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ORIGINAL ARTICLE

Investigation of the Relationship Between Apelin Genetic Variants and COPD Formation

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Abstract

Introduction: Chronic obstructive pulmonary disease (COPD) is a common, preventable, and treatable disease characterized by chronic inflammatory response in the airways and lungs, usually characterized by progressive, persistent airflow limitation. Apelin is a newly discovered peptide with inotropic and vasodilatory properties and is an endogenous ligand of the APJ receptor. The aim of this study is to examine the possible association of variations in the apelin gene (APLN) with COPD in the Turkish population.

Methods: This study was a case-control study, and a total of 341 people aged 40-80 years were included in the study (224 COPD and 117 healthy controls). Genotyping of 2 single nucleotide polymorphisms (SNPs) (rs3115758 and rs3115759) in the APLN was performed by real-time polymerase chain reaction (PCR) method. Genotype and allele frequencies between COPD patients and healthy individuals were compared with Student's t test.

Results: The TT and AA risk genotypes of the rs3115758 and rs3115759 variants in the APLN were found to be associated with a significantly increased risk of COPD at the same rate. Heterozygous and homozygous mutant genotypes (GT, TT, GA, and AA, respectively) of rs3115758 and rs3115759 variations of the APLN were detected at a significantly higher rate, in the same proportion of patients. In the multiple regression analysis, it was determined that the TT genotype of rs3115758 increased the development of COPD by 0.59 times, independent of other COPD risk factors.

Discussion and Conclusion: As the first study in the literature examining the relationship between polymorphisms in the APLN and COPD in the Turkish population, various variations in the APLN were found to be associated with COPD in the Turkish population.

Keywords: Apelin; COPD; gene polymorphism.

Chronic obstructive pulmonary disease (COPD) is a common, important public health problem, but it is a preventable and treatable disease, usually characterized by progressive, persistent airflow limitation, associated with chronic inflammatory response in the airways and lungs as a result of exposure to toxic particles or gases. COPD ranks 4th among the diseases that cause death in the world and its incidence is increasing rapidly. It is a major cause of morbidity and mortality worldwide. This is why many people die at early stage[1]. Adipose tissue is a secretory organ that produces active substances called adipokines. Some of these can be listed as apelin, resistin, adiponectin, leptin,

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chemerin and visfatin. All of these active substances may play an important role in the pathogenesis and prognosis of respiratory diseases such as COPD, asthma, pulmonary hypertension or lung cancer. There are very limited and conflicting data in the literature on the role of adipokines in the development of asthma and COPD. However, visfatin and leptin have been confirmed to be possible markers of inflammation in COPD. Various studies have reported that high leptin and resistin concentrations play a proinflammatory role in cancer development. In addition, in a study published in 2019, it was stated that these active substances have a role in pulmonary hypertension, and that especially apelin-adiponectin imbalance may exacerbate pulmonary hypertension[2]. APJ is a typical G protein-coupled receptor with 380 amino acids, 7 transmembrane domains, with close sequence homology to the angiotensin II receptor type 1 (Fig. 1)[3]. Apelin, an endogenous ligand for the APJ receptor, is a bioactive peptide expressed in a wide variety of tissues. In humans, the apelin gene (APLN) is located on chromosome Xq25-26.1, which encodes a 77

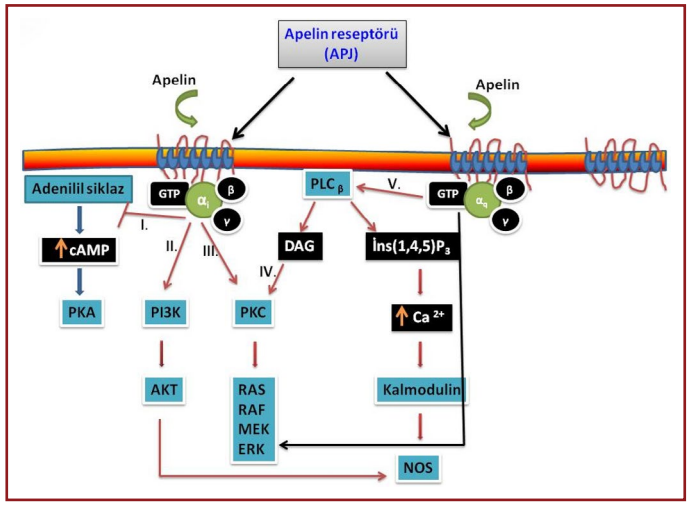

Figure 1. Apelin receptor (APJ)-mediated signaling pathways. Apelin-activated APJ;

I. Matches with Gi and Gq. Gi can inhibit adenylyl cyclase, reduce cAMP production and thereby suppress Protein Kinase A (PKA) activation.

