The Impact of Klotho Gene Polymorphisms on Chronic Obstructive Pulmonary Disease (COPD): A Systematic Review

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is linked to persistent inflammation, repetitive strain, and the accelerated aging of the lungs. The Klotho gene is an anti-aging protein that protects cells from inflammation and alveolar damage in COPD patients. At least 10 mutations in the Klotho gene and single nucleotide polymorphisms (SNPs) have been identified in humans. However, the influence of these polymorphisms is not fully understood in COPD patients. This article aims to determine the influence of Klotho gene polymorphisms on COPD patients.

Method: This study employs a systematic review by analyzing secondary data from scientific research articles. Data search using the Google Scholar database. Done using the terms: Polymorphism, COPD, and Alpha Klotho gene.

Results: Four studies were selected for systematic review. Three studies indicate that Klotho gene polymorphisms can cause alveolar destruction, accelerating emphysema occurrence. There is a relationship between Klotho gene polymorphisms and BMI but not with disease severity parameters.

Conclusion: This review indicates that Klotho gene polymorphisms may accelerate emphysema development in COPD patients. There is a relationship between klotho gene polymorphisms and BMI but not with disease severity parameters.

Keywords: Alpha Klotho gene, COPD, polymorphism

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common and preventable disease characterized by chronic and persistent airway obstruction. The cause of airway obstruction in this disease is a combination of inflammation of the small airways and lung parenchymal damage. COPD results from the interaction between harmful environmental exposures and genetic predispositions. Smoking is the main environmental factor that can cause COPD.[1,2]

Exposure to other factors, such as smoke from burning biomass and pollution, can cause COPD. Cigarette smoke caused chronic inflammation and an imbalance in protease-antiprotease activity, causing lung parenchymal damage. Continued lung parenchymal damage will cause remodeling and fibrosis of the lung tissue. However, only about 20% of individuals exposed to these factors develop COPD, suggesting a significant role of genetic susceptibility. This figure shows that environmental factors are not the only factors that play a role.[1,2]

Genetic factors also have a significant influence on the development of this disease. One of them is the occurrence of genetic polymorphism in the development and progression of COPD. Genetic polymorphism refers to the occurrence of two or more alleles at a particular locus in more than 1% of the population.[1,2]

The Klotho gene was discovered in 1997, when studies showed that expression of the protein in mice caused conditions like aging in humans, such as short stature, arteriosclerosis, and osteoporosis. The secreted protein product or transmembrane protein is expressed primarily in the distal renal tubule, choroid plexus, and pituitary gland. However, its effectiveness in humans is unknown. The secreted form inhibits oxidative stress and signaling factors, including insulin/IGF-1, all of which are associated with longevity.[3,4]

Klotho is an important gene located on chromosome 13 and consists of 5 exons. It is a transmembrane glycoprotein with a molecular weight of 135 kDa. Klotho gene expression is thought to predominantly occur in the kidney and brain. It has also been reported in the reproductive and endocrine systems.[5–7]

Klotho is an anti-aging protein that plays an important role in longevity. It plays an important role in preventing various types of cancer, regulating calcium and phosphorus homeostasis, regulating kidney function, and modulating cellular responses. Some preliminary studies have also suggested a link between Klotho and COPD. People with COPD experience decreased Klotho compared to normal people.[5–7]

Several Klotho mutations and single nucleotide polymorphisms (SNPs) have been identified in humans. However, the impact of each SNP is not fully understood. Human studies have investigated the relationship between aging, longevity, and similar traits. The C1818T polymorphism is in exon 4 and is associated with cardiovascular disease, bone density, coronary heart disease, fasting blood sugar, lipid levels, and blood pressure, all of which are prognostic markers. The G395A polymorphism is a mutation in the Klotho region. This polymorphism is associated with blood diseases, high blood pressure, and glucose metabolism.[8,9]

In addition, several studies have also mentioned that the Klotho gene promoter polymorphism G395A is associated with age-related risk factors for various diseases, including muscle wasting, skin wasting, osteoporosis, vascular disorders, calcium deficiency, and emphysema.[10,11] Electrophoretic analysis showed that G-A substitutions in the promoter region affect DNA-protein interactions. Homozygous mutant mice have defects in Klotho expression and show a syndrome like human aging.[12]

There is still limited research on the influence of Klotho gene polymorphism on COPD. This study aims to investigate the influence of Klotho gene polymorphisms on the development and progression of COPD.

METHOD

This study used a systematic review to map existing literature and identify research gaps. The framework used as a reference in preparing systematic reviews uses PRISMA for systematic reviews, which is a method for increasing quality assurance of the completeness of the systematic review structure and process.

