WILDERNESS & ENVIRONMENTAL MEDICINE, **25,** 392–400 (2014)

ORIGINAL RESEARCH

An EPAS1 Haplotype Is Associated With High Altitude Polycythemia in Male Han Chinese at the Qinghai-Tibetan Plateau

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Background.—Hemoglobin concentration at high altitude is considered an important marker of high altitude adaptation, and native Tibetans in the Qinghai-Tibetan plateau show lower hemoglobin concentrations than Han people who have emigrated from plains areas. Genetic studies revealed that EPAS1 plays a key role in high altitude adaptation and is associated with the low hemoglobin concentration in Tibetans. Three single nucleotide polymorphisms (rs13419896, rs4953354, rs1868092) of noncoding regions in EPAS1 exhibited significantly different allele frequencies in the Tibetan and Han populations and were associated with low hemoglobin concentrations in Tibetans.

Methods.—To explore the hereditary basis of high altitude polycythemia (HAPC) and investigate the association between EPAS1 and HAPC in the Han population, these 3 single nucleotide polymorphisms were assessed in 318 male Han Chinese HAPC patients and 316 control subjects. Genotyping was performed by high resolution melting curve analysis.

Results.—The G-G-G haplotype of rs13419896, rs4953354, and rs1868092 was significantly more frequent in HAPC patients than in control subjects, whereas no differences in the allele or genotype frequencies of the 3 single nucleotide polymorphisms were found between HAPC patients and control subjects. Moreover, genotypes of rs1868092 (AA) and rs4953354 (GG) that were not observed in the Chinese Han in the Beijing population were found at frequencies of 1.6% and 0.9%, respectively, in our study population of HAPC patients and control subjects.

Conclusions.—Carriers of this EPAS1 haplotype (G-G-G, rs13419896, rs4953354, and rs1868092) may have a higher risk for HAPC. These results may contribute to a better understanding of the pathogenesis of HAPC in the Han population.

Key words: high altitude polycythemia, EPAS1, high resolution melting, Qinghai-Tibetan plateau

Introduction

The Qinghai-Tibetan plateau covers a vast area with a harsh natural environment, and millions of people live in this region. Approximately 12 million people were reported to permanently reside in the Qinghai-Tibetan plateau in 2006, and this number increases every year; most of the increase in population has come from Han people emigrating from low altitude areas.[1] For low altitude populations who move to high altitude areas, hemoglobin (Hb) concentration increases at a certain range because of the hypoxic environment, and this response is crucial for them to acclimatize to high altitude. As they stay longer at high altitude, some people, especially Han, are prone to chronic mountain sickness, which is characterized by symptoms of long-term hypoxia.[2,3] The most striking of these features is excessive erythropoiesis, also called high altitude polycythemia (HAPC).[3] Although testosterone level,[4–6], sleep quality,[7,8] oxidative stress,[9] and immune response[10] are involved in the pathogenesis of HAPC, the genetic basis of HAPC has not been studied extensively,[11,12] especially in the Han population.

Of all human populations worldwide, native Tibetans of the Qinghai-Tibetan plateau are regarded as the one best adapted to high altitudes, and they exhibit lower Hb concentrations than Han who have emigrated from low altitudes, even those who have acclimated to the high

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altitude.[13] This phenomenon is considered largely genetic. Moreover, the incidence of HAPC among Tibetans is lower than that among Han,[14] and mounting evidence suggests that genetic factors contribute to the development of altitude-related illnesses.[15–17] Recently, significant progress has been made in the study of the genetic basis of high altitude adaptation in Tibetans, and recent research has highlighted a new aspect of the genetic basis of HAPC. Namely, peroxisome proliferator-activated receptor α (PPARA),[18] egl nine homolog 1 (EGLN1),[18–22] and endothelial Per-Arnt-Sim (PAS) domain protein 1 (EPAS1)[19–24] have been reported to play important roles in high altitude adaptation in Tibetans. Of these 3 candidate genes, EPAS1 has been implicated as making the greatest contribution to genetic adaptation to high altitude and to the low Hb concentrations observed in the Tibetan population.[23,24]

