Journal of the American Society of Hypertension ■(■) (2017) 1–7

Research Article

Interleukin-6 -572C/G polymorphism is associated with serum interleukin-6 levels and risk of idiopathic pulmonary arterial hypertension

Ming Fang, MD[a,b,1], Yueye Huang, MD, PhD[c,1], Yuan Zhang, MD, PhD[d], Zhongping Ning, MCM[b], Luoning Zhu, MCM[b], and Xinming Li, MD[b,*]

[a]Department of Cardiology, Hainan General Hospital, Haikou, Hainan, China;
[b]Department of Cardiology, Shanghai Zhoupu Hospital, Shanghai, China;
[c]Department of Endocrinology and Metabolism, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China; and
[d]Department of Respiratory, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

Manuscript received November 17, 2016 and accepted January 24, 2017

Abstract

Interleukin 6 (IL-6) is a multifunctional proinflammatory cytokine that is elevated in patients with pulmonary arterial hypertension (PAH). Single nucleotide polymorphisms in the promoter region of IL-6 have been reported to transcriptional regulate the expression of IL-6. The aim of the present study is to investigate the roles of two common polymorphisms (-572C/G [rs1800796] and -6331T/C [rs10499563]) of IL-6 in idiopathic PAH (IPAH). A total of 338 IPAH patients and 352 age- and gender-matched healthy controls were enrolled. Genotyping of the two polymorphisms was performed by polymerase chain reaction and direct sequencing. Serum IL-6 levels were determined by ELISA assay. The frequencies of -572C/G genotypes CC, CG, and GG were found to be 63.6%, 32.3%, and 4.1% in IPAH patients group and 51.7%, 39.5%, and 8.8% in the controls, respectively. Compared with the individuals carrying the common genotype CC, the individuals carrying the GG genotype had a decreased risk of IPAH (adjusted odds ratio, 0.40; 95% confidence interval, 0.20–0.77; $P = .006$). The CG genotype and G allele carriers (CG/GG genotypes) were also observed to be associated with decreased risks of IPAH. Moreover, we found that individuals harboring -572GG or GC genotype showed significantly lower IL-6 levels than those harboring the -572CC genotype. No association between -6331T/C polymorphism and risk of IPAH or IL-6 levels was found. These results suggest that IL-6 promoter polymorphism -572C/G, but not -6331T/C, is associated with serum IL-6 levels and risk of IPAH. J Am Soc Hypertens 2017;■(■):1–7.

Keywords: Cytokine; ELISA; genetic alteration; inflammation.

Introduction

Pulmonary arterial hypertension is a progressive and lethal disease, occurs at the small pulmonary arteries, which is characterized by increased pulmonary vascular resistance due to vascular proliferation and remodeling.[1] Although the exact pathogenesis of PAH remains unknown, three factors, including vasoconstriction, remodeling of the pulmonary vessel wall, and thrombosis in situ, are thought to cause the increased pulmonary vascular resistance.[2] With the advances in molecular genetics and cell biology, a number of molecular mechanism have been reported to be involved in the pathogenesis of the disease, including pathways involving metabolic signaling, growth factors, and cytokines.[3–5]

Interleukin 6 (IL-6) is a pleiotropic proinflammatory cytokine with a wide range of functions in immune response, inflammation, hematopoiesis, cell survival, proliferation, and apoptosis.[6] Accumulating studies show that IL-6 involves in the pathogenesis of PAH. First, IL-6 serum levels are consistently elevated in the serum and lungs of

Conflict of interest: The authors declare no conflict of interest.

*Corresponding author: Xinming Li, MD, Department of Cardiology, Shanghai Zhoupu Hospital, 1500 Zhouyuan Road, Pudong New Area, Shanghai 201318, P.R. China. Tel: +86-021-68135590; Fax: +86-021-68130389.
E-mail: xinmingli6@sina.com

[1] Ming Fang and Yueye Huang contributed equally to this work.