II. Activates phosphoinositol 3-kinase (PI3K), leading to Akt activation.

III. Directly activates Protein Kinase C (PKC), resulting in activation of the RAS-RAF-MEK-ERK pathway regulated by mitogenic extracellular signals.

IV. Gq activates Phospholipase C (PLC) to induce the production of Diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate.

V. Gq can inhibit Adenylyl cyclase activity, by activating Phospholipase C (PLC), it increases Diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate (Ins(1,4,5)P3) production and intracellular Ca2+ and Protein Kinase C.

amino acid prepropeptide that is divided into isoforms of different lengths[4,5]. Apelin plays a role in regulating the homeostasis of endothelium and smooth muscle cells. It also modulates endothelial nitric oxide synthase (eNOS) expression, induces eNOS-dependent vasodilation, counteracts angiotensin-II-mediated vasoconstriction, and has positive inotropic and cardioprotective effects. Apelin is highly expressed in pulmonary vessels, but its functions are not entirely clear. The effects of adipocytokines on the pathogenesis of pulmonary arterial hypertension are still not clearly explained[2]. In studies conducted to date, a wide range of common genetic variants of APLN have been studied in diseases, such as hypertension, obesity, coronary artery disease, acute obstructive coronary syndromes, diabetes, metabolic syndrome, diabetic retinopathy, idiopathic dilated cardiomyopathy, preeclampsia, polycystic ovarian syndrome[6-9]. However, there is only one study in the literature investigating the relationship between COPD and APLN.

Materials and Methods

Study Groups

This is an observational case-control study involving 341 people aged 40-80 years (224 COPD and 117 healthy controls). Written informed consent was obtained from all the volunteers involved in the project, and the study was carried out in accordance with the Declaration of Helsinki. The study was started after the ethics committee approval was obtained by the T.R. Ministry of Health Haydarpaşa Numune Training and Research Hospital Clinical Research Ethics Committee with the number HNEAH-KAEK 2021/160-3311. The gene regions on the APLN to be studied were determined by scanning the Ensembl genome database and literature, and considering the results of population genetics.

DNA Isolation and Genotyping

In the study, genotyping of 2 SNPs (rs3115758 and rs3115759) in the APLN was performed by real-time PCR method. Five milliliters of peripheral venous blood samples were collected from volunteers into EDTA tubes for biochemical analysis, DNA isolation and real-time PCR procedure. Samples were stored at -20°C until analysis. After DNA extraction was performed in accordance with the kit protocol (Roche kit; Roche, Manheim), concentration measurements were made with NanoDrop spectrophotometer. Genotyping was done using PCR and reverse hybridization methods. SNP genotyping was performed using the LightCycler 480® system, using hybridization

probes containing a 3'- 5'-LightCycler® red fluorescent-labeled double oligonucleotide probe (TIB MOLBIOL GmbH, Berlin, Germany). Primers for the rs3115758 polymorphism were identified as ggAggACATATTTTATATgTAACAAT and gAgAATgTTgAgCATACACTCTA, and probes as AATCATgCTTAgCCgAAgggA-FL and 640-CCgAACAggAgTAAAAAAATggTCCATggTCCAT. Primers for rs3115759 were identified as AgATgTTTAAATgTCgAATTATTg and AATgTgACTgCTTCTgCAT, probes as ggCTgCTTTTCAACTgTTgA-FL and 640-CATATggTTAgTATg and AggAATgACAgTAgggTp. PCR conditions were optimized as a total PCR volume of 20 µL in a volume containing 2.0 µL HybProbe FastStart DNA Master (Roche Diagnostics, Mannheim, Germany), 1.0 µL Reagent Mix, 3.0 mM MgCl2 and 50 ng genomic DNA.

Statistical Analysis

Continuous data were expressed as mean ± standard deviation in this case-control study. The normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test. Fisher' exact test was used to compare categorical variables and also to test the departure of genotype frequencies from Hardy-Weinberg equilibrium (HWE). Statistical analysis was performed using Student's t-test to compare variables between patient and control groups. All statistical analyzes were performed using SPSS V.20.0 (SPSS for Windows, version 20.0. Chicago, USA). P value <0.05 was considered statistically significant.

