Human Evolution at High Altitude

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Abstract

This chapter reviews evidence that natural selection is acting or has acted on indigenous high-altitude populations of the Andean, Tibetan and East African plateaus and resulted in distinctive biological characteristics conferring vigor and health. It describes the results of classic era and genomic era approaches to detecting natural selection. Genomic era evidence of natural selection on high-altitude populations is accumulating rapidly and broadly supports that from the classic era. An important remaining step is to associate phenotypic with genomic variation and to associate them with survival and reproduction, the demographic currency of natural selection.

> Now, can it be doubted, from the struggle each individual has to obtain subsistence, that any minute variation in structure, habits, or instincts, adapting that individual better to the new conditions, would tell upon its vigour and health? In the struggle it would have a better chance of surviving; and those of its offspring which inherited the variation, be it ever so slight, would also have a better chance. ….. Let this work of selection on the one hand, and death on the other, go on for a thousand generations, who will pretend to affirm that it would produce no effect…? ([1] p. 49)

Introduction

Individuals moving to high altitude gradually encounter the "new condition" of hypobaric hypoxia and their responses vary widely. Darwin would have predicted that descendants from such a population resident at high altitude for a "thousand generations" would differ in "structure, habits, or instincts" from their colonizing ancestors. Consistent with such expectations, the "Andean man" – Quechua and Aymara populations residing on the altiplano – described by Carlos Monge C. and Alberto Hurtado starting in the 1930s had distinctive biological characteristics of [2–4]. The hypothesis that those distinctive characteristics result from evolution by natural selection has been considered formally since the 1960s [5].

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This chapter reviews evidence that natural selection is acting or has acted on indigenous high-altitude populations of the Andean, Tibetan and East African plateaus and resulted in distinctive biological characteristics conferring vigor and health. It examines briefly the state of knowledge up to the end of the last century reviewed by Niermeyer et al. in the predecessor to this volume [6]. They provided an insightful analysis of phenotypic evidence leading to the inference that natural selection has acted or is acting on high altitude populations. Because natural selection works on phenotypes with genetic variance, the rapid growth of genetic data and investigation since then has enabled new advances in our understanding. This chapter presents first the classic strategies—relying largely on phenotypes and protein sequences—and then the genomic strategies relying on DNA variation and sequences alone or in combination with phenotypes.

Background

Some elements of the process of evolution by natural selection are straightforward at high altitude. The unavoidable stress of hypobaric hypoxia is clear and so is the presence of distinctive traits such as the very high hemoglobin concentrations of Andean or exhaled nitric oxide levels of Tibetan highlanders [7, 8]. Other elements of the process are more challenging to detect: identifying inherited variation in traits offsetting high-altitude hypoxia and associating them with survival and reproduction. As a result, the intriguing hypothesis that high altitude hypoxia has been or is an agent of natural selection on highlanders remains the subject of intense work.

The laboratories for testing the natural selection hypothesis are the highland areas of the world populated by the more than 83 million people in 35 countries who live at 2,500 m or above, an altitude commonly used as a threshold for physiological response to hypobaric hypoxia. The estimate is based on census data from 1990 to 2005 matched to a global elevation grid (see Appendix). The majority of those people live between 2,500 and 3,000 m (Figs. 19.1 and 19.2). The relatively few residents of the highest altitudes where the altitude difference from sea level is largest and the stress is most severe are particularly informative for studies of natural selection.

Another important consideration is the length of time, and number of generations, that natural selection has had the opportunity to act on resident populations. Long-term human occupation of high-altitude environments varies globally. People have been using the Tibetan Plateau for a very long time. A recent authoritative review stated that "Although the data are sparse, both archaeology and genetics suggest that the plateau was occupied in the Late Pleistocene, perhaps as early as 30,000 yr ago, and that these early peoples have left a genetic signature in modern Tibetans. ….. Three areas of the plateau…have evidence of permanent settlements dating from ca. 6500, 5900, and 3750 yr ago, respectively" ([9] p. 141). The Andean Plateau has been occupied for some 11,500 years [10]. Maternal and paternal DNA link Andean skeletal remains from 650 to 1100 AD to modern highlanders [11]. The large, deep chest morphology of pre-contact skeletons also link ancient inhabitants to modern highlanders [12]. Less is known about the East African plateau where there is evidence of occupation at 2,300–2,400 m in Ethiopia as long ago as 70,000 years ago although only as recently as 5,000 years ago at 2,500 m or more [10, 13]. East Africa could have the longest or the shortest period of human adaptation of the three plateaus. There is insufficient evidence to reliably link these sites with any of the many modern ethnic groups in Ethiopia.