Expression of EPAS1 is limited to organs that are involved in oxygen transport and metabolism, such as the lung, placenta, and vascular endothelium,[25] and it also contributes to many biological processes and diseases related to metabolism,[26] angiogenesis,[27,28] inflammation,[29,30] and cancer,[31–33] indicating that EPAS1 plays a key role in oxygen sensing and metabolism. Moreover, it was also found that EPAS1 was associated with high altitude pulmonary edema,[17] which is an idiopathic disease of high altitude[34] in the Han population. Genetic studies of high altitude adaptation in Tibetans suggest that EPAS1 has been subjected to strong natural selection by the high altitude environment. Noncoding DNA sequences in or near EPAS1 are significantly different in the Tibetan and Han populations, and these regions are associated with low Hb concentration in Tibetans.[23,24]

Three single nucleotide polymorphisms (SNPs) in noncoding regions—rs13419896, rs4953354, and rs1868092—not only show significant differences in frequencies between the Tibetan and Han populations,[20] but also are associated with low Hb in Tibetans.[24] To better understand the relationship between EPAS1 and HAPC in the Han population, we analyzed the genotypes and allele frequencies of the 3 SNPs, rs13419896 (A/G), rs4953354 (A/G), and rs1868092 (A/G), using the high resolution melting (HRM) method[35,36] in male HAPC patients and control subjects from the Han population in the Qinghai-Tibetan plateau.

Methods

STUDY GROUPS

In all, 318 HAPC patients and 316 healthy control subjects were recruited from the Han population at the Qinghai-Tibetan plateau in China. All patients and control subjects who participated in this research had lived at an altitude above 4000 m for at least 3 months. According to the standard diagnostic criteria for chronic mountain sickness, we selected male HAPC patients with excessive polycythemia (Hb ≥210 g/L) and without chronic pulmonary diseases, which are the same as the inclusion criteria for chronic mountain sickness.[37] Characteristics of all participants are listed in Table 1. This research was approved by the Ethics Committee of the Third Military Medical University of China.

ALLELES FREQUENCIES OF TIBETAN AND CHINESE HAN BEIJING POPULATION

Alleles frequencies of rs13419896, rs4953354, and rs1868092 in the Tibetan population were obtained from Peng et al.[20] Genotypes and alleles of these 3 SNPs in the Chinese Han Beijing population were downloaded from HapMap (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/; HapMap data rel28 phaseII+III,August 10, on NCBI B36 assembly, dbSNP b126). These data were also included in further analysis.

Table 1. Characteristics of healthy control subjects and high altitude polycythemia patients

Characteristics	*n*	*Controls*	*n*	*HAPC*	*P value*
Age, years	315	25.50 ± 6.50 (18–46)	280	24.73 ± 5.58 (18–51)	.192
Hemoglobin, g/L	316	181.08 ± 10.48 (150–203)	318	221.97 ± 11.3 (210–282.5)	< .001
Sao_2, %	255	89.43 ± 3.19 (53–95)	243	87.90 ± 3.52 (70–97)	< .001
Heart rate, beats/min	255	82.52 ± 13.63 (52–146)	243	88.15 ± 16.38 (58–144)	< .001
SBP, mm Hg	255	123.42 ± 11.89 (90–158)	241	124.56 ± 12.79 (92–176)	.307
DBP, mm Hg	255	74.15 ± 10.07 (52–115)	241	77.76 ± 11.18 (51–124)	< .001
Smoking status	159	Yes	141	Yes	.435
	102	No	78	No	
Drinking status	26	Yes	23	Yes	.582
	202	No	151	No	

Values are mean ±SD (minimum–maximum). Comparisons used unpaired Student's *t* test.
HAPC, high altitude polycythemia; SBP, systolic blood pressure; DBP, diastolic blood pressure.