Genetic Adaptation of the Hypoxia-Inducible Factor Pathway to Oxygen Pressure among Eurasian Human Populations

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Abstract

Research into the mechanisms of human adaptation to the hypoxic environment of high altitude is of great interest to the fields of human physiology and clinical medicine. Recently, the gene EGLN1, from the hypoxia-inducible factor (HIF) pathway, was identified as being involved in the hypoxic adaptation of highland Andeans and Tibetans. Both highland Andeans and Tibetans have adapted to an extremely hypoxic habitat and less attention has been paid to populations living in normoxic conditions at sea level and mild-hypoxic environments of moderate altitude, thus, whether a common adaptive mechanism exists in response to quantitative variations of environmental oxygen pressure over a wide range of residing altitudes is unknown. Here, we first performed a genome-wide association study of 35 populations from the Human Genome Diversity-CEPH Panel who dwell at sea level to moderate altitude in Eurasia (N = 691; 0–2,500 m) to identify the genetic adaptation profile of normoxic and mild-hypoxic inhabitants. In addition, we systematically compared the results from the present study to six previously published genome-wide scans of highland Andeans and Tibetans to identify shared adaptive signals in response to quantitative variations of oxygen pressure. For normoxic and mild-hypoxic populations, the strongest adaptive signal came from the mu opioid receptor-encoding gene (OPRM1, 2.54 × 10^-6), which has been implicated in the stimulation of respiration, while in the systematic survey the EGLN1-DISC1 locus was identified in all studies. A replication study performed with highland Tibetans (N = 733) and sea level Han Chinese (N = 748) confirmed the association between altitude and SNP allele frequencies in OPRM1 (in Tibetans only, P < 0.01) and in EGLN1-DISC1 (in Tibetans and Han Chinese, P < 0.01). Taken together, identification of the OPRM1 gene suggests that cardiopulmonary adaptation mechanisms are important and should be a focus in future studies of hypoxia adaptation. Furthermore, the identification of the EGLN1 gene from the HIF pathway suggests a common adaptive mechanism for Eurasian human populations residing at different altitudes with different oxygen pressures.

Key words: adaptive evolution, human genetic variation, oxygen pressure, hypoxia, hypoxia-inducible factor pathway, Eurasian populations.

Introduction

Physiological adaptation to hypobaric hypoxia is an important area of human research and does not only reflect the physiology of high-altitude adaptation, but also may yield benefit for both sports medicine and the treatment of patients with chronic hypoxia resulting from various forms of clinical illness (Martin and Windsor 2008). Physiological responses in altitude acclimatization have already been investigated in highland newcomers (Houston and Riley 1947; Vogel and Harris 1967; Reynafarje and Hurtado 1971; Hannon and Sudman 1973). The successful climb of Mount Everest by Messner and Habeler without the use of supplemental oxygen led to research into the physiological adaptation mechanisms to high-altitude hypoxia (Oelz et al. 1986). Various acclimatization responses were identified including: 1) cardiovascular responses which increase cardiac output,
both acutely and chronically exposed newcomers (West 1998; therefore, they are believed to explain the acclimatization of these responses are capable of restoring, or even surpassing, unexpected findings (Beall et al. 2010; Yi et al. 2010). To date, studies of the mechanisms that lead to success of highland newcomers have revealed many new and unexpected findings (Beall et al. 2010; Yi et al. 2010). To date, two populations, Andeans and Tibetans, who have functionally successfully adapted to high-altitude hypoxia, have been physiologically well characterized (Beall 2007). Due to improved maternal vascular adaptation, they both give birth to heavier babies than acutely exposed lowlanders, and this phenomenon yields better fetal outcomes (Moore et al. 1986, 2001, 2004). Despite this similarity, Andean and Tibetan populations, however, exhibit different adaptation profiles for numerous physiological traits, including circulation, respiration, and hematopoiesis (Beall 2007). Highland Tibetans have higher resting ventilation (VE) and hypoxic ventilatory response (HVR) than their Andean counterparts (Beall et al. 1997), but with lower oxygen saturation and hemoglobin concentrations (Beall and Goldstein 1987; Beall et al. 1990, 1998). Interestingly, highland Tibetans do not adapt by hypoxia-induced erythropoiesis leading to polycythemia as observed in highland Andeans and newcomers; instead, their hemoglobin levels are comparatively lower than those of their lowland Han Chinese counterparts (Adams and Strang 1975; Beall and Reichsman 1984; Beall et al. 2010; Yi et al. 2010). These findings contradict the long-held belief that the restoration of arterial oxygen content and flux by polycythemia is the best adaptation option for humans under hypobaric hypoxia (West 1998; Hornbein and Schoene 2001). These results emphasize that additional research is needed to identify and distinguish common as well as population-specific physiological mechanisms for hypoxia adaptation. Although the physiological mechanisms of adaptation to high altitude have been extensively characterized, the underlying genetic basis has not been fully understood. Previous studies found that genetic factors accounted for a high proportion of the phenotypic variance in hemoglobin concentration among highland Tibetan and Andean populations (Beall et al. 1998). Quantitative genetic analyses of the familial patterning of different adaptive traits between the two populations also provided evidence of population-specific adaptation mechanisms (Beall 2000). Recently, EGLN1 and EPAS1, two genes coding for molecular components of the hypoxia-inducible factor (HIF) pathway, were found to have contributed to high-altitude adaptation in Tibetans (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011); while EGLN1, PRKAA1, and NOS2A, of the same pathway, were suggested to underlie the high-altitude adaptation in Andeans (Bigham et al. 2010). The sharing association with the EGLN1 gene by both populations suggests that a common adaptive mechanism in the HIF pathway occurred in both highland Andeans and Tibetans. On the other hand, genetic polymorphisms of the EPAS1 gene were significantly associated with hemoglobin concentrations in highland Tibetans (Beall et al. 2010; Yi et al. 2010), which partially explains the different adaptation mechanisms of the two highland populations. Notwithstanding the above information, for these two highland populations, whether other common and populations-specific adaptation mechanisms exist is still uncertain and their underlying genetic basis has not yet been fully characterized.

