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Original Article

Lack of Association between the *Klotho* Gene and COPD

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Background: Although the aging process and features of chronic obstructive pulmonary disease (COPD) have several similarities, the relationship between aging and COPD pathogenesis remains incompletely understood. The *klotho* gene was found to be related to premature aging and emphysematous changes in an animal model. We investigated whether *klotho* gene polymorphisms are related to COPD susceptibility and emphysema severity.
Methods: A total of 219 COPD subjects from the Korean Obstructive Lung Disease Cohort and 305 control subjects were genotyped for two single nucleotide polymorphisms (SNPs) of the *klotho* gene associated with coronary artery disease. Logistic regression was performed to determine the association of these SNPs with COPD susceptibility and linear regression was performed to investigate their association with emphysema severity in COPD subjects.
Results: The mean age of the COPD subjects was 66 years and their mean FEV1 was 1.46 L. There were no associations between either SNP or COPD susceptibility (p=0.6 and 0.2, respectively) and there were no associations with emphysema severity.
Conclusion: Genetic polymorphisms of the *klotho* gene were not associated with COPD in a Korean population.

Key Words: Pulmonary Disease, Chronic Obstructive; Polymorphism, Genetic

Introduction

The age-dependent increase in the prevalence of chronic obstructive pulmonary disease (COPD) has suggested a relationship between the aging process and COPD development[1,2]. Although this interrelationship remains incompletely understood, aging and COPD share several similarities, including cardiovascular mortality, obstructive pulmonary physiology, and enlargement of airspaces[3]. In addition, inflammation associated with aging and COPD have several similarities, including neutrophil accumulation, NF-κB activation, and increased expression of markers of oxidative stress[4].

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Furthermore, the expression of antiaging molecules, such as sirtuins (SIRT), histone deacetylase (HDAC), senescence marker protein-30 (SMP30), and *klotho*, are reduced in COPD[4]. Among these, the *klotho* gene was incidentally discovered during the development of a hypertensive transgenic murine model[5]. The *klotho* protein has several functions, including the regulation of several growth factor signaling pathways, including those associated with fibroblast growth factor-23, insulin/insulin-like growth factor and Wnt, its activity in multiple ion channels, and its suppression of oxidative stress[6]. Homozygous mutant *klotho* (KL−/−) mice are regarded as a good animal model of human aging, since these mice have a short life span and exhibit multiple disorders resembling human premature-aging syndrome. Moreover, they exhibit emphysematous changes, characterized by the enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of normal architecture[5,6].

To our knowledge, the association between *klotho* gene polymorphisms and COPD has not been determined. We therefore performed genotype analyses of 2 *klotho* gene SNPs, G-395A in the promoter region and C1818T in exon 4, in Korean COPD patients and control subjects.

Materials and Methods

1. Subjects

Individuals with COPD were recruited, beginning in June 2005, from those in the "The Korean Obstructive Lung Disease cohort", a longitudinal prospective study of COPD in patients from the pulmonary clinics of 11 hospitals in Korea[7]. All COPD subjects included in this study had post-bronchodilator forced expiratory volume in one second [FEV_1]/forced vital capacity [FVC] values of <0.7 and had more than 10 pack-years of smoking history. Complete CT scanning data, blood and other clinical information were obtained from all patients. All pulmonary function tests were performed as recommended by the American Thoracic Society/European Respiratory Society. Emphysema severity was measured quantitatively by determining the volume fraction of the lung below −950 Hounsfield units. Control subjects consisted of 305 smokers or ex-smokers with normal lung function registered in the Korean Genome Epidemiology Study (KoGES)[8].

2. Genotyping

Genomic DNA was prepared from blood of all patients, and SNPs (rs1207568 and rs564481) were genotyped by the TaqMan method using ABI Prism 7300 (Applied Biosystems, Foster City, CA, USA).

3. Statistics

The association between COPD susceptibility and SNP genotype was tested using logistic regression, adjusting for age, gender, and smoking pack-years, assuming an additive genetic model. The associations between emphysema severity and SNP genotypes were tested using linear regression, after adjusting for age, gender, and smoking pack-years, assuming an additive genetic model in Korean obstructive lung disease (KOLD) subjects. All statistical analyses were performed using SAS (SAS Institute, Cary, NC).