Considering a conservative model of 11,000 years or 440 human generations of 25 years each, a mutation occurring at a frequency of 1/10,000 or 0.0001 could reach a frequency of 1/2 or 0.5 if it increased in frequency by ~2 % each generation. Alternatively, if a founding population arrived with a pre-existing allele having a frequency of 1/100 or 0.01 and if the allele were beneficial at high altitude, it could reach a frequency of 0.5 if it increased by ~1.5 % each generation. These increases are in the range reported for the lactase persistence allele that reached a frequency of 77/100 or 0.77 among northern Europeans in 5–10,000 years [14–16]. Therefore, there has probably been sufficient length of residence on all three plateaus for a new mutation or

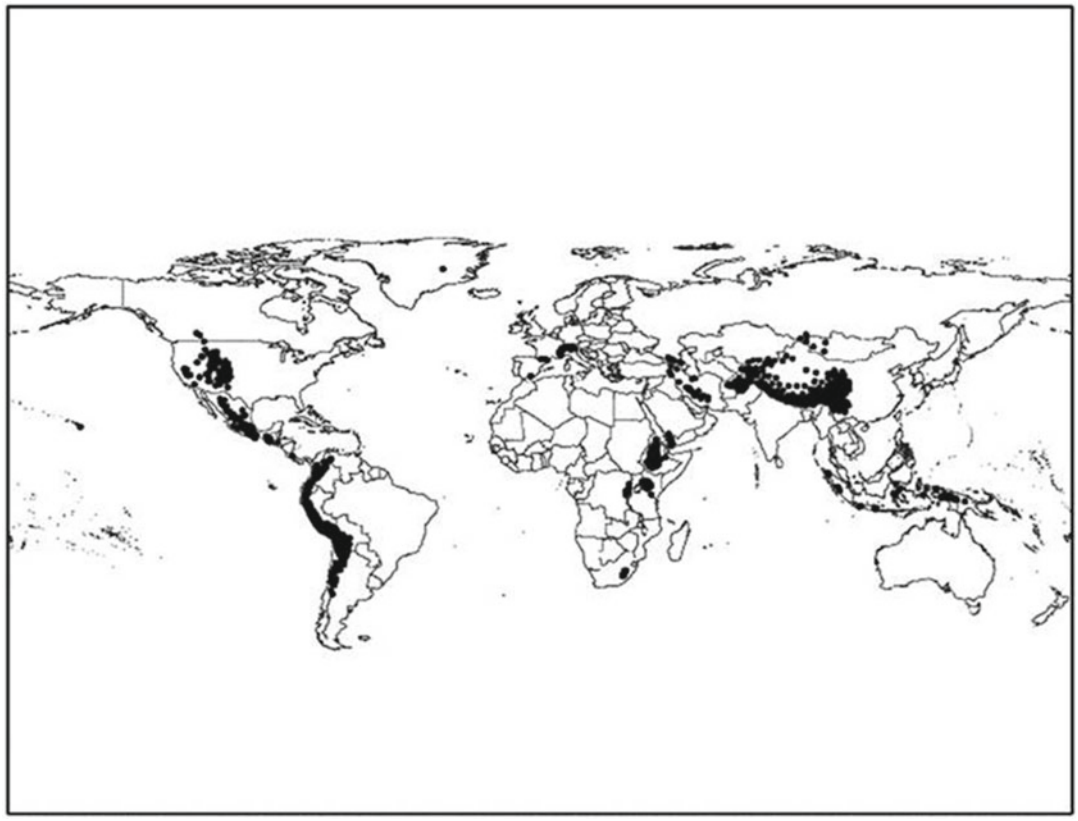

Fig. 19.1 Map of areas of the world where people live at 2,500 m or higher

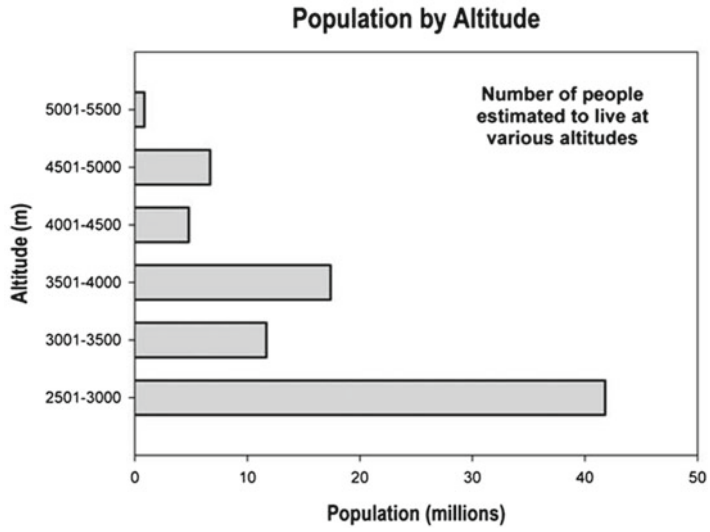

Fig. 19.2 The number of people estimated to live at various altitudes

an existing allele at a polymorphic locus to reach high frequencies in the indigenous populations.

Adaptive traits at high altitude, at least those we know about, are quantitative and continuously varying rather than present or absent as in the case of the lactase persistence phenotype. Some continuous traits are influenced by a single gene with a large quantitative effect, while others are influenced by many loci with small effects as well as by environmental factors. An example of the former is the protein level and activity of angiotensin-converting enzyme (ACE). About 45 % of the variance in ACE levels at sea level can be explained by an insertion/deletion polymorphism at a single locus [17, 18]. Height exemplifies the latter, complex, type of trait. Just 3 % of the variance in height is explained by the top 20 of 54 identified associated chromosomal regions [19]. In practice with natural populations having long lifespans and generations, such as humans, it is easier to detect natural selection on traits influenced by a single or a few loci with large effects because the potential change in a trait from one generation to the next is directly proportional to the large heritable variance.

Research Strategies

Classic Era

1. A classic strategy to detect natural selection correlates variation in traits with variation in the selective factor to infer that it is or was operating [20]. A large altitude range providing a wide range of the selective factor is desirable for such studies. Most studies implicitly or explicitly consider that hypobaric hypoxia is the selective factor although temperature, ultraviolet radiation, and infectious disease are among other potential selective factors that generally vary