Genetics Of Human Origin and Evolution: High-Altitude Adaptations

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Word Count: 2,090

Abstract (116/120):

High altitude, defined as elevations lying above 2,500 m sea level, challenges human survival and reproduction. This environment provides a natural experimental design wherein specific populations, Andeans, Ethiopians, and Tibetans, have lived in a chronic hypoxia state for millennia. These human groups have overcome the low ambient oxygen tension of high elevation via unique physiologic and genetic adaptations. Genomic studies have identified several genes that underlie high-altitude adaptive phenotypes, many of which are central components of the Hypoxia Inducible Factor (HIF) pathway. Further study of mechanisms governing the adaptive changes responsible for high-altitude adaptation will contribute to our understanding of the molecular basis of evolutionary change and assist in the functional annotation of the human genome.

Introduction

High-altitude hypoxia, experienced at elevations $\geq 2,500$ meters (m) presents numerous challenges to human health, survival, and reproduction as a result of decreased oxygen availability brought on by lowered barometric pressure at high elevations. High-altitude sojourners often suffer from acute mountain sickness whereas residents may experience chronic mountain sickness (CMS). Nonetheless, humans have resided for millennia in three high-altitude zones of the globe: the Qinghai-Tibetan Plateau, the Andean Altiplano, and the Semien Plateau in Ethiopia. Populations from each of these regions have adjusted to the rarified air via unique physiological adaptations that aid in the biological response to cellular hypoxia. Changes in pulmonary function, arterial oxygen saturation (SaO_2) hemoglobin concentration, and maternal physiology during pregnancy among others have permitted high-altitude natives to thrive in the harsh conditions (Table 1) [1]. Recent research indicates a genetic basis for these physiological differences, highlighting the contribution of genetic adaptation not simply acclimatization in high-altitude adaptive phenotypes [1-3]. Moreover, it has been proposed that high-altitude adaptation is one of the strongest instances of natural selection acting on humans [4]. Thus, these settings afford an unprecedented opportunity to study the effects of selective pressures on human variation and identify the underlying genetic mechanisms governing these populations' physiological adaptations. Here, I discuss what is known about the major genetic adaptations discovered in each of the three human groups. I then address avenues for productive future research.

Genetic adaptations to high-altitude hypoxia

Emerging genetic data support an evolutionary origin for high-altitude adaptive phenotypes. Furthermore, these data indicate local adaptation via population specific genetic changes wherein different genes have been acted upon by natural selection in each of the three high-altitude human populations. One genetic pathway has been implicated widely in human

high-altitude adaptation, the Hypoxia Inducible Factor (HIF) pathway. HIF is an evolutionarily ancient oxygen regulator that controls hundreds of downstream genes in response to cellular hypoxia. Andean Quechua and Aymara genomic scans for selection have revealed multiple candidate gene targets for natural selection including at least 40 HIF pathway genes as well as other non-HIF genes [5,6]. From these data, several genomic regions emerge as compelling candidates contributing to Andean altitude-adaptive phenotypes: the HIF genes *EGLN1*, *PRKAA1*, *NOS2*, *TGFA*, and *CXCR4* as well as a region on chromosome 12 falling between 109 and 113 megabase (mb) pairs [5,7]. In total, 47 genes are encoded by the chromosome 12 region and include those involved in cellular housekeeping and immunity. It is important to note that genes in this region have not been previously implicated in oxygen sensing or other altitude adaptive phenotypic pathways. Genome scans performed among high-altitude Argentinian Colla and an intermediate altitude population, Argentinian Calchaquíes, implicate HIF (*VEGFB*) and non-HIF genes (*ELTD1*, *PRKG1*) [8,9]. These findings point to the nitric oxide pathway as well as genes involved in cardiac reinforcement as part of the Andean adaptive toolkit. They further highlight that regional populations across the Andes may have experienced population specific genetic changes to hypoxia.