Multiple environmental factors vary with an increase in altitude, including ambient temperature, circadian rhythm (Virues-Ortega et al. 2004), oxygen pressure (Beall 2007), ultraviolet radiation (Boucher 2010), and rainfall (Brunsdon et al. 2001). Among these, since the effect of other environmental factors can be mitigated or prevented, the loss of oxygen pressure due to the exponentially decreasing barometric pressure with altitude is deemed as a determining factor in high-altitude adaptation (Beall 2007). Quantitative variations of oxygen pressure, thus, form a continuous gradient of altitude-specific habitats, where human physiological responses also demonstrate a clinal pattern. In normoxia at sea level, the human physiological variability is within normal limits and is considered the reference condition. When exposed to mild hypoxia of moderate altitude, peripheral chemoreceptors are stimulated and activity in the carotid sinus is increased, although no obvious increase in ventilation can be observed (Mason 2000). In addition, a well-characterized impairment of cognitive function due to exposure to hypobaric hypoxia can first be detected at a moderate altitude (2,440 m) (Farmer et al. 1992). Furthermore, a significant decline in birth weight could be observed at the range of 1,500–1,999 m, suggesting that fetal growth is sensitive to uterine O2 delivery even at moderate altitude (Yip 1987; Zamudio et al. 1995; Mortola et al. 2000). Work in animal models also found clinal patterns in both phenotypic and genetic variations associated with the range of moderate altitudes (0–2,500 m) (Bitner-Mathe et al. 1995; Sorensen et al. 2005; Collinge et al. 2006; Soto et al. 2010), providing support for observations in humans. As the ascent in altitude continues, the human body gradually shows significant physiological changes of the respiratory, circulatory, and hematopoietic systems (West 1998; Hornbein and Schoene 2001). From the above information, therefore, it is reasonable to hypothesize that human populations residing over a wide range of altitudes, not just those that live at high altitude, have undergone natural selection due to environmental oxygen pressure.

Most studies on the adaptive response to reduced oxygen pressure, however, have been conducted in Andeans and...
Tibetans who inhabit altitudes above 3,500 m, a level where the environment is extremely hypoxic and their results are not conductive for normal human physiological function. Less attention has been paid to adaptation to oxygen pressure in populations that live in normoxia at sea level or mild-hypoxic environments of moderate altitude. In particular, whether a common adaptive mechanism that responds to quantitative variations of oxygen pressure over a wide range of resident altitudes is unknown. Since ~95% of human populations live at low and moderate-altitude habitats (0–2,500 m) (Penaloza and Arias-Stella 2007), it is important to understand oxygen pressure adaptation in normoxic and mild-hypoxic populations as this will help illuminate the underlying adaptive mechanisms used by highland inhabitants, thus, help interpret the overall oxygen pressure adaptation mechanisms used by human beings.