with altitude. Altitude gradients of a phenotype can be impractical for a single investigator to assemble. As a result, many studies contrast one or two high altitudes with a low altitude control or combine the results of studies at various altitudes to produce a composite gradient. Phenotypic variation across altitude gradients is well-documented for many traits. For example, birth weight declines linearly [21] while hemoglobin increases exponentially with altitude in the Andes [22, 23].

In contrast to the many phenotypic examples, decades of attempts to identify altitude gradients in allele frequencies were unsuccessful. For example, analyses of 22 protein coding loci and seven loci involved in red blood cell glycolysis assayed in nearly 2,100 people along an altitude gradient from 300 to 4,000 m in the Chilean Andes found no allele frequency gradient [24, 25]. Similarly, there was no gradient in genetic variance of four quantitative red blood cell traits (hemoglobin concentration, hematocrit, 2,3-diphosphoglycerate (DPG), and adenosine triphosphate (ATP) levels) as would be expected if natural selection had reduced variance at high altitude [26].

The authors' suggested reasons for failing to detect an altitude gradient remain relevant for designing and interpreting studies nowadays and bear repeating. They wrote "Not finding evidence for genetic selection may be because of several factors. The physiological responses seen in residents of high altitudes and assumed to be adaptive may simply be within the normal range of physiological response in human populations. The Aymara migration from the altiplano [Andean plateau] to lower altitudes may be too recent for genetic changes to be detected. Finally, the particular genetic loci studied may be too remotely related to the physiology of high altitude adaptation to be used in studying adaptation to hypoxia" ([24] p. 101).

Two recent successful instances of identifying an altitude gradient in allele frequency showed that important genes in crucial pathways can evolve. These genes were in a pathway that regulates oxygen homeostasis in multicellular animals [27]. The HIF1 pathway is named after the master regulator of oxygen homeostasis, the hypoxia inducible factor 1 (HIF) discovered a decade or more after those early studies of altitude gradients. One study reported that certain variants in a key oxygen sensor *EGLN1* in the oxygen homeostasis system were more frequent among high- than low-altitude Tibeto-Burman samples in India [28]. *EGLN1* is also known as *PHD2*. Table 19.1 lists

Table 19.1 Genetic loci mentioned in this chapter and identified by the HUGO gene nomenclature committee (HGNC) (available at http://www.genenames.org)