Results

1. Demographics characteristics of KOLD subjects

We assessed 219 subjects with COPD (mean age, 66.5 years) and 305 control subjects (mean age, 60.5 years)

Table 1. Demographic characteristics of analyzed subjects of the KOLD cohort and the control subjects

	COPD (n=219)	Control (n=305)
Male, %	210 (96)	258 (85)
Age, yr	66.2±7.3	60.5±6.7
Somking, py	47.7±27.1	33.7±20.2
FEV_1, L	1.46±0.5	2.76±0.3
FEV_1, % of predicted	51.9±18.6	113.5±15.5

Data are presented as mean±SD unless otherwise indicated. COPD: chronic obstructive pulmonary disease; KOLD: Korean obstructive lung disease; PY: pack-year; SD: standard deviation.

Table 2. Genetic association between the *klotho* gene and COPD. Covariates in the regression models included age, sex, and pack-years of smoking

Subject number		COPD (n=219)	Control (n=305)	p-value
rs1207568	GG	147	213	
	GA	66	89	
	AA	3	3	0.61
rs564481	CC	145	212	
	CT	65	84	
	TT	9	9	0.20

COPD: chronic obstructive pulmonary disease.

registered in the KOLD study. The mean FEV1 of the COPD subjects was 1.46 L (Table 1).

2. Genetic association with COPD susceptibility in the KOLD study

There were no genetic associations between either SNP and COPD susceptibility (p=0.61 and 0.20) (Table 2).

3. Genetic association with emphysema in the KOLD study

CT measurements were available for 210 COPD subjects. Their mean emphysema index (%) was 21.7± 15.7. SNPs in the *klotho* gene were not associated with emphysema severity (β=−1.03; p=0.63 and β=−1.48; p=0.43).

Discussion

We observed no associations between *klotho* gene polymorphisms and COPD susceptibility in subjects with COPD and controls. In addition, we found no association between *klotho* gene polymorphisms and emphysema severity in COPD subjects.

Accelerated aging of the lungs has recently been suggested as a pathogenic mechanism in COPD. Aging and emphysematous lungs have many similarities, including structural changes, with premature aging enhancing susceptibility to develop emphysema. However, differences in the extent of alveolar destruction have been observed in aging and emphysematous lungs[9,10]. To elucidate the mechanisms of aging and emphysema, senescence-accelerated mice, which possess mutant *klotho* genes and the senescence marker protein-30, have been utilized as an animal model. The *klotho* gene has been related to premature aging, a shorter life span, arteriosclerosis and osteoporosis, and has shown effects on air space enlargement. However, the association between the *klotho* gene and alveolar destruction is unclear[11-13].

The *klotho* gene maps to chromosome 13q12 and several studies have assessed the relationships between *klotho* gene polymorphisms and the aging process. For example, the KL-VS allele of the *klotho* gene, containing a functional variant, has been found in Bohemian Czechs, Caucasians and African-Americans, and has shown an association with age-related phenotypes, including coronary artery disease, and blood pressure[14-16]. In an Italian population, the *klotho* KL-VS allele was not associated with longevity, although another Italian group reported that the *klotho* gene was associated with longevity, but only during a specific time period[17,18].

To date, the KL-VS variant has not been observed in Asian subjects[19]. Rather, the polymorphisms G-395A and C1818T were associated with coronary artery disease[20], stroke in women[21,22], and hypertension[23,24]. Because these SNPs may be related to aging in Asian subjects, we investigated the association between COPD and these polymorphisms. In contrast to findings in cardiovascular diseases, we found no association between these SNPs and COPD. Since aging of the lungs involves a process similar to that of emphysema, we evaluated the relationship between emphysema severity and *klotho* genetic polymorphisms in the KOLD cohort, for which emphysema severity had been assessed using CT. However, we found no association between emphysema severity and *klotho* genetic polymorphisms.

This study had several limitations. First, both COPD cohorts consisted of mostly males. The genetic association of the *klotho* gene with cardiovascular risk in Koreans was observed only in females[21,22], suggesting that the *klotho* gene may interact with gender. Because our cohort included mostly male subjects, any association in female subjects may not have been detected.

Given that there is evidence of gender differences in emphysema[25], the association of the *klotho* gene and emphysema should be studied separately in women.

A second limitation was that the numbers of subjects were relatively small. A third limitation is that we analyzed only two SNPs and we did not genotype the KL-VS variant. However, other studies in Asians have failed to detect the KL-VS variant, and the two genotyped SNPs have been associated with aging phenotypes in other studies. Lastly, our COPD subjects were recruited from multiple centers with different CT scanners that may influence the association. However, adjusting for center effect did not alter the results of association analysis.

In conclusion, the *klotho* gene was not associated with COPD susceptibility or emphysema severity in Korean subjects with COPD.

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