To examine the genetic adaptation profile of normoxic and mild-hypoxic inhabitants, we conducted a genome-wide association study for 35 Eurasian populations in the Human Genome Diversity-CEPH Panel (HGDP-CEPH, N = 691, dwelling from sea level to 2,500 m), which have extensive SNP genotyping data (Li et al. 2008). We then compared the results from our study to several recently published genome-wide scans of highland Andeans and Tibetans to systematically identify adaptive mechanisms in response to quantitative variations of oxygen pressure. Genome-wide significant signals found in normoxic and mild-hypoxic populations and shared signals over a wide range of altitudes were further investigated in a replication study with highland Tibetans (N = 733) as well as lowland and moderate-altitude Han Chinese (N = 748). Our study, thus, for the first time reports the genetic profile of normoxic and mild-hypoxic adaptation in populations residing at low and moderate altitudes, and demonstrates that the HIF pathway operates in oxygen pressure adaptation over a wider range of altitudes than previously recognized.

Materials and Methods

Studied Populations and Genotype Data
Population genotype data was retrieved from the HGDP-CEPH data set that included 952 unrelated individuals from 53 populations genotyped by the Illumina 650Y platform (Li et al. 2008), which is freely available at ftp://ftp.ceph.fr/hgd_supp1. In all previous studies of Tibetan adaptation to high altitude, lowland Han Chinese subjects were usually used as controls (Beall et al. 2010; Yi et al. 2010). Similarly, lowland Europeans were usually used as controls in studies of Andean adaptation to high altitude (Moore et al. 2004). Using genetically closely related highland Tibetans and lowland Han Chinese (Zhao et al. 2009) as well as the highland Andeans and lowland European residents (Hunley and Healy 2011) avoids possible confounding effects due to potential population structure and thus should effectively detect genetic polymorphisms under altitude selection. Accordingly, all 35 Eurasian populations (Burusho and Cambodian populations were excluded due to lack of specific altitude data for these populations) were included. Genotype data from these 35 Eurasian populations that inhabit an extensive altitudinal range of Europe and Asia (N = 691, supplementary fig. S1, Supplementary Material online) were used for this study.

Geographic Information
Altitude data was used as a surrogate for ambient oxygen levels and were obtained from the World Meteorological Organization publication “1961-1990 Global Climate Normals,” which was compiled by National Climatic Data Center of the United States and was also freely available from Hong Kong Observatory website (http://gb.weather.gov.hk) (Ji et al. 2010). Geographic coordinates of the populations were from the HGDP-CEPH data set. Altitude data for the Chinese Han populations were extracted from “The Annual Surface Climate Normals of International Exchanging Stations of China (1971-2000)” through the China Meteorological Data Sharing Service System (http://cdc.cma.gov.cn). Altitude data were transformed to natural logarithms, which follow a normal distribution.

Subjects and Samples for Replication Study
To replicate the results from the lowland and moderate-altitude Eurasian populations, samples were collected from highland Tibetan as well as lowland and moderate-altitude Han Chinese populations, both of which are genetically closely related (Zhao et al. 2009). A total of 733 native Tibetan individuals were sampled from two regions of Tibet (Naqu and Rikaze, inhabiting at >3,500 m), and 748 Han Chinese individuals were collected from Liaoning, Shandong, Sichuan, Yunnan, and Guangdong Provinces (0–2,000 m), representing northern, central, and southern Han Chinese populations. Blood samples were obtained with informed consent. The protocol for this study was reviewed and approved by the ethics committee of the Kunming Institute of Zoology, Chinese Academy of Sciences.