Of these candidate loci, *EGLN1*, displays compelling overlap with a second high-altitude adapted population, Tibetans [5,10]. *EGLN1* regulates the HIF transcriptional pathway via decreased hydroxylation of the HIF-1 α subunit (HIF-1 α) during cellular hypoxia [11-13]. This permits HIF1 to continually target downstream genes to maintain cellular oxygen homeostasis. Support for phenotypic consequence of *EGLN1* mutations comes from humans who possess loss of function mutations wherein heterozygous individuals display erythrocytosis (elevated hemoglobin concentration) [14,15]. Among Andeans, however, no associations between putative functional alleles at this locus and Andean altitude-adaptive phenotypes, including hemoglobin concentration, have been identified to date [16].

Variation near a second candidate locus, *PRKAA1*, has been linked to fetal growth and birth outcomes among Andeans [17]. Hypoxic stress restricts fetal growth and increases infant morbidity and mortality [18,19]. Indeed, birth weight declines an average of 102 g per 1000 m gain in altitude [20]. However, this effect is mitigated in women who are multi-generational residents of altitude. For example, Tibetan and Andean women give birth to larger babies than Han Chinese or European women, respectively, living at the same elevation regardless of socioeconomic status or gestational age [21-23]. Maternal variation at a SNP upstream of *PRKAA1* associates with birth weight as well as uterine artery diameter, an intermediate phenotype influencing fetal growth [17]. Furthermore, pointing to a functional link between variation at this site and advantageous Andean phenotypes, SNP genotype is associated with mRNA expression of pathways implicated in the pathophysiology of fetal growth restriction.

In addition to genome wide SNP scans for selection, targeted candidate-gene SNP typing and whole-genome sequencing (WGS) have identified loci contributing to high-altitude Andean phenotypes. One gene in particular, *ACE*, has been implicated in numerous studies of high-altitude adaptation. *ACE* is a central component of the renin-angiotensin-aldosterone (RAS) system that is involved in the regulation of cardiovascular homeostasis. The insertion (I) allele of a 47-bp insertion-deletion (I/D) polymorphism contributes to higher resting and exercise SaO₂ among Peruvian Quechua [24]. However, the I-allele frequency is as high or higher in several sea-level populations compared to high-altitude groups [25] and compelling evidence of natural selection operating at this locus is lacking. WGS of an Andean CMS cohort identified *ANP32D*, an oncogene, and *SENP1*, an enzymatic regulator of erythropoiesis, as possible targets of natural selection [26]. Functional work performed in drosophila indicated that down regulation of these two genes leads to increased hypoxia tolerance. These data indicate the potential for pharmaceutical intervention in treating CMS by targeting protein products of naturally selected genes. Combined, Andean genomic data that includes candidate gene studies, SNP selection screens, and WGS indicate Andean genetic adaptation to high altitude.

At this time, it is imperative to integrate genotype with phenotype data by assessing the impact of candidate SNPs on protein function. Only then will we be able to reveal how genetic changes translate into Andean altitude-adaptive phenotypes.

Among Tibetans, a wealth of data support genetic adaptation to high-altitude. Early studies demonstrated that SaO₂ and hemoglobin concentration show significant heritability in this population [27,28]. Subsequent genome-wide scans for natural selection identified several key HIF pathway genes involved in the Tibetan pattern of adaptation including *EPAS1* and *EGLN1* [4,5,10,29-32]. Both genes exhibit compelling evidence of natural selection and variants in these genes contribute to the low hemoglobin concentrations observed among Tibetans [4,10,29]. In particular, two, nonsynonymous *EGLN1* SNPs, D4E and C127S, are implicated in functional adaptive consequence [33-35]. In vitro assays indicate that the Tibetan (D4E/C127S) *EGLN1* haplotype is defective in its hydroxylation of HIF- α , leading to diminished down regulation of the HIF pathway [36]. Tibetans, therefore, may overcome hypoxic stress via a loss of function allele that permits continued activation of the HIF pathway.

