical significance in completely CAG group,

and no statistical significance in incompletely CAG group (**Table 3**). Sensitivity analyses were conducted to evaluate the effect of each study on the overall estimation. We performed sensitivity analysis in different methods, including using random-effects model, and removing maximum weighted study or the study, in which the difference of allele was the largest. Our sensitivity analysis suggested that the result of our Meta-analysis was stable (**Table 4**).

Publication bias

We performed the funnel plot using Stata 12.0 to test the publication bias. The graph showed both sides were almost symmetrical, which suggested that all these studies had little publication bias (**Figure 2**). Moreover, the result of Begg's test, which was used to calculate the

Table 3. Results of different groups about the relation between polymorphism of Klotho G-395A gene and CAD

Diagnose methods	Num of Studies	Heterogeneity test		Model	OR (95% CI)	P
		I^2	P			
Completely CAG	4	37.2%	0.189	Fixed-effect model	1.38 (1.12-1.70)	0.003
Incompletely CAG	2	0	0.747	Fixed-effect model	1.07 (0.84-1.37)	0.587

Note: Compared model is (GA + AA) vs. GG.

Table 4. Results of different research scopes about the relation between polymorphism of Klotho G-395A and CAD

Different research scope	Heterogeneity test		OR (95% CI)	P
	I^2	P		
All studies				
Fixed-effect model	30.8%	0.204	1.24 (1.06-1.45)	0.009
Random-effect model	30.8%	0.204	1.24 (1.02-1.51)	0.028
Removing maximum weighted study[a]	39%	0.160	1.29 (1.07-1.55)	0.008
Removing the study of the largest difference in alleles[b]	39%	0.160	1.29 (1.07-1.55)	0.008

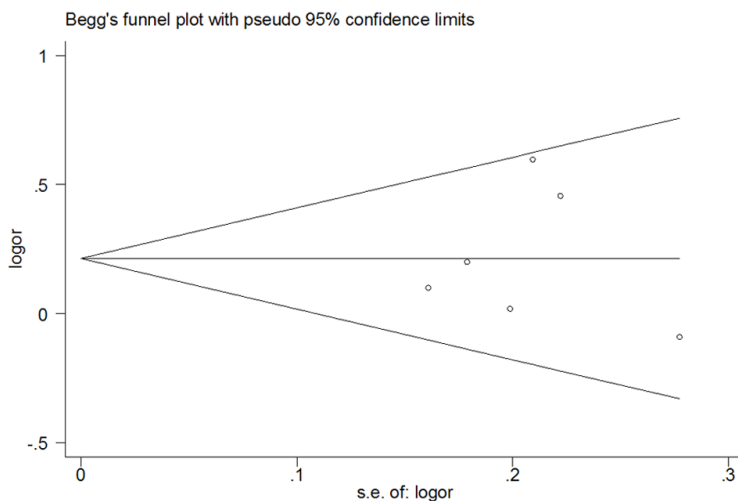Note: Compared model is (GA + AA) vs. GG; [a,b]: excluded literature is [15].

Figure 2. Funnel plot: Polymorphism of Klotho G-395A gene and susceptibility of CAD (GA + AA) vs. GG.

publication bias, also supported our conclusion (P = 1.0).

Discussion

It is well known that Klotho protein can protect the vascular endothelial cells and the mechanism including: inhibiting the active oxygen product which is induced by Ang II, slowing down the cell aging and apoptosis, regulating the inflammatory response and maintaining the integrity of endothelial cell, increasing intracellular NO concentration and improving the compliance of artery [17-21]. The mice of which Klotho gene can't be expressed appropriately will present the performance of atherosclerosis, including the extensive calcification in aorta or arteriole, middle artery thicken together with calcification, which is similar to that in human aging [1]. Navarro et al. suggested that the decline of both Klotho protein in serum and expression of Klotho gene would promote the onset of CAD, and was positively correlated with severity. Moreover, it might be one of independent risk factors of CAD [22]. Human's Klotho gene located in chromosome 13q12 owning 5 exons, among which there were 11 polymorphisms of SNPs [23]. Few reports found that genes associating with the onset of CAD mainly include: KL-VS mutant [24] and C1818T, which is located in exon 4 [12, 25]. Klotho G-395A gene, which is located in the promoter region of Klotho gene, in Europe, has not been reported that its polymorphism was associated with CAD. However, in Asian countries, for example, in Japan, G-395A gene is regarded as a risk factor of CAD. In Korea, Rhee et al. reported that in people over age 60, the allele of G-395A performed negatively correlated with the susceptibility of

CAD. Jo et al. reported that G-395A gene was positively correlated with the susceptibility of CAD, and further subgroup-analysis revealed a better correlation with the susceptibility of CAD in people under age 60 and no correlation in people over age 60 [13]. In China, You et al. reported that homozygote of G-395A gene was associated with CAD, and further subgroup-analysis revealed only in male subgroup there was a correlation between G-395A gene and CAD [15]. Differences might result from the discordant of studies' sample size, diagnostic criteria of CAD and the age of recruited people.