Genotyping for Replication Study
Five SNPs (rs17084501 of OPRM1, rs6605044 of EGLN1-DISC1, rs2495718 of HIF1AN, rs11881124 of EGLN2, and rs1809461 of EGLN3) identified in the Eurasian HGDP-CEPH populations were genotyped in the Tibetan and Han Chinese replication samples. Genomic DNA was extracted from whole blood by the standard phenol/chloroform method. SNP rs11881124 of the EGLN2 gene was genotyped by PCR–RFLP using mismatched primers (forward primer 5′-CTCCAGCTTCCCTC CGGAAT-3′, reverse primer 5′-GAAGAGTCAGGGTGGGGG ·3′) with the products digested with the restriction enzyme SspI (New England Biolabs). The other four SNPs were genotyped by the GenomeLab SNPstream Genotyping System (Beckman Coulter Inc.) according to the protocol provided by the manufacturer. Among them, rs6605044 of the EGLN1-DISC1 locus was replaced by a nearby SNP (rs10797564, D′ = 1.000) due to lack of available appropriate primers (www.autoprimer.com, Beckman Coulter Inc.).
Data Analyses
Among the 644,258 genotyped autosomal SNPs, 503,698 were defined as common polymorphisms and were used for subsequent analysis (MAF ≥ 0.1). Nei’s measurement of the average gene diversity per locus $H_2$ was calculated for each of the 35 populations (Nei 1973). A genome-wide association study was performed by using additive effect with a dominance deviation model implemented in PLINK (Purcell et al. 2007). To adjust for inflation due to population stratification, principal components ( PCs) were used as covariates. PCs, which explain the intrinsic population structures, were calculated by principal components analysis (PCA) of all the autosomal genotype data (Price et al. 2006). WGAViewer software was used to evaluate the genomic inflation factor and to draw Manhattan plots to show the locations of the significant signals (Ge et al. 2008).

SNPs with significant association were mapped to specific genes through the dbSNP dataset (Sherry et al. 2001). For SNPs within intergenic regions, the most adjacent gene was assigned. Genes were then mapped to their Gene Ontology annotation terms (Ashburner et al. 2000) and KEGG pathways (Kanehisa et al. 2006) via Entrez Gene, using the publicly available program Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Huang da et al. 2009a,b). Like many other similar publicly available tools, DAVID adopts a common core strategy to systematically map a list of genes to the associated biological annotation, and then statistically highlight the most enriched biological annotation out of thousands of linked terms and contents. Therefore, it can help identify biological processes most pertinent to the biological phenomena under study (Huang da et al. 2009b).

A one-way ANOVA was used in the comparison of habitat altitude with different genotypes for the OPRM1 SNP in the Eurasian HGDP-CEPH populations. Statistical analyses of the allele frequencies, sex and age differences were performed by χ²-test. The associations of the allele frequencies and the residing altitudes of the five confirmed signals among the Han Chinese populations were tested through Pearson correlation analysis, and among Tibetan populations were tested by Spearman rank correlation analysis (due to the lack of specific dwelling altitude data). All of these procedures were performed using SPSS for Windows, 13.0. Hardy–Weinberg equilibrium (HWE) was tested with the software PEDSTATS V0.6.8 (http://www.sph.umich.edu/csg/abecasis/).

Results
Candidate Genes for Normoxic and Mild-hypoxic Adaptation in Eurasian Populations from Genome-wide Association Study
To examine the genetic adaptation profile of normoxic and mild-hypoxic inhabitants, we conducted a genome-wide association study with the extensive SNP genotyping data from 35 Eurasian HGDP-CEPH populations ($N = 691$, living at sea level to 2,500 m). No significant association of average gene diversity with residing altitude was observed (Pearson $P > 0.05$, supplementary table S1, Supplementary Material online) indicating that the effect of potential founder effects or population bottlenecks on the following association study is low. The 35 studied populations were comparatively more homogeneous than a worldwide sample from the full HGDP as shown for their genotypes using the first two PCs (fig. 1), similar to what has been previously described (Reich et al. 2008). Among the first 68 significant PCs ($P < 0.001$), according to the TW statistic (Patterson et al. 2006), the amount of variation explained reaches a leveling-off by PC20, therefore, the inflation factor ($\lambda_{GC}$) due to population structure was normalized by adjustment through the top 20 PCs ($\lambda_{GC} = 1.0759$, fig. 2). The list of candidate SNPs was also more stable when approximately 20 PCs, rather than only few PCs (e.g., 3 PCs), were used (supplementary tables S2–S3, Supplementary Material online).