The PRISMA framework was chosen to enhance the quality and completeness of the systematic review. This review follows these steps: 1) identification of relevant articles; 2) screening of articles by title and abstract; 3) full-text eligibility assessment; 4) critical appraisal; and 5) combine data, summarize, and present results.

In this systematic review, the article search uses the PICO (Population, Intervention, Comparison and Outcomes) framework. This framework assists in identifying relevant populations, interventions, comparisons, and outcomes related to Klotho gene polymorphisms and COPD. The description for the PICO framework is as follows:

Table 1. Framework PICO

Framework	Information
Patient/Population/ Problem	Chronic Obstructive Pulmonary Disease
Intervention/ Prognostic factor/ Exposure	The Influence of Klotho Gene Polymorphism on Chronic Obstructive Pulmonary Disease
Comparison/Control	-
Outcome	Klotho Gene Polymorphism on Chronic Obstructive Pulmonary Disease

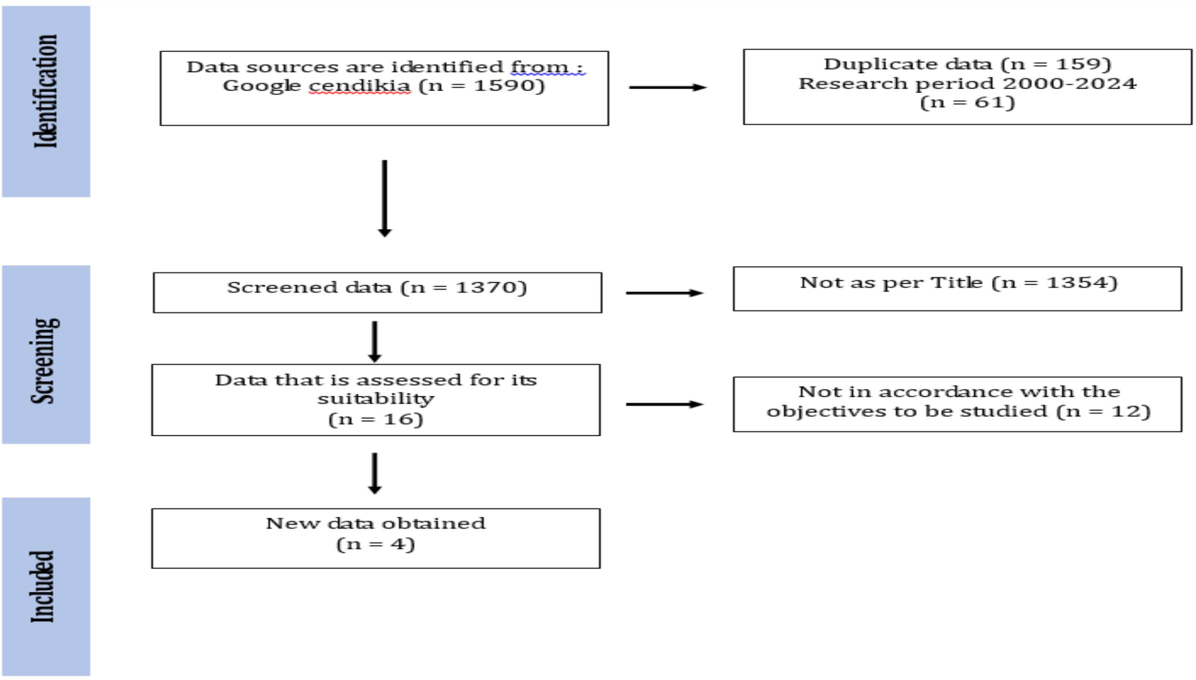

Figure 1. PRISMA for systematic review

The eligibility criteria for articles are specified using inclusion and exclusion criteria. The inclusion criteria used are original articles, published in English, discussing the effect of Klotho gene polymorphism on chronic obstructive pulmonary disease, journals published in 2000-2024, and free full text. The exclusion criteria are review/comment articles, opinion articles, report documents/draft policies/guidelines from WHO/certain formal organizations/thesis reports. Article searches use the Google Scholar database. This review uses the keywords (Polymorphism) AND (COPD) AND (Alpha Klotho Gene).

At the article selection stage, the articles obtained are then screened based on the number of duplicates and the suitability of the article related to the research objectives by paying attention to the title and abstract, analyzing full-text articles according to the inclusion and exclusion criteria.

The search results obtained 4 out of 1590 articles that met the inclusion and exclusion criteria. The search results obtained amounted to 4 studies, then entered a charting data table with the criteria of researchers, research titles, research subjects, methodology, and research results.

Critical appraisal is the process of assessing articles carefully, systematically and relevantly. The author assesses the article using PICO by giving a score of 0=NO, 1=Not Applicable, 2=Unclear, 3=Yes.