Approved symbol for gene	HGNC gene ID #	Name or alias	Chromosome location	General role in hypoxia sensing or responding to HIF
ACE	2707	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	17q23	HIF induced
HIF1A	4910	Hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	14q23.2	Hypoxia stabilizes
EPAS1	3374	*HIF2A*, endothelial PAS domain protein 1	2p21-p16	Hypoxia stabilizes
EGLN1	1232	*PHD2*, egl nine homolog 1 (*C. elegans*)	1q42.1	Tags HIF-alpha for binding to VHL
VHL	2687	VHL1, von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	3p25.3	Targets HIF1A for degradation
PRKAA1	9376	*AMPKa1*, protein kinase, AMP-activated, alpha 1 catalytic subunit	5p12	Along with HIF1A regulates energy metabolism
NOS2A	7873	*iNOS*, nitric oxide synthase 2, inducible	17q11.2-q12	HIF1 induced
NOS3	7876	*eNOS*, nitric oxide synthase 3 (endothelial cell)	7q36	Hypoxia down-regulates
EPO	3415	Erythropoietin	7q21	HIF1 induced
EDN1	3176	Endothelin-1, *ET1*	6p24.1	HIF1 induced
CBARA1	1530	Mitochondrial calcium uptake 1	10q22.1	No known association with HIFs
VAV3	12659	vav 3 guanine nucleotide exchange factor	1p13.3	No known association with HIFs
ARNT2	16876	Aryl-hydrocarbon receptor nuclear translocator 2	15q25.1	Protein forms dimer with HIF1A
THRB	11799	Thyroid hormone receptor, beta	3p24.2	No known association with HIFs

the genes mentioned in this chapter along with their names and chromosomal locations. The second recent study confirmed variation in *EGLN1* along an altitude gradient among East Asian samples and added variation in *EPAS1* (also known as HIF2a or hypoxic inducible factor 2) [29]. *EPAS1* binds to another protein to form HIF2, a second transcription factor regulating the expression of hundreds of loci involved in the response to hypoxia [30] such as the erythropoietin (*EPO*) locus.

2. A second classic strategy involves perturbing natural populations in order to infer the past action of natural selection. This is implemented in human populations with the "migrant model" comparing residents and migrants from one altitude to another [31]. It interprets differences between high altitude residents and upward migrants in the mean values of a trait associated with good function or offsetting hypoxia as evidence for a genetic basis for the trait in the high-altitude population. This strategy is somewhat like comparing the phenotypes of contemporary high-altitude populations after thousands of years of opportunity for natural selection with those of the early colonists adapting to high altitude by acclimatization or development. An informative illustration is a comparison of two samples of highland Andean ancestry

Table 19.2 Differences in adaptive success between lifelong high-altitude natives and acclimatized newcomers (updated from ([6], p. 84)), types of supportive evidence for the past action of natural selection, and disease indicators of unsuccessful adaptation. The differences favor better function among natives and seem to differ from the normal range of homeostatic response to hypoxia. These patterns form a major body of evidence that natural selection has modified the biological characteristics of high-altitude natives

Altitude natives—acclimatized newcomer differences	Altitude gradient evidence	Altitude extremes evidence	Diseases indicating unsuccessful adaptation
Source: Niermeyer et al. [6]			
Less intrauterine growth retardation	Present	Present	Low birthweight, pre-eclampsia [131, 132]
Better neonatal oxygenation and involution of fetal cardiopulmonary characteristics	Not enough data	Present	Elevated pulmonary artery pressure, increased prevalence of patent ductus arteriosus [133, 134]
Enlarged lung volumes and deceased alveolar-arterial oxygen diffusion gradients	Not enough data	Present	–
Higher maximal exercise capacity	Present	Present	–
Better maintained increase in cerebral blood flow during exercise	Not enough data	Present	–
Lower hemoglobin concentration (Tibetans only)	Present	Present	Andean excessive erythrocytosis [135]
Less susceptibility to chronic mountain sickness (CMS) (Tibetans only)	Not enough data	Present	Chronic mountain sickness
Update since [6]			
Higher exhaled nitric oxide (Tibetans only)	Not enough data	Present	Excessive hypoxia pulmonary vasoconstriction [38]
Physiological responses to pregnancy favor good outcome	Not enough data	Present	Higher neonatal mortality [39, 136]

born and raised at high and at low altitude with two samples of lowland European ancestry born and raised at high and at low altitude. It revealed that both populations had larger forced vital capacities if they grew up at high altitude. However, a larger effect among those of Andean descent indicated that both developmental exposure and genetic ancestry contributed to the trait of large forced vital capacity at high altitude [32].