Like *EGLN1*, *EPAS1* is a major upstream transcriptional regulator of the HIF pathway. In addition to associations between SNPs in this gene and hemoglobin concentration, recent data suggest that *EPAS1* promoter variants may contribute to birth weight among Tibetan newborns [37]. However, the *EPAS1* functional allele and its genetic mechanism remain to be determined. Further work identifying the causative mutation(s) contributing to Tibetan high-altitude adaptive phenotypes is necessary to characterize the molecular basis of the allele. Intriguingly, the functional Tibetan *EPAS1* allele may be derived from Denisovan, an extinct species of *Homo* discovered in Denisova Cave, Siberia, or Denisovan-related individuals [38].

Emerging genomic data suggest a role for additional HIF-related genes in the Tibetan adaptive landscape. *HMOX2* recently has been proposed as a modifier of hemoglobin metabolism [39]. Other long-term resident populations of the Qinghai-Tibetan plateau (Sherpa and Ayurveda) as well as recent migrants to the area (Mongolians) show patterns of natural

selection that are consistent with Tibetan adaptation [40-43]. Collectively the Tibetan pattern of genetic adaptation to high altitude indicates a suite of genes in the hypoxia pathway that contribute to the Tibetan pattern of high-altitude adaptation. Furthermore, Tibetan high-altitude adaptive variants have been shown to derive from an ancestral high-altitude population most closely related to modern day Sherpa [44]. Together with the Denisovan data, these findings indicate ancient human and archaic introgression to be important evolutionary sources for adaptive variation.

Ethiopian studies of high-altitude adaptation present a unique opportunity to work with closely related ethnic groups that dwell at both high and low altitude in the same geographic region. Among Ethiopian Amhara and Oromo highlanders, genomic scans have been performed on both SNP and WGS data to identify high-altitude adaptive genes [45-48]. These studies revealed distinct genes from the hypoxia pathway as well as genes in unique pathways of adaptation compared to Andean and Tibetan signatures of selection. Genes involved in the hypoxia response showing evidence of adaptive change among Ethiopians include *BHLHE41*, *ARNT2*, and *THRB* [46,47]. A gene-rich region on chromosome 19 containing several genes that are involved in vascular physiology, *CXCL17* and *PAFAH1B3*, or have been linked to hypoxia, *LIPE*, also exhibits a strong signature of selection [46-48]. Strong signatures of natural selection also have been identified for genes involved in pathogen defense (HLA-DR), angiogenesis (*VAV3*), and calcium uptake (*CBARA1*), as well as alcohol dehydrogenase genes [45,46]. Of interest, convergent evolution for alcohol dehydrogenase genes has been demonstrated between Tibetan and Andean populations [7]. This points to the potential of convergent evolution across all three high-altitude adapted human groups and possibly indicates adaptive phenotypes yet to be identified.

Linking genotype to phenotype, the phenotypic consequence of several SNPs on hemoglobin concentration has been demonstrated. Two genes, *ARNT2* and *THRB*, show suggestive relationships with hemoglobin concentration also among Amhara [46] and a region

on chromosome 1 significantly contributes to hemoglobin levels also among Amhara [45]. Furthermore, SNPs in *EGLN1* and *EPAS1* with demonstrated hemoglobin associations among Tibetans do not contribute to Ethiopian hemoglobin levels [45]. Together these results suggest a third pattern of genetic adaptation wherein Ethiopian highlanders responded to high altitude stress via a different set of genetic loci.

Future Directions

Recent advancements in the field of high-altitude genomics have begun to answer long-standing questions in high-altitude and evolutionary biology. It is imperative to move forward with research linking genotype to phenotype by identifying the causal mutation(s) responsible for the observed phenotypic adaptations and functionally characterizing the selected alleles. Substantial progress has been made to understand the functional consequence of *EGLN1* variation among Tibetans, but more effort is needed to expand these findings beyond a single locus in one high-altitude population. Until then, genes showing evidence of positive selection will remain putatively adaptive. Functional experimentation will be critical for answering evolutionary questions regarding the mode and tempo of these changes, helping to distinguish whether Andeans, Tibetans, and Ethiopians display convergent/parallel evolution in response to the same environmental pressure, gaining insight into the molecular mechanisms of adaptive change, and determining whether functionally different changes in the same gene lead to distinct altitude-adaptive phenotypes.