The source of data and information for the literature study was obtained from Google Scholar, which collected 1590 literature studies. This literature study consists of articles or journals that have been collected and have been selected based on the title and related abstract information to see whether the article or journal has met the author's inclusion criteria to be used as literature in the literature review, 16 journals were analyzed and 4 selected journals were obtained with the number of literature studies published in 2000, 2010, 2011, 2019, each of which is 1 article or journal.

With the large amount of material that has been collected and reviewed, there is a relationship between the articles or journals obtained, so this literature study can be used as information material and recommendations to determine the polymorphism of the alpha klotho gene in chronic obstructive pulmonary disease (COPD).

RESULT

A total of 1590 articles were identified using the PICO method and specific keywords on Google Scholar. Articles were screened using inclusion and exclusion criteria and assessed for relevance by reviewing titles and abstracts. Four journals were included in this review.

Table 2. Journal Summary

Researcher	Title	Subject	Methodology	Result
Kim WJ, Oh YM, Kim TH, Lee JH, Kim EK, Lee JH, et al[13]	Lack of Association between the Klotho Gene and COPD	• This study included subjects with COPD recruited from "The Korean Obstructive Lung Disease cohort", a prospective longitudinal study of COPD in patients from lung clinics at 11 hospitals in Korea. All COPD subjects in this study had a post-bronchodilator FEV_1/FVC of less than 0.7 and a smoking history of more than 10 packs per year. Complete CT scan data, blood, and other clinical information were obtained from all patients. • In addition, this study also included 305 control subjects consisting of smokers or former smokers with normal lung function who were enrolled in the Korean Genome Epidemiology Study (KoGES)	Data Collection • Data collected included complete CT scans, blood counts, and other clinical information from all patients. • All pulmonary function tests were performed according to the recommendations of the American Thoracic Society/European Respiratory Society. Genotyping • Genomic DNA was isolated from the blood of all patients and SNPs (rs1207568 and rs564481) were genotyped using the TaqMan method with an ABI Prism 7300. Statistical Analysis • The association between COPD susceptibility and SNP genotypes was tested using logistic regression, with adjustment for age, sex, and pack-years of smoking, assuming an additive genetic model. • The association between emphysema severity and SNP genotypes was tested using linear regression after adjustment for age, sex, and pack-years of smoking, assuming an additive genetic model. Data Analysis • All statistical analyses were performed using SAS software (SAS Institute, Cary, NC).	• No association was found between klotho gene polymorphisms and COPD susceptibility in subjects with COPD and controls. • This study has limitations. - First, both COPD groups were predominantly male. The genetic association of the Klotho gene with cardiovascular risk in Koreans was only observed in women, suggesting that the Klotho gene may interact with gender. Given the evidence of gender differences in emphysema, the association of the Klotho gene and emphysema should be studied separately in women. - The second limitation is the relatively small number of subjects. - The third limitation is that we only analyzed two SNPs and we did not genotype the KL-VS variant.

Researcher	Title	Subject	Methodology	Result
Sotiriou I, Kukuvitis A, Chatzikyriakidou A, Tryfon S, Froudarakis ME, Georgiou I, et al[14]	Klotho Gene Polymorphism -395 G>A in Chronic Obstructive Pulmonary Disease (COPD)	Patients included in this study were those who had a confirmed diagnosis of COPD through medical history and pulmonary function tests, had a smoking history of at least 25 packs per year, were of Greek origin, and had no genetic relationship to other patients in the study.	Patient Selection ▪ Patients included in the study had to meet the following criteria: confirmed diagnosis of COPD by medical history and pulmonary function tests, a smoking history of at least 25 pack-years, Greek origin, and no genetic relationship to other patients in the study. ▪ Patients with lung diseases other than COPD, collagen diseases, cancer, metabolic disorders, or non-Greek origin were excluded from the study. Clinical Data Collection ▪ Medical history and tobacco exposure were recorded for each screened patient. ▪ Pulmonary function tests were performed, including measurement of forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and blood gas measurements (PaO_2, $PaCO_2$, pH). ▪ Body mass index (BMI) was calculated by dividing body weight (in kg) by height squared (in m^2). Genetic Analysis ▪ DNA was extracted from peripheral white blood cells using standard salt extraction procedures. ▪ Polymorphism of -395 G>A genotype was screened using the PCR-SSCP (Polymerase Chain Reaction-Single Strand Conformation Polymorphism) method followed by sequence analysis using specific primers. ▪ PCR products were further analyzed by non-denaturing polyacrylamide gel electrophoresis and silver staining to detect SSCP patterns corresponding to each -395 G>A genotype.	• There was a Klotho gene polymorphism (-395 G>A SNP) in COPD patients and it was associated with BMI but not with various parameters of disease severity. • 99 (59.3%) showed the wild-type allele -395 G, 62 (37.1%) were heterozygous (allele –395 G>A), and 6 (3.6%) showed the non-wild-type allele –395 A. • No association of Klotho polymorphism with demographic parameters such as age or gender was observed, although other studies have shown an association of the Klotho gene with advanced age or female gender. • A negative finding of this study is that the COPD patients in this study were all in a small age range and only 5 were female (3%) due to the low incidence of female smokers in Greece