Relying largely on evidence obtained using these two strategies, Niermeyer et al. [6] identified seven "differences in adaptive success between natives and newcomers…." To the extent possible, those authors took care to consider the alternative hypothesis that developmental adaptation or acclimatization accounted for the differences. The differences in all traits were in the direction of better function for the indigenous high-altitude populations. The traits were less intrauterine growth retardation, better neonatal oxygenation and involution of fetal cardiopulmonary characteristics, enlarged lung volumes and decreased alveolar-arterial oxygen diffusion gradients, higher maximal work capacity, better maintained increase in cerebral blood flow during exercise, lower hemoglobin concentrations (Tibetans only), and less susceptibility to chronic mountain sickness (CMS) (Tibetans only) among natives than among newcomers (Table 19.2).

Subsequent work has added two more traits to the list: higher nitric oxide levels among highlanders (Tibetans only) and higher uterine artery blood flow and oxygen delivery during pregnancy. Acute exposure to hypoxia in lowlanders causes a decrease in exhaled nitric oxide followed by a return to baseline or slightly above [33–38], however they do not elevate levels to those observed among Tibetan highlanders [8, 37]. Similarly, women who migrated themselves or

whose recent ancestors migrated, do not increase uterine artery blood flow to the same degree as highland women [39]. Table 19.2 also lists some diseases of the reproductive, cardiovascular, and hematological systems reflecting unsuccessful adaptation that may have beset early colonists such as pre-eclampsia, pulmonary hypertension, congenital heart anomalies, excessive erythrocytosis, and CMS. Genetic variants contributing to the differences in adaptive success would likely have increased in frequency along with those associated with decreased vulnerability to the diseases.

The "admixture model" adapts the migration strategy to take advantage of gene flow occurring in the Andes over the past 500 years of European and indigenous highlanders' co-residence and potential for intermarriage. Blending classic and genomic strategies, it quantifies individuals' proportion of Native American ancestry on a scale ranging from 0 to 100 % as assessed by a panel of ancestry informative single nucleotide polymorphisms (SNPs) [40]. For example, a sample of low-altitude natives with an average estimated "Native American Ancestry Proportion" (NAAP) of 85 % was perturbed by a trip to 4,338 m and tested after 10–12 h there. Higher NAAP correlated with lower hypoxic ventilatory response after 10 min of experimental hypoxia at high altitude and with lower ventilation during exercise, both characteristics of Andean highlanders that differ from those of Tibetans. The authors asserted that this was "the first direct evidence that ventilatory traits, probably unique to Andeans, have a population genetic basis. Our quantification of ancestry as an independent variable has led us to infer both a genetic mechanism and an evolutionary origin for these traits" ([40] p. R232). Continuing with that study design, lowlanders with a high proportion of Andean ancestry experienced a smaller fall in maximal oxygen consumption tested within 24 h at 4,338 m [41]. However, there was no similar evidence for forced vital capacity or maximal oxygen consumption [42]. Those results imply that natural selection produced a distinctive Andean gene pool at unknown loci influencing some although not all distinctive Andean traits.

3. A third classic strategy to detect natural selection is the cross-population strategy comparing multiple indigenous populations exposed to the same environment [43]. If phenotypes differ among populations exposed to the same stress, after taking into account potential confounding factors, it suggests that natural selection favored different responses. Table 19.3 presents the compilation by Niermeyer et al. [6] summarizing phenotypic differences between Tibetan and Andean highlanders. It includes less intrauterine growth retardation, greater reliance on redistribution of blood flow than elevated arterial oxygen content to increase uteroplacental oxygen delivery during pregnancy, higher resting ventilation and hypoxic ventilatory responsiveness, less hypoxic vasoconstriction as measured by lower pulmonary arterial pressure and resistance, less susceptibility to CMS among Tibetan than among Andean highlanders.