This meta-analysis is the first one to assess the correlation between polymorphism of Klotho G-395A gene and the susceptibility of CAD. Our results showed that in dominant genetic model, there was a certain correlation between them in East Asian population. That was to say, the G-395A gene might be one of risk genes of CAD. In our study, recruited people were from eastern Asian countries and all of them were xanthoderm, suggesting that we could remove the interference of race partly. As mentioned earlier, our subgroup-analysis was performed in terms of different diagnostic criteria of CAD and results revealed that incompletely CAG group's outcome didn't have statistical significance, suggesting that differences in diagnostic criteria might one of the reasons of heterogeneity. When we performed random-effects model, there were still significant heterogeneity. Both sensitivity analysis and Begg's test showed that there was little publication bias and our conclusion was stable.

Only a few studies assessed the influence of age, sex factor to the relation of G-395A gene and CAD. In order to analyze the correlation between polymorphism of mononucleotide and disease susceptibility, we should also consider the effect of allele and the linkage disequilibrium of gene, which could cause disease, respectively. Besides, we also need to recruit different races' population and conduct larger sample size, higher quality studies to confirm the correlation between the polymorphism of Klotho gene and CAD.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Ming Yao and Jiayin Li, Department of Cardiology, First Affiliated Hospital of Jiaxing University, No. 1882 South Zhonghuan Road, Jiaxing 314000, Zhejiang, China. E-mail: yaoming_zj@163.com (MY); lijiayin_jiax@126.com (JYL)

References

[1] Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E, Iwasaki H, Iida A, Shiraki-Iida T, Nishikawa S, Nagai R and Nabeshima YI. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 1997; 390: 45-51.

[2] Kuro-o M. Klotho. Pflugers Arch 2010; 459: 333-343.

[3] Wang HL, Xu Q, Wang Z, Zhang YH, Si LY, Li XJ, Yang QH and Xiao H. A potential regulatory single nucleotide polymorphism in the promoter of the Klotho gene may be associated with essential hypertension in the Chinese Han population. Clin Chim Acta 2010; 411: 386-390.

[4] Majumdar V and Christopher R. Association of exonic variants of Klotho with metabolic syndrome in Asian Indians. Clin Chim Acta 2011; 412: 1116-1121.

[5] Majumdar V, Nagaraja D and Christopher R. Association of the functional KL-VS variant of Klotho gene with early-onset ischemic stroke. Biochem Biophys Res Commun 2010; 403: 412-416.

[6] Shimoyama Y, Nishio K, Hamajima N and Niwa T. KLOTHO gene polymorphisms G-395A and C1818T are associated with lipid and glucose metabolism, bone mineral density and systolic blood pressure in Japanese healthy subjects. Clin Chim Acta 2009; 406: 134-138.

[7] Wang HL, Si LY, Sun LY, Meng P and Xiao H. Association of G-395A polymorphism of human Klotho gene with arteriosclerosis. Chin J Geri Hear Brai and Ves Dis 2008; 10: 96-98.

[8] Rhee EJ, Oh KW, Yun EJ, Jung CH, Lee WY, Kim SW, Baek KH, Kang MI and Park SW. Relationship between polymorphisms G395A in promoter and C1818T in exon 4 of the KLOTHO gene with glucose metabolism and cardiovascular risk factors in Korean women. J Endocrinol Invest 2006; 29: 613-618.

[9] Shimamura Y, Hamada K, Inoue K, Ogata K, Ishihara M, Kagawa T, Inoue M, Fujimoto S, Ikebe M, Yuasa K, Yamanaka S, Sugiura T and Terada Y. Serum levels of soluble secreted alpha-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis. Clin Exp Nephrol 2012; 16: 722-729.

[10] Moe SM. Klotho: a master regulator of cardiovascular disease? Circulation 2012; 125: 2181-2183.

[11] Imamura A, Okumura K, Ogawa Y, Murakami R, Torigoe M, Numaguchi Y and Murohara T. Klotho gene polymorphism may be a genetic risk factor for atherosclerotic coronary artery disease but not for vasospastic angina in Japanese. Clin Chim Acta 2006; 371: 66-70.