Researcher	Title	Subject	Methodology	Result
			Statistical Analysis ▪ Statistical analysis was performed using StatView 4.5 software. ▪ Mean values were compared between different study groups using unpaired t-tests to reveal significant differences in the distribution of -395 G>A genotypes among COPD patients. ▪ ANOVA test was used to investigate possible associations between clinical data (age, smoking, BMI, FEV_1, FVC, FEV_1/FVC ratio, PO_2, PCO_2, pH) and three -395 G>A genotypes, using mean values and standard deviations. ▪ The chi-square test was used to compare the genotype distributions among the studied parameters.	
Suga T, Kurabayashi M, Sando Y, Ohyama Y, Maeno T, Maeno Y, et al[15]	Disruption of the klotho Gene Causes Pulmonary Emphysema in Mice: Defect in Maintenance of Pulmonary Integrity during Postnatal Life	This study focuses on the analysis of pathological and molecular changes in the lungs of mice with the KL -/- genotype, which is a model for studying emphysema disease. This study includes electron microscopic observation of the lungs, lung function analysis, and Northern blot analysis to assess gene expression related to the pathophysiology of emphysema.	Tissue Preparation and Analysis ▪ Mice were anesthetized with urethane and killed by cutting the abdominal aorta. ▪ Lungs were fixed by intratracheal instillation of 4% paraformaldehyde at a constant pressure of 20 cm H_2O for at least 24 h. ▪ Lung tissues were sliced at 4 μm thickness and stained with hematoxylin and eosin (H&E) for light microscopy. Serial sections were also examined with Kossa staining to detect calcification. Lung Function Analysis ▪ Physiological parameters were measured in mice anesthetized with urethane and breathing spontaneously. Respiratory flow signals were measured via a Lilley-type pneumotachograph connected to an intratracheal tube.	• Emphysema occurs at 4 weeks of age in homozygous klotho mutant mice due to progressive destruction of normal alveolar architecture after normal lung development. Despite impaired respiratory function, the partial pressures of oxygen and carbon dioxide in the arterial blood of homozygous klotho mutant mice are normal, indicating that homozygous klotho mutant mice do not experience respiratory failure at rest. • Ultrastructural analysis of the lungs of KL-/- mice detected calcium deposits on type I collagen fibers in the alveolar septa and degeneration of type II pneumocytes.

Researcher	Title	Subject	Methodology	Result
			▪ Lung volumes were obtained by electrical integration of the flow signals, and intraesophageal pressure was used as the intrathoracic pressure. ▪ Parameters measured included respiratory rate, tidal volume, minute ventilation, expiratory time, dynamic compliance, and total lung resistance. ▪ Northern Blot Analysis ▪ Total RNA was extracted from the lungs of KL -/- mice at 7 to 9 weeks of age, from WT mice at 7 to 9 and 120 weeks of age, and from KL +/- mice at 120 weeks of age. ▪ RNA was separated on a 1% formaldehyde agarose gel, transferred to a nylon membrane, and fixed by ultraviolet exposure. ▪ The membrane was hybridized with a DNA probe labeled with [32P]dCTP using a random primer labeling technique, then washed and exposed to Kodak XR film for autoradiography. Electron Microscopic Observations ▪ Lung specimens were fixed in 3% glutaraldehyde at 4°C and then refixed with 1% osmium tetroxide at room temperature. ▪ Specimens were stained with 1% uranylacetate in 50% ethanol, dehydrated in a graded ethanol series, and finally embedded in agar 100 epoxy resin. ▪ Ultrathin sections (60 nm) were contrasted with lead citrate and examined by electron microscopy.	• Northern blot analysis revealed that the expression of type IV collagen and SP-A was markedly upregulated in homozygous klotho mutant mice at 7 to 9 weeks of age as a compensatory response to the destructive changes in the lung. • Emphysema occurs at 120 weeks of age in heterozygous klotho mutant mice indicating that klotho gene expression is essential for maintaining normal alveolar architecture in adulthood.

