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

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ABSTRACT. Background: Recently, klotho has been proposed as a link between cardiovascular diseases and premature aging, but the relationship between KLOTHO genes and cardiovascular risk factors, especially glucose metabolism, in humans is unclear. Objectives: We investigate the 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. Material and methods: In 251 women (mean age 51.3±6.9 yr), body mass index (BMI), waist circumference, blood pressure, fasting plasma glucose, insulin and lipid profiles were measured. The genotyping of polymorphisms G395A in promoter and C1818T in exon 4 of the KLOTHO gene was performed by allelic discrimination using a 5′ nuclease polymerase chain reaction assay. Results: Allele frequencies of G395A polymorphism was 0.829 for the G allele and 0.171 for the A allele and allele frequencies of C1818T polymorphism were 0.804 for the C allele and 0.196 for the T allele, both of which were in compliance with Hardy-Weinberg equilibrium and the two polymorphisms were in linkage disequilibrium (D′=0.43, p<0.01). Mean systolic blood pressure was significantly higher in A allele carriers of G395A polymorphism compared with non-carriers, and the significance was persistent even after adjustment for age and BMI. Mean fasting plasma glucose was significantly higher in T allele carriers of C1818T polymorphism compared with non-carriers, and the significance was persistent even after adjustment for age and BMI. Subjects without any minor allele from either single nucleotide polymorphisms (SNP) had significantly lower mean values for systolic, diastolic blood pressure and fasting plasma glucose levels compared with subjects with both minor allele from either SNP. Conclusions: We observed that KLOTHO G395A polymorphism was associated with blood pressure and KLOTHO C1818T polymorphism was associated with glucose metabolism in Korean women. Further studies are needed to clarify this relationship. (J. Endocrinol. Invest. 29: 613-618, 2006) ©2006, Editrice Kurtis

INTRODUCTION

A novel mouse mutant, klotho, was discovered incidentally by Kuro-o et al. (1, 2) during the development of hypertensive transgenic mice models. Mice homozygous for the transgene show various phenotypes resembling premature aging syndrome, ie, a short life span, arteriosclerosis, osteoporosis, skin atrophy, pulmonary emphysema, ectopic calcification in various soft tissues, impaired sexual organ maturation, senile atrophy of the skin and defective hearing (2, 3).
In klotho mice, Mönckeberg type atherosclerosis was observed from aorta to small arterioles, and the impairments of angiogenesis and vasculogenesis were observed (2-4). Although klotho mRNA has been shown to be expressed predominantly in the kidney in mice and humans (3), the expression of klotho mRNA was significantly attenuated in the kidneys of rat models of Type 2 diabetes mellitus and diabetic nephropathy (5, 6). The synthesis of nitric oxide was reduced in kl/kl mice, and the delivery of klotho gene to a diabetic rat model ameliorated vascular endothelial dysfunction, increased nitric oxide production and reduced blood pressure (7, 8). Furthermore, klotho mice showed decreased blood glucose levels and increased insulin sensitivity due to enhanced GLUT4 expression in skeletal muscle, despite reduced pancreatic insulin secretion (2, 9), suggesting the involvement of klotho in glucose metabolism. In a recent report, Kurosu et al. (10) revealed that the overexpression or administration of klotho protein increased insulin resistance (IR) and inhibited intracellular insulin signaling in mice through the inhibitory effect of autophosphorylation of receptor tyrosine kinases, and from these findings there has been an interesting suggestion that the IR induced by klotho might be a protection against metabolic syndrome (11).

Several single nucleotide polymorphisms (SNP) in the human KLOTHO gene have been reported. A functional KLOTHO allele status, KL-VS, has been reported to be associated with the increased risk of occult coronary artery disease and in advance, lipid profiles and thus the risk of stroke and cognitive ability in Caucasians (12-15). Kawanou et al. (16) reported several SNP in KLOTHO gene in Whites and Japanese, and revealed that G395A in the promoter region and C1818T in exon 4 were associated with bone mineral density (BMD) in post-menopausal women. Although KLOTHO gene is expected to be an interesting model for the link between atherosclerosis and glucose metabolism, the reports in association with these two polymorphisms with atherosclerosis or glucose are scarce.

To determine whether the SNP in human KLOTHO gene might affect glucose metabolism and cardiovascular risk factors, we genotyped two SNP, that is, G395A in the promoter region and C1818T in exon 4, which have been reported to be associated with bone metabolism, but have not been studied in relation with atherosclerosis or glucose metabolism. We then analyzed the association with glucose metabolism and cardiovascular risk factors including blood pressures and lipid profiles in apparently healthy Korean females.

MATERIALS AND METHODS
Subjects
The study population consisted of 251 healthy Korean women (mean age 51.3±6.9; range 37-73 yr), who entered Miz Medi Hospital Healthcare Center for medical checkups from January 1st to December 31st, 2002. Women with documented coronary heart disease, cerebrovascular disease, diabetes mellitus, overt thyroid dysfunction, pituitary disease, chronic liver disease, or renal disease were excluded. The protocol used was approved by the Institutional Review Board of Miz Medi Hospital, and informed consent was obtained from all participants.

Anthropometric measurements and the assessment of cardiovascular risk factors
Height, weight, waist circumference, and systolic and diastolic blood pressures (SBP and DBP) were measured in duplicate and results were averaged. Weight and height were measured in kg and cm, respectively. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared and was used as an index of overall adiposity (kg/m²). Waist circumference was measured midway between the lowest rib and the iliac crest, and hip circumference was taken over the widest part of the gluteal region. Waist-to-hip ratios were used as measures of central obesity.

After 12 h of fasting, blood was sampled, and fasting plasma glucose, total cholesterol, triglyceride (TG), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) were measured. Fasting plasma glucose, serum total cholesterol, TG, HDL-C levels were determined by colorimetry (Vitros, Ortho-Clinical Diagnostics, Puritan, U.S.A) and serum LDL-C levels were calculated using the Friedewald equation.

The assessment of cardiovascular risk factors was carried out according to the third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP-ATP III) (17).

Calculation of insulin resistance indices
Fasting serum insulin level was measured by immunoradiometric assay (RIABEAD II, Abbott, Japan) with an intra-assay coefficient of variance (CV) of 1.2~1.9% and an inter-assay CV of 1.4~3.3%. Homeostasis model (HOMA)-IR and HOMA β-cell were used as indices of IR and secretion (18). In brief, HOMA-IR shows good correlation with IR status, that is, when the value is high, the subject is insulin resistance in proportion to the value. HOMA β-cell stands for the insulin secretory function of pancreatic β-cell, that is, when the value is high, insulin secretory function is good. The formulas used were as follows:

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\text{HOMA-IR} = \frac{[\text{fasting insulin (}\mu\text{U/ml}) \times \text{fasting blood glucose (mmol/l)]}}{22.5}
\]

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\text{HOMA β-cell} = \frac{[20 \times \text{fasting insulin (}\mu\text{U/ml})]}{[\text{fasting glucose (mmol/l)]-3.5]}
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Genotyping of KLOTHO gene polymorphisms using real-time polymerase chain reaction (PCR)
Buffy coat was obtained from blood samples, refrigerated at -70 C, and genomic DNA was extracted using Takara DNA Purification kits. The genotyping of the G395A in promoter and C1818T in exon 4 of the KLOTHO gene was performed using an allelic discrimination assay using TaqMan probe.