

Genetic selection by high altitude: Beware of experiments at ambient conditions

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Evolutionary Adaptation to Hypoxia

The study of evolutionarily determined changes of the human genome and, in particular, the impact of extreme environments, has provided valuable information on human pathophysiology. The report by Stobdan et al. (1) is a nice demonstration of an evolutionary adaptation that would have escaped the prediction of its impact by studies of a mouse model under standard laboratory conditions.

Some human populations have adapted to very high altitudes, where the atmospheric pressure and oxygen are at such low tension that they are challenging to nonadapted humans. The resultant hypoxia can be associated with significant morbidity and even mortality, such as brain and lung edema, the principal characteristics of acute mountain sickness that sojourners venturing to the high mountains may encounter. Four populations of adapted high-mountain dwellers of diverse ethnicities have been investigated in great detail, and the adapted phenotypes and—in some instances—selected haplotypes have been reported (2, 3). These adapted populations include Tibetans (who are thus far the best studied); two separate Andean mountain dwellers, the Quechua and Aymara; and Ethiopians. Other mountain populations that may be adapted to prosper in extreme hypoxia are inhabitants of the Asian high Tian Shan mountain range, such as the Kyrgyz people, but these are as yet incompletely studied and their adaptation characteristics poorly phenotyped.

Much can be gained from the identification of evolutionarily selected genes by extreme hypoxia, definition of their function, and determination of their protection from or proclivity to defined pathophysiological states. Transcriptional modulators of hypoxic responses, transcription factors named hypoxia-inducible factors (HIFs), regulate transcription of an array of genes. HIFs are dimers of three separate α -subunit homologs and a single β -subunit: the constitutively expressed aryl hydrocarbon receptor nuclear translocator. These HIFs (i.e., HIF-1, HIF-2, and HIF-3) have often diverse activities within tissue-specific contexts (4). HIF-1

and HIF-2 are the best studied, and their levels and transcriptional activity are determined by the amount of their α -subunits, which are rapidly degraded by posttranslational regulation in the presence of oxygen, in the first step by prolyl hydroxylases (PHD enzymes). Then, their prolyl hydroxylated form interacts with von Hippel Lindau (VHL) protein, which leads to their ubiquitination and rapid degradation in proteasome. HIFs play essential roles in energy metabolism, erythropoiesis (HIF-1 was discovered from regulation of erythropoietin by hypoxia), development, immune response, cancer (the Warburg effect is largely determined by HIFs), and an array of other essential functions. Thus, ascertainment of hypoxia-modulated phenotypes in the evolutionarily adapted high-mountain dwellers has

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obvious importance in our understanding of common human maladies, with potential development of targeted therapies for many highly prevalent diseases, such as cerebral and pulmonary edema, pulmonary hypertension, and other yet to be determined HIF-regulated pathologies. The article by Stobdan et al. (1) also shows that lessons from Ethiopian adaptations may be harnessed for improved cardiac output in hypoxia, improved delivery of oxygen, and decreased ischemic tissue product lactate in critical tissues, including the brain.

Several inherited abnormalities of hypoxia sensing have been reported, the first described being Chuvash polycythemia that is endemic in the Chuvash Autonomous

Russian Republic and on the Italian island of Ischia, and is sporadic among other populations. It is caused by homozygosity for a hypomorphic-negative regulator of HIFs (i.e., the *VHL* gene) and leads to up-regulation of HIF-1 and HIF-2 (5) and is usually present on an ancient haplotype originating from a single founder 30,000–50,000 y ago (6). Other mutations of the HIF pathway associated with augmented hypoxia sensing are sporadic and caused either by heterozygosity for a loss-of-function allele of *PHD2*, [encoded by the *Egl* nine homolog 1 (*EGLN1*) gene] or a gain-of-function mutation of HIF-2 α [encoded by the endothelial PAS domain-containing protein 1 (*EPAS1*) gene] (7).