At the genome-wide scale, the strongest signal that achieved significance was found near the OPRM1 gene, which encodes the mu opioid receptor (rs17084501, $P = 2.54 \times 10^{-9}$, fig. 3), with a $P$-value that reached the commonly accepted genome-wide significant level of $< 1 \times 10^{-7}$. In general, the ancestral T allele is significantly enriched with an increase in altitude (ANOVA $P < 0.001$, fig. 4). To further survey for any other significant associations using a pathway approach, the top 500 SNPs with the highest genome-wide association scores were examined as they likely include additional selection signals (supplementary table S4, Supplementary Material online). These 500 SNPs map to 337 unique genes. Applying the GO annotation and KEGG pathway analysis through DAVID, we failed to identify any significant gene categories (Bonferroni $P > 0.05$).

Evidence for Shared Adaptation Mechanism Over a Wide Range of Altitudes
Similar to the approach used to reveal shared adaptation mechanism at extremely high altitude by using a systematic survey for common genes (Simonson et al. 2010), we combined results from six recent genome-wide scans in Andeans and Tibetans with our present study to identify shared genes showing adaptation signals over a wide range of altitudes. Since the recently published genome-wide scans in Tibetans and Andeans usually proposed only a list of candidate genes, instead of specific SNPs, therefore, the adaptation signals were compared through a gene-based analysis though some detailed information would be lost due to this procedure.

We first reappraised six recent genome-wide scans to identify any shared high-altitude adaptation signals in highland Tibetan and Andean populations (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011), resulting in the identification of eight candidate gene lists from highland Tibetans and one from highland Andeans (supplementary table S5, Supplementary Material online). The gene EGLN1 was most reproducible, as it was present in all of the Tibetan and Andean candidate gene lists while EPAS1 was highly reproducible in the Tibetans, appearing in seven of the eight candidate gene lists. The exclusivity of the EPAS1 gene to the highland Tibetans together with the finding that its genetic polymorphisms are
FIG. 1. Principal component analysis of the 35 Eurasian HGDP-CEPH populations (N = 691) by the first two principal components. Among the first 68 significant principal components (PCs, \( P < 0.001 \)), the amount of variation explained levels off by PC20 (\( \lambda_{GC} = 1.0759 \)). The 35 studied populations were comparatively more homogeneous than a worldwide sample from the full HGDP as shown by their genotypes using the first two PCs.

FIG. 2. QQ plot of the genome-wide association study for normoxic and mild-hypoxic adaptation. Among the first 68 significant PCs (\( P < 0.001 \)), the amount of variation explained levels off by PC20. After adjustment for the top 20 PCs, the genome inflation is almost normalized (\( \lambda_{GC} = 1.0759 \)).
significantly associated with hemoglobin concentrations suggested that it could underlie the genetic basis for different hypoxia adaptation mechanisms between the two populations.

Next, the top 500 SNPs from the present study were attributed to 337 unique genes. These 337 genes for normoxic and mild-hypoxic adaptation were then mapped to the candidate gene lists for highland adaptation in Tibetans and Andeans. The DISC1 gene was found to be most reproducible which appeared in the present study and four of the eight Tibetan gene lists, followed by HIF1AN which appeared in the present study and three of the eight Tibetan candidate gene lists (fig. 5). The high reproducibility of the DISC1 locus is potentially due to its close genetic linkage with the nearby EGLN1 gene (Yi et al. 2010), which is only about 201 kb upstream. To test this hypothesis, we examined another gene that is closely linked to EGLN1, C1orf124 (Yi et al. 2010), which is about 11 kb upstream of EGLN1. SNP rs2437150, which is located within C1orf124, was found to have a weak association with altitude (P = 0.0018, ranked 1,635th of the 503,698 SNPs, table 1), therefore it is likely that the signal at the EGLN1-DISC1 locus exists in normoxic and mild-hypoxic inhabitants and is probably due to the EGLN1 gene.

EGLN1 is a member of a family of prolyl hydroxylase encoding genes, whose products degrade HIF under normoxic conditions (Lendahl et al. 2009). The two other members of this gene family, EGLN2 and EGLN3, also appeared as top candidate signals (rs11881124 of the EGLN2 gene ranked 514th, while rs1809461 of EGLN3 ranked 1,947th of the 503,698 SNPs, table 1), though neither was statistically significant at the GWAS level. These results suggest that a genuine enrichment of prolyl hydroxylase encoding genes in normoxic and mild-hypoxic adaptation. On the other hand, the enzyme asparaginyl hydroxylase encoded by the highly reproducible HIF1AN is another class of enzymes that can degrade HIF under normoxia (Lando et al. 2002). Together, the genes

**Fig. 3.** Manhattan plot of the genome-wide association study (GWAS) for normoxic and mild-hypoxic adaptation. 503,698 genotyped autosomal common SNPs (MAF ≥ 0.1) were analyzed in GWAS study. The single genome-wide signal rs17084501 is located near the OPRM1 gene on chromosome 6 (P = 2.54 × 10^{-9}).