Researcher	Title	Subject	Methodology	Result
Sugitani A, Asai K, Watanabe T, Suzumura T, Kojima K, Kubo H, Sato K, et al[16]	A polymorphism rs6726395 in Nrf2 contributes to the development of Emphysema-Associated age in smokers without COPD	• The subjects in this study were 273 healthy individuals who underwent an annual health check-up at MedCity21 Clinic, Osaka City University Hospital, between May and December 2017. All subjects completed a medical interview covering medical history and smoking history (pack-years), physical examination, routine blood studies, pulmonary function tests (PFTs), and high-resolution computed tomography (CT) of the chest. • From the PFT data, 28 subjects were excluded from the study because they had been diagnosed with COPD according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines. Finally, 245 subjects were enrolled for further analysis.	Definition of Smoker Category ▪ In this study, nonsmokers were defined as participants who smoked less than 10 packs per year, and smokers were defined as participants who smoked more than or equal to 10 packs per year. %LAA Measurement ▪ Chest CT was performed using the Whole Body X-ray CT System [Supria (16ch), Hitachi, Ltd., Tokyo, Japan]. The scan time was 0.75 seconds, and the image matrix was 512 × 512 pixels. Thin-slice CT images with a thickness of 1 mm were reconstructed for all lung fields, and all images were analyzed using Airway Inspector software. ▪ Areas with attenuation less than -950 Hounsfield units were defined as low-attenuation areas (LAA), indicating emphysema, and the ratio of LAA to total area was defined as %LAA. SNP Genotyping ▪ Genomic DNA was extracted from blood samples obtained from each participant using the Gentra Puregene Blood Kit Plus (Qiagen NV, Venlo, The Netherlands). Genotyping of SNP (rs6726395) in Nrf2 gene was performed for each participant with 50 ng genomic DNA, and a pre-designed TaqMan allele-specific polymerase chain reaction (PCR) assay using GeneAmp PCR System (Applied Biosystems, Foster City, CA). Data Analysis ▪ Statistical analysis compared %LAA in clinical variables and each lung area. Data are presented as mean±SD or n (%).	• There is a correlation between Nrf2 gene SNPs and the degree of emphysema-related aging in the Japanese population. • Suggests a gene-environment interaction in the % of upper lung LAA. • The average % of LAA in smokers was significantly higher than that in light non-smokers. • The level of soluble α-klotho, which is identified as an aging suppressor gene, was significantly lower in smokers with COPD. • SNP rs6726395 with the A allele has been reported to have lower Nrf2 expression compared to the G allele [23, 24], and the AG/AA genotype may be susceptible to oxidant-induced lung damage, namely emphysema. • Genotype showed that 52.7% (AA/AG) of the study population were susceptible to smoking-induced emphysema. • CT Scan and Spirometry can only assess current lung conditions, but this study may contribute to the development of COPD prevention concepts.

DISCUSSION

Chuang et al demonstrated an association between circulating α-klotho levels and demographic factors such as ethnicity. The study population had a mean age of 56.1 years, with the majority being White (73.1%), followed by Black (9.1%), Hispanic (11.3%), and other ethnic groups (6.5%). Multiple linear regression analysis showed that women and non-Hispanic Black individuals had higher α-klotho levels compared to non-Hispanic White individuals. Individuals with higher BMI and older ages had lower α-klotho levels.[17]

During follow-up, cox regression analysis suggested that individuals with higher α-klotho levels had a lower mortality risk, with significant differences observed after adjusting for ethnicity. Additionally, multinomial logistic regression analysis showed that individuals with low α-klotho levels were more likely to be current or former smokers, Black, and have a lower BMI and reduced eGFR.[17]

Kim et al found no association between Klotho gene polymorphisms and COPD susceptibility or emphysema severity in COPD patients. Structural changes in lung tissue have been found in aging conditions and pulmonary emphysema. Premature aging reduces patient life expectancy, and atherosclerosis, osteoporosis, and alveolar wall expansion and damage have been associated with Klotho genes. However, the relationship and impact between Klotho genes and alveolar damage are still unclear.[18]

The Klotho gene has been mapped to chromosome 13q12, and several studies have investigated the relationship between Klotho gene polymorphisms and the aging process. For example, the KL-VS allele of the Klotho gene, which contains functional variants, has been found in Czechs, Caucasians, and African Americans and is associated with age-related phenotypes such as coronary heart disease.[18] The Klotho KL-VS allele in the Italian population suggests that the Klotho gene is associated with longevity, but only for a limited period.[19] In contrast, the -395 G>A and C1818T polymorphisms are associated with coronary heart disease, stroke in women, and hypertension.[20–22]

In contrast to the findings in cardiovascular disease, no association was found between SNPs and COPD in the Korean population in the study by Kim et al. The study by Kim et al investigated the association between emphysema severity and Klotho gene polymorphisms in the KOLD cohort study, in which emphysema severity was evaluated using CT.[21]