Additions to that list include evidence of higher nitric oxide levels among Tibetans than among Andean highlanders and the direct association of nitric oxide levels with pulmonary and systemic blood flow among Tibetans [38, 44, 45]. Another addition may be higher cerebral blood flow among Tibetan than among Andean highlanders [46]. The addition of lower percent of oxygen saturation of hemoglobin reflects findings of studies using the same protocol [47, 48]. A possible deletion is lower pulmonary artery pressure among Tibetans than Andean highlanders. Two recent studies of minimally elevated pulmonary artery pressure in samples of healthy, non-miners of Andean ancestry reported values very similar to the low values among Tibetans [49, 50]. The explanation for the difference between those and earlier studies of pulmonary hypertension among Andean highlanders could be that many, although not all, early studies of this trait were conducted with samples of miners in the Central Andes. There they may have been confounded by non-altitude-related environmental factors. Later studies found elevated cobalt levels in the water that exaggerated the hemoglobin concentration levels and have caused cardiomyopathy and elevated pulmonary artery

Table 19.3 Differences in adaptive success between Tibetan and Andean highlanders (updated from [6]), and relevant measures of genetic variance and findings from genomic studies. The measures inform about the possibility of ongoing selection for the traits because selection works on variation than can be inherited. This information guides study interpretation by directing attention to information needed to study ongoing natural selection

Tibetan and Andean highlanders—differences	Presence of genetic variance
Source: Niermeyer et al. [6]	
Tibetans have less intrauterine growth retardation	No data
Tibetans have greater reliance on redistribution of blood flow than elevated arterial oxygen content to increase uteroplacental oxygen delivery during pregnancy	No data
Tibetans have higher levels of resting ventilation and hypoxic ventilatory responsiveness	h^2 for resting ventilation is 0.32 and insignificant; for hypoxic ventilatory responsiveness it is 0.35 and 0.22, for Tibetans and Andean highlanders respectively; admixture studies identify Andean adaptations [40, 41, 91]
Tibetans have less hypoxic vasoconstriction as measured by lower pulmonary arterial pressure and resistance	No data
Tibetans have lower hemoglobin concentration	h^2 is 0.61 and 0.89 for Tibetans and Andean highlanders; respectively; Tibetans have high frequencies of alleles associated with lower hemoglobin concentration [89–91]
Tibetans are less susceptible to chronic mountain sickness	No data; variance in some loci associated with hemoglobin are excluded for Andean highlanders, genome-wide analyses found no associations [114]
Update since [6]	
Tibetans have lower percent of oxygen saturation of hemoglobin when measured using the same protocol	h^2 ranges from 0.33 to 0.47 for Tibetans and is insignificant for Andean highlanders; ACE and other genotypes contribute to variation among Andean highlanders [41, 118]
Tibetans have higher exhaled nitric oxide	No data

pressure at low altitude [51, 52]. Similarly, experimental evidence shows that iron status influences hemoglobin levels at high altitude [53] and that iron supplementation may reduce pulmonary artery pressure under hypoxia [54]. Those findings emphasize the need for considering a growing number of known confounding factors in order to have the most informative phenotypic data.

Information on East African highlanders, specifically of the Amhara ethnic group of Ethiopia, is sparse although accumulating. Consider the evidence for differences in adaptive success between high-altitude natives and acclimatized newcomers (Table 19.2), Amhara highlanders have large lung volumes, lower hemoglobin concentrations and higher oxygen saturations of hemoglobin [55, 56]. Considering the cross-population differences among highlanders (Table 19.3), the evidence remains sparse. With respect to intrauterine growth retardation as measured by birthweight, publications from Ethiopia are not very informative about altitude. They generally do not distinguish among ethnic groups, are usually put into the context of problems and interventions to raise birthweights and are available for the relatively low and narrow altitude range of 1,300–2,300 m. Mean birthweights for healthy singleton hospital deliveries were in the 3.1–3.3 kg range [57–63]. A focused study of altitude effects on intrauterine growth in East African would be valuable. The birthweights are 100–200 g lower than predicted for Tibetan, Andean or European populations at the corresponding altitudes although they are higher than predicted for Han Chinese [64].

The Amhara have the highest systolic pulmonary artery pressures among samples of the three populations along with high pulmonary blood flow and low pulmonary vascular resistance [65]. That pattern of high pressure accompanied by high flow and low resistance is different from the classic pattern based on the Andean model of high pressure and resistance owing to pulmonary vasoconstriction.

Continuing a trend of investigating blood flow, a study found that the cerebral circulation of Amhara was relatively insensitive to hypoxia as compared with Andean highlanders and concluded that such a response could contribute to high cerebral blood flow at altitude rather than the usual response of reducing cerebral blood flow [66].