Results

Both rs3115758 and rs3115759 variants in the APLN showed a statistically significant difference between patient and control groups. The rs3115758 and rs3115759 genotype frequencies were consistent with Hardy-Weinberg equilibrium between patients and controls (p> 0.05). Among the variations in the APLN, GG genotype and G allele showed a significant difference for rs3115758 (p value: 0.0353 and p value: 0.0008, respectively) (Table 1). It was determined that GG genotype and G allele showed a significant difference for rs3115758 (p value: 0.0419 and p value: 0.0012, respectively) (Table 2). In the multiple regression analysis, it was determined that the TT genotype of rs3115758 increased the development of COPD by 0.59 times, independent of other COPD risk factors. The mean ages of COPD patients and healthy controls were 58.45±7.43 and 56.32±8.51, respectively. No statistically significant difference was found between patients and controls in terms of age and gender (p>0.05).

Table 1. Genotype and allele frequencies in COPD patients and control groups for the APLN1 gene rs3115758 gene variant

APLN1 rs3115758 Genotype	Control, n (%)	COPD, n (%)	p
GG	116 (99.1)	211 (94.1)	*0.0353
GT	1 (0.9)	1 (0.44)	
TT	0 (0)	12 (5.46)	
ALLEL			
G	233 (99.58)	423 (94.42)	*0.0008
T	1 (0.42)	23 (5.58)	

APLN: Apelin gene; COPD: Chronic obstructive pulmonary.

Table 2. Genotype and allele frequencies in COPD patients and control groups for the APLN1 gene rs3115759 gene variant

APLN1 rs3115759 Genotype	Control, n (%)	COPD, n (%)	p
GG	109 (99.1)	211 (94.19)	*0.0419
GA	1 (0.91)	1 (0.45)	
AA	0 (0)	12 (5.36)	
ALLEL			
G	219 (99.55)	423 (94.42)	*0.0012
A	1 (4.45)	1 (5.58)	

APLN: Apelin gene; COPD: Chronic obstructive pulmonary.

Discussion

It is known that apelin has the physiological function of increasing heart contraction as well as lowering blood pressure. In addition, it has been shown in various publications that APLN expression has an anti-obesity and anti-diabetic effect, and an increase in apelin is observed in cases of inflammation or oxidative stress[10-13]. In recent studies, the importance of the apelin/APJ system in the respiratory system has been emphasized. In addition to enrichment of apelin/APJ in lung tissue, strong evidence also confirms that apelin/APJ signaling is impaired in the pulmonary vasculature of nitrophen-induced congenital diaphragmatic hernia[3,14]. In addition, it has been shown that apelin serum level is significantly increased in lung tissue and plasma in patients with acute respiratory distress syndrome (ARDS), while it is decreased in patients with pulmonary arterial hypertension (PAH)[15,16]. In hemodialysis patients with PAH, serum apelin levels are dramatically lower than in patients with normal arterial pressure and are not affected by hemodialysis[17]. All these data reveal that the apelin/APJ system is responsible for the development of respiratory diseases. Patients with COPD have signs of increased oxidative stress. It has been determined that reactive oxygen metabolites found

in cigarette smoke and released from inflammatory cells contribute to the pathophysiology of COPD. Various markers of oxidative stress are found in increased amounts in the lungs, exhaled breath condensate and urine of smokers and patients with COPD. These markers are nitric oxide and lipid peroxidation products such as hydrogen peroxide, 8-OHdG, isoprostane F2a-III. Oxidative stress oxidizes many biological molecules, causing cell dysfunction and death. It may also contribute to the pathogenesis of COPD by disrupting the structure of the extracellular matrix, inactivating the antioxidant defense mechanism, and activating proteinases or facilitating histone acetylation. In addition, it is known that patients with COPD have a significant decrease in apelin levels. This suggests that apelin can also be used as a predictive biomarker of COPD formation. Mutations in the APLN rs3115758 and rs3115759 gene regions lead to a decrease in serum apelin, which may be one of the causes of exacerbation of COPD. From this point of view, in this study, we investigated the relationship of variations in the APLN with COPD in the Turkish population and investigated whether the expression levels of inflammation and oxidative stress were affected. We concluded that 2 different variations are associated with COPD in the Turkish population due to the possible pleiotropic effects of mutations in different regions on the APLN that may lead to inflammation and COPD development. We think that the results we obtained, in addition to more comprehensive studies, in which the number of patients are increased and other clinical parameters affecting the pathogenesis of COPD were added, can be used effectively in the diagnosis and treatment of COPD in the future.

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