However, this study failed to find an association and impact between emphysema severity and Klotho gene polymorphisms. This study has several limitations. First, both COPD groups were predominantly male. The genetic association of the Klotho gene with cardiovascular risk in Koreans was only observed in women.[21] Given the evidence for gender differences in emphysema.[23] The second limitation is the relatively small number of subjects. A third limitation is that we only analyzed two SNPs and did not genotype the KL-VS variant.[13]

The study by Sotiriou et al provides information on the frequency of klotho genotypes in COPD patients and their possible association with age, lung function, GOLD stage, and BMI. No association was found with age, lung function, or GOLD stage, but an association with BMI was observed. This is the first study to report the identification of the Klotho gene in a large group of COPD patients. The heterozygosity for the -395 G>A allele phenotype was 39.1%, which may indicate emphysematous changes of the Klotho gene in the development of lung lesions.[24]

Possible explanations for the shortcomings of this study are that all COPD patients were in a small age range and only 5 were female (3%) and there is a low incidence of female smokers in Greece. None of the disease severity parameters studied, such as GOLD stage, FEV_1, FEV_1/FVC, or blood gases, showed a significant association with the distribution of the klotho -395 G>A genotype. A possible explanation for the lack of association is that most patients in this study had advanced diseases and had very similar lung function parameters. An animal cohort study by Sato et al showed that when emphysema appeared in Klotho mice, their total lung capacity (TLC) was significantly reduced compared with control mice.[24]

Apart from this study, there has been no report on the assessment of klotho gene polymorphism and lung function parameters in patients with COPD. In this study, no association was found between the distribution of Klotho -395 G>A genotypes and smoking habits. In Imamura's study, smoking was shown to be an independent risk factor for coronary heart disease associated with the -395 A allele.[25]

Among the limitations of this study are the lack of confirmation of emphysema by HRCT in the study patients and the absence of a control group. In this study, the Klotho -395 G>A polymorphism was detected in COPD patients. Except for BMI, no other associations were found with clinical parameters, especially those assessing the severity of COPD, including pulmonary function tests, GOLD staging, patient age and smoking history. Given that the Kotho gene is a metabolic gene, the question arises as to whether the mechanism of induction of emphysema by the Klotho deficiency gene in COPD patients is via a possible metabolic pathway.[14]

Research by Suga et al found that the lungs of mice with Klotho homozygous polymorphism (KL-/-) developed normally until the age of 2 weeks. Emphysematous changes first appeared at the age of 4 weeks in KL-/- mice and progressed with age until they died around 8 to 10 weeks. This is a result of progressive damage to normal alveolar architecture after normal lung development.[26]

In addition, Klotho heterozygous polymorphism KL+/- mice that survived more than 120 weeks also showed pulmonary emphysema due to gene changes in the Klotho gene in lung lesions. These observations indicate that klotho gene expression is essential for maintaining normal alveolar architecture in adulthood.[26]

The respiratory function of KL+/+ mice also matches the respiratory function of patients with pulmonary emphysema. Lung damage in Klotho homozygous polymorphism (KL-/-) mice occurs because the lung ultrastructure of KL-/- mice detects calcium deposits on type I collagen fibers in the alveolar septa and degeneration of type II pneumocytes. The degeneration of type II pneumocytes may impair alveolar cell regeneration in KL-/- mice since type II pneumocytes are known to play an important role in repairing damaged alveoli.[26]

However, northern blot analysis revealed that the expression of type IV collagen and SP-A were markedly upregulated in KL+/+ mice at 7 to 9 weeks of age, when pulmonary emphysema was fully developed. These proteins are thought to exert beneficial effects in preventing emphysematous changes because type IV collagen fibers are an important component of the extracellular matrix and because SP-A has been reported to have a protective function against the development of elastase-induced pulmonary emphysema.[15]

These findings may support the hypothesis of Suga et al that increased expression of the type IV collagen gene is a compensatory genetic response to lung injury. The mitochondrial β-ATPase gene was identified as one of the genes selectively upregulated in the lungs of KL-/- mice by differential screening. Mitochondrial β-ATPase is a subunit of adenosine triphosphate (ATP) synthase, an essential enzyme for ATP synthesis. Northern blot analysis of 13 KL+/+ mice revealed that mitochondrial bATPase expression was increased in most individuals. Mitochondrial β-ATPase plays a critical role in ATP synthesis, and it is likely that decreased expression of this gene ultimately leads to cell death.[15]

The study of Sugitani et al determined that Nrf2 gene polymorphism accelerates the development of aging-related upper pulmonary emphysema in smokers without COPD. Showing a correlation between Nrf2 gene SNPs and the degree of aging-related emphysema in the general Japanese population, and our results suggest a gene-environment interaction in % LAA of the upper lung fields. In several studies of healthy individuals from the general population, Nrf2 SNPs have been shown to correlate with FEV_1.[27]