**Fig. 4.** Boxplot of the rs17084501 genotype distribution at different altitude levels among the 35 Eurasian HGDP-CEPH populations (N = 691). The ancestral T allele of rs17084501 is significantly enriched with an increase in altitude (ANOVA P < 0.001).
that encode these two classes of enzymes which degrade HIF under normoxia (Lendahl et al. 2009), EGLN1-DISC1/ EGLN2/ EGLN3 and HIF1AN, appear to be candidates for normoxia and mild-hypoxia adaptation.

Replication Study with Highland Tibetans as well as Lowland and Moderate-Altitude Han Chinese (Total \( N = 1,481 \))

The distributions of the most significant hit OPRM1, highly reproducible genes (EGLN1-DISC1 and HIF1AN) and related genes in the EGLN1 gene family (EGLN2 and EGLN3) were examined in a replication sample of highland Tibetans (\( N = 733 \)) as well as lowland and moderate-altitude Han Chinese (\( N = 748 \)) who were genotyped for their SNPs using the GenomeLab SNPstream Genotyping System (Beckman Coulter Inc.) and PCR–RFLP methods. The Genetic Power Calculator (available online at http://pngu.mgh.harvard.edu/~purcell/gpc/) indicated that our sample size was large enough to do a case–control analysis with 80% power for all five SNPs (Purcell et al. 2003). No significant deviation from HWE was observed for these SNPs (\( P > 0.001 \)), nor were any significant sex or age differences for these populations found (\( P > 0.05 \)).

For the OPRM1 SNP, the frequencies of the rs17084501T allele were nearly fixed in both populations, and no significant differential signal was detected in the Han and Tibetan populations (table 3). However, if the two populations were divided into different altitude range subgroups then the OPRM1 SNP showed a significant T allele frequency increase with an increase in altitude in the highland Tibetan populations (\( N = 3 \), Spearman rank correlation \( P < 0.01 \), fig. 6 and supplementary table S6, Supplementary Material online). This trend is consistent with the pattern derived from the lowland and moderate-altitude Eurasian HGDP-CEPH populations in which T allele was associated with higher altitude.

The highly reproducible EGLN1-DISC1 and HIF1AN genes both showed a significant allelic differentiation between Tibetan and Han Chinese populations (\( P < 0.05 \), table 3). When the subgroups were divided by altitude, the EGLN1-DISC1 locus showed a significant increase in the frequency of the rs10797564A allele with an increase in altitude in both Tibetan and Han Chinese subpopulations (\( N = 3 \), Spearman rank correlation \( P < 0.01 \) in Tibetan populations; \( N = 5 \), Pearson correlation \( P = 0.0012 \) in Han Chinese populations; fig. 7 and supplementary tables S7–S8, Supplementary Material online). On the other hand, the EGLN2 SNP rs11881124 was monomorphic (A allele only) in both the

![Fig. 5. Intersection results of the top 337 genes for normoxic/mild-hypoxic adaptation and the eight gene lists for extremely-hypoxic adaptation from six previously published studies. Results from recent genome-wide scans in Andean and Tibetan populations are compared to those of the present study to identify shared adaptive signals. The most reproducible genes are DISC1 and HIF1AN. Different colors represent candidate lists as follows: dark blue for iHS candidates (PMID 20466884, 426 genes); green for XP-EHH candidates (PMID 20466884, 433 genes); red for candidates from the present study (337 genes); yellow for candidates from PMID 20961960 (29 genes); light blue for candidates from PMID 20595611 (35 genes); and orange for candidates from PMID 2053544 (105 genes).](image-url)
Tibetan and Han Chinese populations, and no significant difference in the distributions of the EGLN3 alleles was seen ($P > 0.05$).

