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Klotho gene polymorphism may be a genetic risk factor for atherosclerotic coronary artery disease but not for vasospastic angina in Japanese

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Abstract

Background: The klotho gene, originally identified by insertional mutagenesis in mice, suppresses multiple aging phenotypes, including atherosclerosis. We tested the hypothesis that the G–395A polymorphism of the klotho gene is associated with increased risk for 2 types of ischemic heart disease in Japanese.
Methods: The study population consisted of 197 patients with coronary heart disease (CAD) who had >75% luminal diameter narrowing, 77 patients with vasospastic angina (VSA) without significant fixed coronary artery disease, and 331 healthy control subjects.
Results: The frequency of the A allele carriers of the klotho gene was significantly higher in the CAD group than in the control group (29.9% vs. 19.0%). The unadjusted odds ratio for CAD in the A allele carriers compared with the control group was 1.82 ($p=0.004$) and a traditional risk-adjusted logistic regression model revealed that the A allele was an independent predictor of CAD (odds ratio, 1.76; $p=0.03$). In contrast, the frequency of the A allele carriers was not significantly different in the VSA group (23.4%; adjusted odds ratio, 1.18.
Conclusions: The –395A polymorphism of the human klotho gene may be a genetic risk factor for IHD and not for VSA.

Keywords: DNA; Klotho gene; Atherosclerosis; Coronary artery disease; Vasospastic angina

1. Introduction

Defects of klotho gene expression in mice result in a syndrome that is similar to human aging, and that includes such effects as arteriosclerosis, osteoporosis, infertility, emphysema, and ectopic calcification [1]. One of the most notable histological changes in klotho-deficient mice is the presence of arteriosclerosis. Klotho-deficient mice display extensive and accelerated arteriosclerosis in association with medial calcification of the aorta and both medial calcification and intimal thickening of medium-sized muscular arteries. Furthermore, it has been reported that mice deficient for the klotho gene showed endothelial dysfunction as manifested by an attenuated response of aortic relaxation in response to acetylcholine stimulation, and this endothelial dysfunction was prevented by parabiosis between wild type mice and mice heterozygously deficient for the klotho gene [2,3]. It has also been reported that adenovirus-mediated transfer of the klotho gene into rats with multiple risk factors for atherosclerosis and endothelial dysfunction could ameliorate vascular endothelial dysfunction and increase nitric oxide (NO) production [4]. These findings suggest that the klotho gene is involved in the regulation of endothelial function through a pathway mediated by NO.

It has been demonstrated that a functional variant of klotho (termed KL–VS), which harbors 2 amino acid substitutions in complete linkage disequilibrium, was an independent risk factor for occult coronary artery disease in 2 independent high risk groups [5]. This finding implies that the klotho gene may contribute to the pathophysiology of coronary artery disease. Recently, the G–395A polymorphism in the promoter region of

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the human klotho gene was reported to be significantly associated with bone density in aged postmenopausal women in a Caucasian and a Japanese population. An electrophoretic mobility shift analysis revealed that the G–A substitution in the promoter region affected DNA–protein interaction in cultured human kidney 293 cells, and that this polymorphism may change the expression of the klotho gene [6].

We conducted a case–control study in a Japanese population to investigate whether G–395A polymorphism in the promoter region of the human klotho gene is an independent risk factor for atherosclerotic coronary artery disease (CAD), which is mainly caused by established coronary arteriosclerosis, and vasospastic angina (VSA), which is mainly caused by coronary endothelial dysfunction.

2. Materials and methods

2.1. Subjects

All subjects were Japanese living in Aichi prefecture, Japan. They were categorized into 3 groups—those with VSA, those with CAD and a control group.

2.2. CAD group

A total of 197 patients (150 men and 47 women; mean age, 63.4 years) had undergone percutaneous transluminal coronary intervention because of effort angina or myocardial infarction. They had stenosed coronary arteries with ≥75% luminal diameter narrowing in at least one main branch of the coronary artery.

2.3. VSA group

77 patients (50 men and 27 women; mean age, 58.5 years) had attacks of chest pain and had no significant fixed coronary stenosis (<50% lumen diameter stenosis) as detected by coronary angiography. They had no history of myocardial infarction. VSA was diagnosed by using an acetylcholine provocation test. Patients were considered to have VSA if they met both of the following criteria: [1] total or 99% occlusion with a delayed image as revealed angiographically; and [2] a significant ST segment alteration (at least 0.1 mV of elevation or 0.2 mV of depression from at least 2 contiguous leads) as revealed by a standard 12-lead electrocardiogram [7].

Table 2
Genotype frequencies of the klotho gene G–395A polymorphism in the control, atherosclerotic coronary artery disease (CAD) and vasospastic angina (VSA) groups

	Control, *n* (%)	CAD, *n* (%)	VSA, *n* (%)
GG	268 (81.0)	138 (70.1)	59 (76.6)
AG	62 (18.7)	59 (29.9)	18 (23.4)
AA	1 (0.3)	0 (0.0)	0 (0.0)
AG+AA	63 (19.0)	59 (29.9)	18 (23.4)

2.4. Control group

A total of 331 subjects (238 men and 93 women; mean age, 52.4 years) were assigned to a control group. This group included 298 subjects, who were apparently healthy and visited the hospitals for their annual physical examinations, and that their results of physical examinations revealed that there were no evidence of having coronary artery disease, and 33 subjects, who underwent cardiac catheterization for atypical chest pain and had angiographically documented normal coronary arteries, normal ventriculography, and negative results on an acetylcholine provocation test. Written informed consent was obtained from all of the subjects. The present study was approved by the Ethics Committee of Nagoya University.

2.5. Baseline data collection

Information on smoking habits was obtained during subject interviews. Smokers of ≥20 cigarettes/day over 2 years were defined as having a smoking habit. Subjects with a history of taking medication for hypertension or those with an average blood pressure of ≥90 mm Hg in diastole or ≥140 mm Hg in systole by 2 or more measurements were labeled as having hypertension. Diabetes mellitus was diagnosed by the criteria of the American Diabetes Association [8] and was assumed to be present in subjects with a history of diabetes taking antidiabetic medication. Hyperlipidemia was diagnosed if plasma total cholesterol >220 mg/dl, plasma LDL cholesterol >130 mg/dl, plasma triglycerides >200 mg/dl, or if the patient used lipid-lowering drugs.

2.6. Genotyping of the klotho gene G–395A polymorphism

Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp DNA blood minikit (Qiagen, Valencia,

Table 1
Traditional risk factors in the control, atherosclerotic coronary artery disease (CAD) and vasospastic angina (VSA) groups

	Control (*n*=331)	CAD (*n*=197)	*P* value vs. Controls	VSA (*n*=77)	*P* value vs. Controls	*P* value vs. CAD
Age (years)	53.6±11.4	63.5±9.8	<0.001	58.5±11.2	<0.001	<0.001
Male/Female	238/93	150/47	NS	50/27	NS	NS
BMI (kg/m^2)	21.7±2.6	23.1±2.6	0.005	22.9±3.1	NS	NS
Hypertension (%)	21.3	42.9	<0.001	32.5	0.038	NS
Diabetes mellitus (%)	14.1	31.3	<0.001	14.3	NS	0.008
Hypercholesterolemia (%)	18.3	19.7	NS	16.9	NS	NS
Smoking (%)	32.7	45.3	0.004	51.9	0.002	NS

Mean±S.D. BMI=body mass index. NS=not significant.