The hypothesis in the study by Sugitani et al is that Nrf2 SNPs may play a role in the development of aging-related emphysema due to smoking.[28] Cigarette smoke contains a range of harmful substances, including elevated levels of oxidants. Oxidants cause local inflammation, which causes apoptosis in airway epithelial cells and vascular endothelium and causes emphysema. This study found that the average %LAA in smokers was much higher than in nonsmokers.[16]

In this study, the average %LAA in the upper lungs was higher than in the lower lungs, even in smokers without COPD. This study reported that the levels of soluble α-klotho identified as an aging suppressor gene were significantly lower in smokers with COPD. This study focused on the SNP (rs6726395), which has a relatively high minor allele frequency, as the target SNP.[16]

The rs6726395 SNP is in the first intron of the Nrf2 gene. The study found that the %LAA of the upper lung field was significantly correlated with age in smokers with the GG genotype but not in those with the AG/AA genotype. In individuals who are homozygous for the major allele (GG), this reflects the concept that cigarette smoke accelerates the development of aging-related upper lung emphysema, in contrast to the AG/AA genotype with the minor A allele. The A allele of SNP rs6726395 has been shown to result in lower Nrf2 expression compared to the G allele, and the AG/AA genotype may be more prone to oxidant-induced lung damage, such as emphysema.[16]

In this study, genotyping revealed that 52.7% (AA/AG) of the population was susceptible to smoking-induced emphysema. Pulmonary function testing (PFT) is recommended for diagnosing COPD and for early screening of potential or unidentified COPD cases during health check-ups. PFT is a relatively simple and non-invasive method compared to other tests as it does not involve radiation.[16]

In contrast, Nrf2 gene genotyping is less invasive than PFT and CT, requiring only a genomic DNA sample for screening. In addition, genotyping can assess the risk of future emphysema/COPD, whereas PFT and chest CT can only assess the current lungs. Because genotyping can be used to determine susceptibility, genotyping can be a tool that can be used in early prevention efforts.[16]

The results of this study can contribute to the development of this prevention concept. This study population had a limited sample size. Due to this limited sample size, we only identified a small number of smokers with the AA genotype, so this group was combined with the AG genotype group. For this reason, we have not evaluated other SNPs in the Nrf2 gene.[16]

However, in this study, we demonstrated a correlation between SNP (rs6726395) and the degree of aging-related upper pulmonary emphysema in Japanese smokers. This may contribute to personalized treatment or prevention of COPD progression, and Nrf2 may be an intervention target for COPD prevention and treatment.[16]

CONCLUSION

This review suggests that Klotho gene polymorphisms may accelerate emphysema development in COPD patients. Klotho gene polymorphisms were associated with BMI but not with disease severity parameters.

REFERENCES

1. Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease 2017 Report: GOLD Executive Summary. Am J Respir Crit Care Med. 2017;195(5):557–82.
2. García-Sanz MT, Pol-Balado C, Abellás C, Cánive-Gómez JC, Antón-Sanmartin D, González-Barcala FJ. Factors associated with hospital admission in patients reaching the emergency department with COPD exacerbation. Multidiscip Respir Med. 2012;7(1):6.
3. Xu X, Liang X, Hu G, Zhang J, Lei H. Renal Function and Klotho Gene Polymorphisms among Uygur and Kazak Populations in Xinjiang, China. Medical Science Monitor. 2015;21:44–51.
4. Di Bona D, Accardi G, Virruso C, Candore G, Caruso C. Association of Klotho Polymorphisms with Healthy Aging: A Systematic Review and Meta-Analysis. Rejuvenation Res. 2014;17(2):212–6.
5. Zhu Z, Xia W, Cui Y, Zeng F, Li Y, Yang Z, et al. Klotho gene polymorphisms are associated with healthy aging and longevity: Evidence from a meta-analysis. Mech Ageing Dev. 2019;178:33–40.
6. Olejnik A, Franczak A, Krzywonos-Zawadzka A, Kałużna-Oleksy M, Bil-Lula I. The Biological Role of Klotho Protein in the Development of Cardiovascular Diseases. Biomed Res Int. 2018;2018:5171945.
7. Elghoroury EA, Fadel FI, Elshamaa MF, Kandil D, Salah DM, El-Sonbaty MM, et al. Klotho G-395A gene polymorphism: impact on progression of end-stage renal disease and development of cardiovascular complications in children on dialysis. Pediatric Nephrology. 2018;33(6):1019–27.
8. Akbari H, Asadikaram G, Aria H, Fooladi S, Vakili S, Masoumi M. Association of Klotho gene polymorphism with hypertension and coronary artery disease in an Iranian population. BMC Cardiovasc Disord. 2018;18(1):237.
9. Mengel-From J, Soerensen M, Nygaard M, McGue M, Christensen K, Christiansen L. Genetic Variants in *KLOTHO* Associate With Cognitive Function in the Oldest Old Group. J Gerontol A Biol Sci Med Sci. 2016;71(9):1151–9.
10. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature. 1997;390:45–51.
11. Hayashi Y, Okino N, Kakuta Y, Shikanai T, Tani M, Narimatsu H, et al. Klotho-related Protein Is a Novel Cytosolic Neutral β-Glycosylceramidase. Journal of Biological Chemistry. 2007;282(42):30889–900.