**Discussion**

Previous studies have extensively examined the mechanisms of adaptation to hypoxia in highland human populations, especially those of Tibetans (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011) and Andeans (Bigham et al. 2009, 2010). Reviewing these genome-wide scans indicates that convergent evolution may have occurred in highland Tibetan and Andean populations as suggested by the shared association with the EGLN1 gene, which encodes a component of the HIF pathway (Bigham et al. 2010). Different physiological adaptation traits and the exclusively adaptive signals between these two populations also suggested population-specific adaptation mechanisms (Beall 2007). While a considerable number of studies have examined the adaptation of Andeans and Tibetans to an extremely hypoxic habitat, less attention has been paid to populations living in normoxic and mild-hypoxic environments, especially in mild hypoxia of moderate altitude where many physiological responses to reduced oxygen pressure can be first observed (Farmer et al. 1992; Mason 2000; Mortola et al. 2000). Thus, whether a general adaptive mechanism exists in response to quantitative variations of oxygen pressure over a wide range of residing altitudes is unknown.

A SNP near the OPRM1 gene was identified as the only genome-wide significant signal for normoxic and mild-hypoxia adaptation in this study. More importantly, the highly reproducible EGLN1-DISC1 locus was identified among the highland Tibetans and Andeans populations (inhabiting $> 2,500$ m) as well as the lowland and moderate-altitude Eurasian human populations (inhabiting $< 2,500$ m), providing evidence for a common adaptive mechanism for human populations over a wide range of altitudes.

Through the use of PCs as covariants in our genome-wide association study we were able to identify a signal near the OPRM1 gene, a mu opioid receptor gene, thus nominating it as a candidate for normoxia and mild-hypoxia adaptation. Activation of the mu opioid receptor could cause respiratory...
stimulation/depression and thus may contribute to differences in breathing patterns, including frequency and amplitude, of high and low altitude populations (Shook et al. 1990; Greer et al. 1995; Johnson et al. 2008). Administration of μ-opioids can cause abnormal ventilatory responses to hypoxia and hypoxia (Wang and Teichah 2007), a clinical phenomena that is common when lowlanders travel to a highland environment. Recent studies in animals (Moss et al. 2006) and children with obstructive sleep apnea (Brown et al. 2004) suggest that hypoxia can enhance the analgesic effects of exogenous opioids, and perioperative opioid requirements are significantly decreased in hypoxic children living at a high altitude (Rabbitts et al. 2010). A mechanism involving respiration is thus suggested for this gene. OPRM1 acts pleiotropically and other responses may also explain its contribution to adaptation to hypoxia. Among its pleiotropic effects, paraventricular nucleus (PVN) expressed mu-receptors regulate the heart rate during stress, and endogenous opioids released during stress may modulate adrenomedullary responses (Kiritsy-Roy et al. 1986). These observations suggest that OPRM1 acts through both a respiratory mechanism as well as a cardiovascular response to underlie mild-hypoxia adaptation at moderate altitude.

In 35 lowland and moderate-altitude Eurasian populations, the ancestral T allele of the OPRM1 rs17084501 locus is significantly enriched with an increase in altitude. In the replication study, the rs17084501T allele frequency also increases significantly with altitude in highland Tibetan subpopulations (>3,500 m, P < 0.01). Thus, the OPRM1 signal derived from lowland and moderate-altitude Eurasian populations was confirmed in the highland Tibetan populations, indicating that OPRM1 might function over a wide range of altitudes. We failed, however, to observe a significant allelic differentiation between the Tibetan and Han Chinese populations or find a significant association of T allele frequency with altitude in Han Chinese subgroups living within a moderate range of altitudes. The lack of significant population differentiation or negative association of rs17084501T allele with altitude in Han Chinese populations may be explained by the close genetic relationship between the populations and the differences in the selective pressures acting on these two populations. The C allele of rs17084501 is rare in both populations; therefore it is even more difficult to detect a differential signal between these two closely related populations than in populations where this SNP is more prevalent. This putative hypothesis may be supported as Highland Tibetans have lived in the Qinghai-Tibet Plateau for thousands of years (Zhao et al. 2009), and considering their extremely hypoxic environment and resultant strong selection pressure, this time period may have been long enough for selection to significantly increase the frequency of the rs17084501T allele in different subpopulations. In contrast, as a rare SNP it is hard to detect allelic differences among Han Chinese subpopulations when they reside within a moderate altitude range where the selective advantage of this allele is relatively very small. However, we do not have data to document such differential selection occurred, further examination of this SNP in other populations such as highland Andean as well as lowland and moderate-altitude European populations, where it is more prevalent, and thus will have more statistical power to reveal difference in frequencies, should enhance our understanding of the function of this gene in hypoxia.