1933-1711/$ - see front matter
http://dx.doi.org/10.1016/j.jash.2017.01.011

patients with PAH,[7] and the increased levels of IL-6 associated with poor prognosis of patients with idiopathic and familial PAH.[8] Second, evidence from lung-specific IL-6 overexpressing transgenic mice showed that IL-6 overexpression replicates the pathologic lesions observed in advanced PAH, including both distal arteriolar muscularization and plexogenic arteriopathy, and leads to increased pulmonary vascular resistance and PAH.[9] Third, IL-6 is sufficient to cause pulmonary hypertension in mice, and its effects are augmented by hypoxia.[10] Fourth, in the mice with hypoxia-induced pulmonary hypertension, IL-6/IL-21 signaling axis played a critical role in the development of the disease in association with M2 macrophage polarization.[11]

It is well accepted that common genetic variants especially the polymorphisms located in the regulatory region of candidate genes that may regulate the gene's expression and correlated with risk of diseases.[12] Three single nucleotide polymorphisms, including -174G>C (rs1800795), -572C>G ([rs1800796], also named as -634C>G in somewhere), and -6331T>C (rs10499563), have been identified in the promoter region of IL-6.[13–15] All the three polymorphisms modified the transcription activity of IL-6 and associated with IL-6 expression, and they contributed to the pathogenesis of multiple inflammation-related disease, such as cancer and hypertension.[13–17] In the present study, we tested the hypothesis that IL-6 polymorphisms are associated with the risk of idiopathic PAH (IPAH).

Materials and Methods

Study Subjects

A total of consecutive 338 IPAH patients and 352 normal subjects were enrolled in the present study. The diagnosis of IPAH was according to WHO criteria. Patients with lung disease and chronic thromboembolic pulmonary hypertension were excluded. IPAH patients were collected from Hainan General Hospital (n = 92), Shanghai Pulmonary Hospital (n = 181), and Shanghai Zhoupu Hospital (n = 65). Samples from 352 healthy volunteers served as normal controls. The patients and control subjects were geographically matched with very closely age range and gender proportion. All the subjects were ethnic Han Chinese population and were genetically unrelated. Informed patient consent was obtained from all subjects before entering the study, and the study was approved by the Institutional Ethics Committees at the Hainan General Hospital and Shanghai Zhoupu Hospital.

Genotyping

Genomic DNA from blood samples was isolated using the standard salting-out method. The rs10499563 (-6331T>C) polymorphism region in IL-6 promoter was amplified by polymerase chain reaction (PCR) as previously described.[18] The PCR was performed in a total volume of 25 μL containing 20–50 ng genomic DNA, 1 unit Taq polymerase (Takara, Japan), 1 X Taq polymerase buffer (Mg^{2+} plus), 0.2 mM of each dNTP, and 0.4 μM each of primers. The forward primer was 5′-GCCTGGTCTGGCCTGTATAA-3′, and the reverse was 5′-CCCAAGGACCTGTTAGTGGA-3'. The cycling conditions for PCR consisted of an initial denaturation at 95°C for 5 minutest and then amplified by 30 cycles of 94°C for 45 seconds, 60°C for 40 seconds, and 72°C for 45 seconds, followed by a terminal extension phase at 72°C for 5 minutes. The rs1800796 (-572C>G) polymorphism was amplified using the same PCR conditions as did for -6331T>C except the primers and the annealing temperature. The primers for the -572C>G polymorphism were 5′-TGGCAAAAAGGAGTCACACA-3' (forward) and 5′-CCCAAGCCTGGATTATGAAG-3' (reverse); and the annealing temperature was 62°C. The PCR products were then subject to direct sequencing on an Applied Biosystems 3700 DNA Analyzer at Sangon Biotech (Shanghai, China).

ELISA Assay for IL-6

Blood samples from 86 IPAH patients and 86 controls were randomly selected and used for assessing the serum levels of IL-6. The collected blood samples were first centrifuged at 1000*g* for 15 minutes at 4°C, and then serum samples were aliquot and stored at −80°C until to be used. Serum levels of IL-6 were determined by the Human IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's protocol.

Statistical Analysis

For continuous variables, the data were presented as means ± standard deviation, and the comparisons were performed by independent-samples *t* test. For categorical variables, the data were presented as numbers (percentage), and the comparisons were performed by chi-square test. Hardy-Weinberg equilibrium (HWE) analysis for genotype distribution in control group was carried out by Pearson's goodness-of-fit chi-square test. To assess the effects of genotypes and alleles on risk of IPAH, the logistic regression was done to calculate odds ratio (OR) and 95% confidence interval (CI), which were adjusted for age, gender, and smoking status. All the statistical analyses were performed by Stata for Windows (version 10.1; Stata Corp, USA), and the graphs and figures were done by GraphPad Prism software (version 6.0; GraphPad Software, Inc, USA). *P* value less than .05 was regarded as statistically significant.