Interestingly, haplotypes of *EGLN1* and *EPAS1* genes were evolutionary-selected in Tibetans (8–11). Tibetan *EGLN1* has a Tibetan-specific missense variant of the *EGLN1* gene, c.12C > G (D4E), that is in linkage disequilibrium with a widely polymorphic missense variant, *EGLN1*:c.380G > C (C127S). This *EGLN1* variant has increased hydroxylase activity under hypoxic conditions (10). However, another group studying the *EGLN1* D4E/C127S variant demonstrated decreased interaction with the HIF-1 α co-chaperone p23 that would be expected to result in decreased hydroxylation and up-regulation of HIF α -subunits (12). Intriguingly, the Tibetan *EPAS1* haplotype is unique among modern humans and appears to have introgressed from the Neanderthal-related Denisovan genome (11); however, the functional significance of this haplotype remains to be determined. In contrast to Tibetans, in another three selected highland populations, the evolutionarily selected gene functions have not been interrogated and appear to be enriched compared with other populations, but do not appear to be unique to the Andean or Ethiopian highlanders.

It has been argued that in direct comparison of Tibetans and Andean highlanders with Ethiopians, the Ethiopians are best adapted to chronic hypoxia. Ethiopians have unique

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features, in addition to almost complete absence of chronic mountain sickness, such as: (i) enhanced vasodilatation in response to hypoxia with features of increased oxygen delivery to the brain, (ii) an improved ability to fully metabolize pyruvate in the Krebs cycle, and (iii) protection from high lactate-associated metabolic acidosis (13). Because the evolutionarily selected haplotypes, with the exception of an *EGLN1* missense mutation in Tibetans (10), are not associated with changes of exons that would directly impact on protein function, more can be learned from analysis of whole-genome sequencing. The ENCODE (Encyclopedia of DNA Elements) database provides information about those sequences that have been shown—or are likely—to change gene activity by epigenetic modulation, opening the chromatin, or by interfering with binding of transcription factors. Indeed, whole-genome sequencing was performed in Tibetan (11) and Ethiopian (14) highlander populations and reported in two 2014 papers. In the 2014 Ethiopian adaptation paper (14) published by the same group that has published their findings in PNAS (1), several Ethiopian selected genes were found to be evolutionarily selected and tested in *Drosophila* for survival benefit in hypoxia. One of these genes, which could not be tested for a role in hypoxic adaptation, as it did not have a fly ortholog, was *Endothelin receptor B (EDNRB)*. *EDNRB* encodes a receptor for endothelin 1, a vasoactive peptide that regulates vascular constriction. Endothelin 1 is a direct HIF target. Stobdan et al. (1) focused on the *EDNRB* gene because virtually all Ethiopian highlanders had several polymorphisms that were less likely to occur among Ethiopians living at lower altitudes,

and by ENCODE analyses these were predicted to interfere with binding of several transcription factors. Furthermore, an *EDNRB* inhibitor was shown to ameliorate pulmonary hypertension in high-altitude residents (15) and these authors argued that the decreased *EDNRB* activity may be beneficial in hypoxic adaptation of Ethiopian highlanders. They generated a *EDNRB* knockout mouse and found that homozygotes died as neonates. The testing of heterozygous mice revealed that at normoxia, or moderate hypoxia, no discernable phenotype was observed. However, at severe hypoxia at 5% or 1% O₂, the cardiac performance of heterozygous mice was superior and even at moderate hypoxia blood lactate levels were lower and, in contrast to control mice, the heterozygotes were not acidotic. In addition, the direct measurements of tissue oxygen revealed superior oxygenation in the heart, brain, and renal tissue of the heterozygous mice.

More Work Needed

Stobdan et al.'s (1) report is an elegant example of high-altitude evolutionary selection that has not yet been directly demonstrated to involve the HIF pathway but appears to exercise its benefits at hypoxia. The authors of this paper show that the testing of this gene in a mouse model at ambient laboratory conditions, or even interrogation of human cardiovascular and metabolic performance at sea level, would likely fail to detect the striking hypoxic advantage of the hypomorphic *EDNRB* allele. However, caution needs to be exercised to avoid overinterpretation of these results. As yet there is no evidence that this *EDNRB* Ethiopian-selected haplotype has decreased, or that there is even any modulation, of its receptor activity. More work will be needed to elucidate the function of *EDNRB* in Ethiopian hypoxic adaptation.

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