WILDERNESS & ENVIRONMENTAL MEDICINE, **24,** 315–320 (2013)

ORIGINAL RESEARCH

Endothelial PAS Domain Protein 1 Chr2:46441523(hg18) Polymorphism Is Associated With Susceptibility to High Altitude Pulmonary Edema in Han Chinese

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Objective.—The purpose of this study was to test the hypothesis that polymorphisms in the endothelial PAS domain protein 1 (EPAS1) gene are associated with the susceptibility to high altitude pulmonary edema (HAPE) in Han Chinese.

Methods.—This study enrolled 153 HAPE patients (HAPE-p), matched with Han Chinese resistant to HAPE (HAPE-r) and local highland Tibetans from Yushu earthquake construction population in Qinghai where the altitude is more than 3500 m above sea level. The polymorphism of EPAS1 chr2:46441523(hg18) was genotyped by polymerase chain reaction restriction fragment length polymorphism and confirmed by DNA sequencing.

Results.—The frequencies of EPAS1 chr2:46441523(hg18) polymorphism C allele were significantly higher in the HAPE-p group than in the HAPE-r group ($P < .001$), but the frequencies of heterozygous C/G were significantly higher in the HAPE-r group than in the HAPE-p group ($P < .001$). Moreover, the frequencies of the EPAS1 chr2:46441523(hg18) polymorphism G allele were significantly higher in the highland Tibetan group than in the HAPE-p and HAPE-r groups.

Conclusions.—The EPAS1 chr2:46441523(hg18) polymorphism C is strongly associated with susceptibility to HAPE in Han Chinese, and the EPAS1 chr2:46441523(hg18) polymorphism G is present at high frequency and may be associated with high altitude adaptation in the Tibetans.

Key words: EPAS1, high altitude pulmonary edema, susceptibility, single nucleotide polymorphism

Introduction

High-altitude pulmonary edema (HAPE) is noncardiogenic pulmonary edema that usually occurs at altitudes above 3,000 m in rapidly ascending, nonacclimatized persons within the first 2 to 5 days after arrival.[1,2] HAPE is characterized by high pressure in pulmonary arteries, with edema in pulmonary interstitial tissue and alveoli, leading to pulmonary capillary stress failure and a high permeability type of edema.[3] Although hypoxia is a major trigger factor, the pathogenesis of HAPE remains unclear because some persons are more susceptible to HAPE than others when exposed to the same hypoxia conditions. Growing evidence suggests that genetics plays an important role in the risk of HAPE.[4,5] Recently, several candidates have been proposed as HAPE susceptibility genes.[6–8] However, the exact genetic mechanism responsible for the development of HAPE is largely unknown.

The endothelial PAS domain protein 1 (EPAS1) gene is one of the most notable candidate genes that have been identified as contributing to evolutionary adaptation to high altitude in Tibetans, probably by some unknown molecular pathways that delicately control erythropoietic response to hypoxia.[9–11] The EPAS1 gene is located on the short (p) arm of chromosome 2 between positions 21 and 16, from base pair 46,524,540 to base pair 46,613,841 on chromosome 2. Human EPAS1 encodes the oxygen-sensitive alpha subunit of hypoxia-inducible factor-2 (HIF-2) transcription factor, which is a key regulator of chronic hypoxia by regulating a large number of genes involved in the cellular and systemic responses to hypoxia including erythropoiesis, angiogenesis, vascular regulation, and anaerobic metabolism.[10] The expression of HIF-2 is induced by hypoxia, and it demonstrates a selective pattern of expression in

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microvascular endothelial cells, lung epithelial cells, cardiac myocytes, and the brain; and an EPAS1 gene knockout mouse model revealed a significant reduction in hematocrit levels and peripheral blood counts.[10] Recent genetic studies have provided evidence for natural selection on EPAS1 gene in the Tibetan population during their adaptation to living on the Tibetan Plateau.[11–14]