The identification of the highly reproducible EGLN1-DISC1 locus and HIF1AN gene in adaptation to moderate altitude provides additional information on adaptation mechanisms for normoxic and mild-hypoxic human populations. During ascent to ~2,500 m, oxygen pressure decreases to ~70% of that of sea level (Beall 2007). At this moderate altitude, peripheral chemoreceptors are stimulated and activity in the carotid sinus increases although no obvious increase in ventilation is observed (Mason 2000). In addition, a well-characterized impairment of cognitive function due to exposure to hypobaria hypoxia can be first detected at this altitude (Farmer et al. 1992). Generally, however, at this altitude the decrease in oxygen pressure should not generate too much difficulty in acclimatization for daily activities. Given the pivotal role of HIF in hypoxia-induced pulmonary hypertension (HPH) (Chen et al. 2006; Fu et al. 2008), an enhancement of the transcriptional activities of HIF should not be necessary; instead, enhancement may compromise fitness under normoxic and mild hypoxic conditions. Consistent with this hypothesis, we did not observe any genes that enhance the transcriptional activity of HIF in our study of populations at moderate altitudes. In contrast, HIF1AN and EGLN1/EPAS1 and EGLN2/EGLN3, representing two groups of enzymes that degrade HIF under normoxia (asparaginyl hydroxylase and prolyl hydroxylase) (Lendahl et al. 2009), were found among the candidate gene lists generated from sea-level and moderate-altitude populations. The association of EGLN1-DISC1 and HIF1AN were confirmed in our replication study that used highland Tibetan and moderate-altitude Han Chinese populations and these results further emphasize the contributions of these genes to normoxia and mild-hypoxia adaptation.

With the identification of EGLN1-DISC1 and HIF1AN from the HIF pathway having a role in normoxia and mild-hypoxia adaptation from the present work, we continued to systematically compare genome-wide adaptation signals, especially those for the HIF pathway genes, in response to quantitative variations of oxygen pressure over a wide range of altitudes. A careful reappraisal of six recent genome-wide scans in highland Tibetans and Andeans (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011) together with the current adaptation study for lowland and moderate-altitude Eurasian populations, we identified EGLN1 as a gene that existed on the candidate gene lists for all of these populations (table 2). In addition to EGLN1, each population also had unique genes from the HIF pathway that were involved in altitude adaptation (table 2). Examples include, EPAS1 for highland Tibetans (Beall et al. 2010; Bigham et al. 2010; Simonson et al. 2010; Yi et al. 2010; Peng et al. 2011; Xu et al. 2011), NOS2A and PRKAA1 for highland Andeans (Bigham et al. 2010), and HIF1AN for lowland and moderate-altitude Eurasian human populations. These results suggest that the HIF pathway tends.
to be inhibited under normoxia and mild-hypoxia, while activation of the HIF pathway would be preferable under extremely hypobaric hypoxia. Thus, in addition to the shared adaptation signal of \( \text{EGLN1} \), it is interesting to note that different mechanisms from the same HIF pathway are probably involved in adaptation to different altitudes with different oxygen pressures.

In summary, this study has shown that both the oxygen-sensing pathway and cardiopulmonary function underpin adaptation in Eurasian lowland and moderate-altitude human populations. Furthermore, the shared genetic adaptation signal of \( \text{EGLN1} \) emphasizes the role of the HIF pathway in hypoxia adaptation to the continuous gradient of altitude-specific habitats, while other HIF pathway genes, which are unique to each population, suggest different mechanisms of altering the HIF pathway were used. The present results provide new insights for future high altitude adaptation studies as well as sports medicine and the clinical treatment of patients with chronic hypoxia that resulted from various forms of clinical illness.

Links to Data Sets Used in This Study

HGDP-CEPH data set: ftp://ftp.cephb.fr/hgdp_supp1. Also geographic coordinates of the populations were from the HGDP-CEPH data set.

Altitude data were obtained from the World Meteorological Organization publication “1961–1990 Global Climate Normals,” which is also available from Hong Kong Observatory website (http://gb.weather.gov.hk).

Supplementary Material

Supplementary figure S1 and tables S1–S8 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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