With respect to hematological traits, early evidence suggested that Amhara adapted like the Tibetans in the sense of having little altitude-associated increase in hemoglobin concentration and uniquely in the sense of having little decrease in oxygen saturation of hemoglobin. Later work has shown that Amhara can increase hemoglobin concentration and decrease oxygen saturation somewhat at altitudes above 3,500 m [55, 65, 67]. That suggests that the Amhara threshold for hematological response lies in between Andean and Tibetan populations. However, excessive erythrocytosis measured in terms of very high hemoglobin concentration has not been observed.

Although measures of exhaled nitric oxide have not been made, urinary measures of total body nitric oxide synthesis show that Amhara at high and low altitude [65] have lower synthesis than Tibetans. Finally on the list evaluating possible population differences, a report on physiological responses to pregnancy found pre-eclampsia at about 5 % [68] in a hospital at 2,300 m. The rate in the US varies from roughly 1 to 5 % depending on ethnicity [69]. Because pre-eclampsia is generally more prevalent in developing countries [70], it is probably inappropriate to infer with these data any association with altitude. Overall, although data are sparse, they suggest the possibility that Amhara high-altitude natives in Ethiopia may represent a third pattern of population adaptation to high altitude, one with distinctive hematological and cardiovascular characteristics.

4. Quantitative and statistical genetics techniques are additional classic strategies. Quantitative genetics estimate the heritability (h^2), the proportion of total variance in a trait that is accounted for by biological kinship among individuals in a population. It may range from 0 to 1.0 with zero indicating no genetic contribution and one indicating no non-genetic contribution to variation in the trait. Because natural selection requires genetic variance, a significant h^2 indicates the potential for ongoing natural selection. A lack of genetic variance indicates no current potential for ongoing selection, perhaps because a past selective sweep removed it or perhaps another trait is preventing its expression. Tibetan samples generally have higher genetic variance, in the range of 0.3–0.7 commonly considered moderate to high levels, than Andean samples with the exception of hemoglobin concentration for which both have high variance (Table 19.3). Research using genomic techniques described below confirmed and extended that evidence of genetic variance.

Complex segregation analyses test the hypothesis that levels of a quantitative trait are inherited in Mendelian fashion. Such analyses detected a major gene at an unknown locus with an autosomal dominant mode of inheritance among Tibetans associated with 6–10 % higher percent of oxygen saturation of hemoglobin. Tibetan women estimated with high probability to have one or two copies of the inferred autosomal dominant allele had more than twice as many living children as compared with women estimated to be homozygous recessive for the low saturation allele [71]. That evidence linking inferred genotypes with offspring survival suggests that very strong natural selection is increasing the frequency of the inferred allele at the unknown locus.

The cross-species strategy compares multiple species exposed to the same environment [43]. Traits common to a wide range of organisms with long histories of high-altitude habitats may indicate expression of a common inherited response. The few data about our closest biological relatives, other primate species, come from Old World monkeys. Upon acute exposure, pig-tailed macaques increase hemoglobin concentration [72], suggesting an inherited response in common with human visitors to altitude and Andean highlanders. Mitochondrial DNA shows evidence of altitude differences in allele frequencies among snub-nosed macaques resident at 4,000 m [73].

Data are relatively abundant for more distantly related species. An illustration is deer mice populations resident along an altitude gradient in the Rocky Mountains of the U.S. that exhibit a

Table 19.4 Characteristics of genotypically and phenotypically adapted high-altitude animals (categories identified by [80]) compared with cross-population evidence about Tibetan and Andean high-altitude native phenotypes. Tibetans share several traits in the genotypically adapted category while Andean highlanders share all but one of the traits in the phenotypically adapted category. This was further evidence that past natural selection might be more evident among Tibetans

Trait	Genotypically adapted high-altitude animals	Tibetan high-altitude natives	Andean high-altitude natives	Phenotypically adapted high-altitude animals
High hemoglobin affinity (low p50)	Present	Absent	Absent	Absent
Moderate or absent polycythemia	Present	Present	Absent	Absent
Low venous pO_2	Present	No data	Present	Absent
Thin-walled pulmonary vascular tree that responds moderately to hypoxia	Present	Present	Absent	Absent
Absence of chronic mountain sickness (CMS)	Present	Present	Absent	Absent

parallel gradient of higher frequency at higher altitude of alleles in the beta chain of hemoglobin that increase oxygen affinity [74]. High oxygen affinity of hemoglobin has been reported for numerous high-altitude species and is accomplished by at least two different mechanisms, although people on the Andean and Tibetan Plateaus do not have the trait [75–78]. Instead, visitors, Tibetan and Andean highlanders achieve lower oxygen affinity as a result of high levels of the red blood cell enzyme 2, 3 DPG that lowers oxygen binding to hemoglobin [79]. High affinity enhances pulmonary loading while low affinity enhances tissue offloading of oxygen. That response, however, is offset by higher ventilation [76].