Notably, the EPAS1 gene has a single nucleotide polymorphism (SNP), chr2:46441523 (hg18), 5 base pairs from the beginning of exon 6, and this SNP has a 78% frequency difference between highland Tibetan and lowland Han Chinese.[13] Furthermore, our preliminary experiments on the sequencing of EPAS1 gene in HAPE patients and healthy controls showed that there were no changes in the sequences of all exons, but the frequency of SNP chr2:46441523 (hg18) was different between HAPE patients and healthy controls. Therefore, we hypothesized that SNP chr2:46441523 (hg18) of EPAS1 is associated with the susceptibility to HAPE in Han Chinese. To test our hypothesis, in this study we detected the EPAS1 chr2:46441523 (hg18) polymorphism in HAPE patients, matched Han Chinese and local Tibetans from Yushu earthquake construction population in Qinghai where the altitude is more than 3,500 m above sea level.

Methods

SUBJECTS

A total of 153 HAPE patients (HAPE-p) had been hospitalized in Yushu People's Hospital between March 2010 and June 2012 owing to the onset of HAPE after arriving 1 to 2 days at Yushu (3760 m). The diagnosis of HAPE was based on chest radiographs and standard diagnostic criteria.[15] A total of 298 healthy controls resistant to HAPE (HAPE-r) were randomly selected from among the coworkers of HAPE-p, matching the patients in age, sex, ethnicity, and working conditions. These subjects remained healthy after working at Yushu for at least 3 months, without having HAPE or high altitude cerebral edema. As controls, 245 healthy highland Tibetans (HLT) from the Yushu area were randomly selected. The study protocols were approved by the Ethics Committee of Qinghai University (Xining, China). All participants in this study signed informed consent.

SAMPLE COLLECTION

Venous blood samples, 5 mL, were collected from each participant. The whole blood was separated into blood cells and plasma by centrifuging at 1000*g* for 10 minutes, and then the blood cells and plasma were transported to Xining for biochemical assays and genetic polymorphism analysis.

DNA EXTRACTION AND GENOTYPING ASSAYS

Genomic DNA was extracted from venous blood by Gentra Puregene Blood Kit (158389; Qiagen GmbH, Hilden, Germany) according to standard procedures. The EPAS1-[ch2:46441523(hg18)] polymorphism was examined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) with the following primers: 5'-AGGCTCTGGTTTTGGGAA-3' (forward) and 5'-TGGGATGGGTGCTGGATT-3' (reverse). The PCR mixture contained 12.5 μL Phusion High-Fidelity PCR Master Mix (F-532L; Thermo Fisher Scientific, Waltham, MA), 1 μL each primer (final concentration 10 μM), and 50 ng genomic DNA in a final volume of 25 μL. The PCR cycles consisted of denaturation at 96°C for 1 minute, 35 cycles of 96°C for 10 s, 60.5°C for 30 s, and 72°C for 20 s, and a final elongation at 72°C for 10 minutes. Amplified fragments were digested by BsmAI (New England Biolabs, Ipswich, MA), and the digested PCR products were resolved on 1.8% agarose gels stained with ethidium bromide. The fragments run as 247 and 130 bp fragments in the presence of the C nucleotide or as uncut 377 bp fragment in the presence of the G nucleotide. The polymorphism was confirmed by DNA sequencing with ABI 3730 DNA analyzer.

STATISTICAL ANALYSIS

Statistical analyses were performed using the SPSS software (version 17.0; SPSS Inc, Chicago, IL). Continuous values were expressed as means ± SD. Deviation of genotype frequency from Hardy-Weinberg equilibrium was assessed by χ^2 test with 1 degree of freedom. Allele frequencies were calculated based on genotype frequencies in the HAPE and control groups, and the intergroup difference was estimated with the χ^2 test.

Results

CLINICAL CHARACTERISTICS

The average age, heart rate, arterial oxygen saturation, and concentration of hemoglobin plus hematocrit for the HAPE-p, HAPE-r, and HLT groups are listed in Table 1. The incidence of HAPE was much higher among men than among women, consistent with the predisposition of men to HAPE.[1] We found that arterial oxygen saturation was significantly lower whereas heart rate was significantly higher in the HAPE-p group compared to the HAPE-r and HLT groups. Compared to the HLT group, hematocrit was at a similar level in