[12] Rhee EJ, Oh KW, Lee WY, Kim SY, Jung CH, Kim BJ, Sung KC, Kim BS, Kang JH, Lee MH, Kim SW and Park JR. The differential effects of age on the association of KLOTHO gene polymorphisms with coronary artery disease. Metabolism 2006; 55: 1344-1351.

[13] Jo SH, Kim SG, Choi YJ, Joo NR, Cho GY, Choi SR, Kim EJ, Kim HS, Kim HJ and Rhim CY. KLOTHO gene polymorphism is associated with coronary artery stenosis but not with coronary calcification in a Korean population. Int Heart J 2009; 50: 23-32.

[14] He GP, Jin MF, Gao L, Yang J, Wang JH, Shen LJ, Yang D and Zhang JY. KLOTHO gene promoter G-395A polymorphisms of acute coronary syndrome in the Chinese Han population of Suwan region. Acta Univ Med Nanjing: Nat Sci 2010.

[15] You N, Liu XL, Tian XC and Ma HX. Correlation between single nucleotide polymorphisms at three sites of Klotho gene with coronary heart disease. J Chongqing Med Univ 2012; 37: 579-584.

[16] Cai MY, Yang XL, Cen JM, Jing X, Liu XG and Xiong XD. Association analysis between Klotho G-395A polymorphism and the risk of coronary artery disease. Guangdong Med J 2013; 34: 1832-1835.

[17] Saito Y, Yamagishi T, Nakamura T, Ohyama Y, Aizawa H, Suga T, Matsumura Y, Masuda H, Kurabayashi M, Kuro-o M, Nabeshima Y and Nagai R. Klotho protein protects against endothelial dysfunction. Biochem Biophys Res Commun 1998; 248: 324-329.

[18] Rakugi H, Matsukawa N, Ishikawa K, Yang J, Imai M, Ikushima M, Maekawa Y, Kida I, Miyazaki J and Ogihara T. Anti-oxidative effect of Klotho on endothelial cells through cAMP activation. Endocrine 2007; 31: 82-87.

[19] Maekawa Y, Ishikawa K, Yasuda O, Oguro R, Hanasaki H, Kida I, Takemura Y, Ohishi M, Katsuya T and Rakugi H. Klotho suppresses TNF-alpha-induced expression of adhesion molecules in the endothelium and attenuates NF-kappaB activation. Endocrine 2009; 35: 341-346.

[20] Kusaba T, Okigaki M, Matui A, Murakami M, Ishikawa K, Kimura T, Sonomura K, Adachi Y, Shibuya M, Shirayama T, Tanda S, Hatta T, Sasaki S, Mori Y and Matsubara H. Klotho is associated with VEGF receptor-2 and the transient receptor potential canonical-1 Ca^{2+} channel to maintain endothelial integrity. Proc Natl Acad Sci U S A 2010; 107: 19308-19313.

[21] Maekawa Y, Ohishi M, Ikushima M, Yamamoto K, Yasuda O, Oguro R, Yamamoto-Hanasaki H, Tatara Y, Takeya Y and Rakugi H. Klotho protein diminishes endothelial apoptosis and senescence via a mitogen-activated kinase pathway. Geriatr Gerontol Int 2011; 11: 510-516.

[22] Navarro-Gonzalez JF, Donate-Correa J, Muros de Fuentes M, Perez-Hernandez H, Martinez-Sanz R and Mora-Fernandez C. Reduced Klotho is associated with the presence and severity of coronary artery disease. Heart 2014; 100: 34-40.

[23] Kawano K, Ogata N, Chiano M, Molloy H, Kleyn P, Spector TD, Uchida M, Hosoi T, Suzuki T, Orimo H, Inoue S, Nabeshima Y, Nakamura K, Kuro-o M and Kawaguchi H. Klotho gene polymorphisms associated with bone density of aged postmenopausal women. J Bone Miner Res 2002; 17: 1744-1751.

[24] Arking DE, Becker DM, Yanek LR, Fallin D, Judge DP, Moy TF, Becker LC and Dietz HC. KLOTHO allele status and the risk of early-onset occult coronary artery disease. Am J Hum Genet 2003; 72: 1154-1161.

[25] He GP, Jin MF, Gao L, Yang J, Wang JH, Shen L, J, Qi CP, Yang GY, Yang D and Zhang JN. KLOTHO Gene C-1818T Polymorphism in Elderly Patients With Acute Coronary Syndrome. Chin Circu J 2008; 23: 203-206.