Genetic change alone does not fully explain the extent of variation observed in high-altitude adaptive phenotypes. Growth and development under hypoxic conditions also are hypothesized to contribute to physiological adaptations by altering an individual's genetic potential [49]. Andean pulmonary volume and SaO₂, for example, appear to be phenotypically plastic and shaped by hypoxia exposure during early development [50,51]. A second productive arena for future research is thus the characterization of epigenetic change that occurs during

growth and development at high-altitude. To date, a single study of epigenetic change has been performed showing significant CpG methylation differences among high- versus low-altitude Ethiopian Oromo [45]. Identifying epigenetic modifications associated with high-altitude exposure will enable us to mechanistically characterize the effects of growth and development at high altitude on gene expression. This may be particularly fruitful among Andeans, for whom developmental adaptation has been considered the main adaptive strategy [52,53]. Migrant study designs wherein both genetic and epigenetic changes are characterized in long-term residents of high-altitude compared to first and second generation down-migrants of high-altitude ancestry have the power to uncover the genomic and epigenomic architecture that together contribute to altitude-adaptive traits. By harnessing genetic and epigenetic methods to clarify the role of genetic adaptation versus developmental adaptation, altitude studies are poised to dig deeper into the 'omic basis of adaptive phenotypes.

Conclusions

High altitude is an ideal environment in which to study the evolutionary process as it provides scientists with a natural experiment design using separate, highland populations residing on three continents. These conditions afford an unprecedented opportunity to identify the underlying genetic mechanisms governing adaptive physiology. In addition, determining the mechanisms by which an identical environmental pressure results in similar or distinct genetic adaptations will allow evolutionary geneticists to better understand the molecular basis for convergent human adaptations. Such knowledge is likely to profoundly impact our understanding of the genetic control of biological traits as well as provide deeper insight into patterns of human genetic variation, their origins, and the evolutionary forces that maintain them. Furthermore, such knowledge will be important for identifying genetic factors involved in chronic ischemic diseases such as ischemic heart disease, cerebrovascular disease, and chronic obstructive pulmonary disease.

Acknowledgements

This research was supported by funds from the University of Michigan, The National Science Foundation (BCS-1132310), The National Institutes of Health (R33-HL-12075), and the American Association of Physical Anthropologists.

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***This research identifies a novel mechanism, admixture-mediated adaptation, of adaptation**

The modern Tibetan population is an admixed population comprised of ancestral groups related to present day Nepalese Sherpa and Han Chinese. The results indicate that admixture-mediated adaptation has been an important mechanism beyond selection through which Tibetans have adapted to hypoxia.

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Tables

Table 1. Physiologic adaptations to high-altitude hypoxia among high-altitude populations*

Phenotype	Andean	Tibetan	Ethiopian
Resting Ventilation	No increase	50% higher	NR
Hypoxic Ventilatory Response	Blunted (low)	Similar to sea-level	NR
Arterial Oxygen Saturation	Elevated	No increase	Elevated
Hemoglobin Concentration	Elevated	Lowered	Minimal Increase
Birth Weight	Elevated	Elevated	NR

*Adapted from [1].

Figure Legends

Figure 1. HIF pathway candidate genes for human adaptation to high altitude

The three geographic regions where humans have adapted to high altitude are highlighted in blue and include the Andean Altiplano, Semien Plateau, and the Tibetan Plateau. HIF pathway candidate genes for each population are listed in each of the population boxes. Genes listed in black are unique to the particular population, blue are shared between all three, green are shared between Tibetan and Andean, red are shared between Andean and Ethiopian, and purple are shared between Tibetan and Ethiopian.

Figure 1