12. Kawano KI, Ogata N, Chiano M, Molloy H, Kleyn P, Spector TD, et al. Klotho Gene Polymorphisms Associated With Bone Density of Aged Postmenopausal Women. Journal of Bone and Mineral Research. 2002;17(10):1744–51.
13. Kim WJ, Oh YM, Kim TH, Lee JH, Kim EK, Lee JH, et al. Lack of Association between the Klotho Gene and COPD. Tuberc Respir Dis (Seoul). 2011;71(4):254–8.
14. Sotiriou I, Kukuvitis A, Chatzikyriakidou A, Tryfon S, Froudarakis ME, Georgiou I, et al. Klotho gene polymorphism -395 G<A in patients with chronic obstructive pulmonary disease (COPD). Pneumon. 2010;4(23):348–54.
15. Suga T, Kurabayashi M, Sando Y, Ohyama Y, Maeno T, Maeno Y, et al. Disruption of the klotho Gene Causes Pulmonary Emphysema in Mice. Am J Respir Cell Mol Biol. 2000;22(1):26–33.
16. Sugitani A, Asai K, Watanabe T, Suzumura T, Kojima K, Kubo H, et al. A Polymorphism rs6726395 in Nrf2 Contributes to the Development of Emphysema-Associated Age in Smokers Without COPD. Lung. 2019;197(5):559–64.
17. Chuang MH, Wang HW, Huang YT, Jiang MY. Association between soluble α-klotho and mortality risk in middle-aged and older adults. Front Endocrinol (Lausanne). 2023;14:1246590.
18. Arking DE, Atzmon G, Arking A, Barzilai N, Dietz HC. Association Between a Functional Variant of the KLOTHO Gene and High-Density Lipoprotein Cholesterol, Blood Pressure, Stroke, and Longevity. Circ Res. 2005;96(4):412–8.
19. Invidia L, Salvioli S, Altilia S, Pierini M, Panourgia MP, Monti D, et al. The frequency of Klotho KL-VS polymorphism in a large Italian population, from young subjects to centenarians, suggests the presence of specific time windows for its effect. Biogerontology. 2010;11(1):67–73.
20. Jo SH, Kim SG, Choi YJ, Joo NR, Cho GY, Choi SR, et al. KLOTHO Gene Polymorphism Is Associated With Coronary Artery Stenosis but Not With Coronary Calcification in a Korean Population. Int Heart J. 2009;50(1):23–32.
21. Kim Y, Kim JH, Nam YJ, Kong M, Kim YJ, Yu KH, et al. Klotho is a genetic risk factor for ischemic stroke caused by cardioembolism in Korean females. Neurosci Lett. 2006;407(3):189–94.
22. Rhee EJ, Oh KW, Yun EJ, Jung CH, Lee WY, Kim SW, et al. 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(7):613–8.
23. Martinez FJ, Curtis JL, Sciurba F, Mumford J, Giardino ND, Weinmann G, et al. Sex Differences in Severe

Pulmonary Emphysema. Am J Respir Crit Care Med. 2007;176(3):243–52.

24. Sato A, Hirai T, Imura A, Kita N, Iwano A, Muro S, et al. Morphological mechanism of the development of pulmonary emphysema in klotho mice. Proceedings of the National Academy of Sciences. 2007;104(7):2361–5.
25. Imamura A, Okumura K, Ogawa Y, Murakami R, Torigoe M, Numaguchi Y, et al. Klotho gene polymorphism may be a genetic risk factor for atherosclerotic coronary artery disease but not for vasospastic angina in Japanese. Clinica Chimica Acta. 2006;371(1–2):66–70.
26. Lwebuga-Mukasa JS. Matrix-driven Pneumocyte Differentiation. American Review of Respiratory Disease. 1991;144(2):452–7.
27. Masuko H, Sakamoto T, Kaneko Y, Iijima H, Naito T, Noguchi E, et al. An interaction between Nrf2 polymorphisms and smoking status affects annual decline in FEV1: a longitudinal retrospective cohort study. BMC Med Genet. 2011;12:97.
28. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2013;187(4):347–65.