An influential cross-species analyses by Monge and Leon-Velarde proposed that high-altitude animals such as llamas and yak are "genotypically adapted" while others such as cows introduced in the past few 100 years are "phenotypically adapted" [80]. According to their analysis, genotypically adapted organisms have high hemoglobin affinity, moderate or absent polycythemia, low venous pO_2, thin-walled pulmonary vascular trees that respond moderately to hypoxia, and the absence of CMS. Table 19.4 presents the five traits characterizing the two categories of adaptations and indicates the phenotypic resemblance of Tibetan and Andean highlanders to one or the other category of adaptation. Tibetans exhibit three of the genotypically adapted phenotypic features—moderate or absent polycythemia, thin-walled pulmonary vasculature, little CMS, do not exhibit high hemoglobin affinity, and there are no data on venous pO_2. In contrast, Andean highlanders exhibit four of the phenotypically adapted phenotypic features but not a fifth, low venous pO_2 [81]. The concept of cryptic adaptive evolution has been proposed to explain the contrast between genotypically and phenotypically adapted species. It reasons that "In cases in which the acclimatization response to hypoxia is maladaptive, selection will favor an attenuation of the induced phenotypic change" ([79] p. 4125). Beneficial outcomes include maintaining adaptability to further stress and avoiding the costs of sustained acclimatization responses. Some of the Tibetan—Andean contrasts can be interpreted with this concept, for example the relatively dampened hemoglobin response and relatively low pulmonary artery pressures of the Tibetans. That is, those phenotypes may resemble those of lowlanders as a result of selection at high altitude.

Thus, a large body of mainly phenotypic data supports the hypothesis that natural selection has acted on indigenous highland populations of the Andean and Tibetan Plateaus. There are links with reproductive success. The situation on the East African Plateau is not clear because of lack of information. These informative studies have generally not dealt with specific genetic loci or variants. Thus it has not been possible to test formal population genetics "null models" of no selection. That situation has changed rapidly with the implementation of genomic strategies [82–85].

Genomic Era

Genomic-Wide Approach

Since the completion of the first survey of the human genome in 2000 and especially since the publication of the first haplotype map of the human genome in 2005 [86], the genomic era is providing an abundance of new strategies to detect "signals of natural selection" in the genome [83, 86–88]. The genomic era burst onto the high-altitude scene in 2010–2011 with the publication of seven articles reporting signals of selection detected in Tibetan samples, three of which also reported specific genotypes that associated with the distinctively low hemoglobin concentration of Tibetans [29, 89–95]. Extensive analysis and commentary followed as did a report on an East African sample [79, 95–103].

It is extraordinary that every one of the seven studies of Tibetans identified *EPAS1* (*HIF2A*) and four identified *EGLN1* (*PHD2*) loci as playing a role that population's genetic adaptation to high altitude. More than a dozen samples of Tibetans across a wide swathe of the plateau provided evidence. It is extraordinary for the degree of consensus and replication among studies with different designs and analyses as well as extraordinary because those two loci are central to the pathways regulating oxygen homeostasis in all vertebrates [104–106]. This ancient homeostatic biochemical system that participates in many biochemical pathways has apparently tolerated adaptive variants [107]. A change in HIF regulation by oxygen could be one route to efficiently cause many adaptive responses. Yet the results of that cascade and others it initiates could be too far-reaching and have maladaptive consequences. So far it appears that the Tibetan population benefits. The causal mutation(s) (and whether it is indeed new or unique to Tibetans) and the functional links between genotype and phenotype remained to be discovered. The extent to which hemoglobin levels are the target of selection or a pleiotropic manifestation of selection on another trait is another important point to address in future work.

Such genomic strategies differ fundamentally from the classic strategies seeking to discover and explain the distinctive phenotypes of high-altitude natives. Genomic strategies are potentially useful for identifying inductively areas of the genome and thus certain loci under selection that may underlie distinctive high-altitude phenotypes traits or even suggest new phenotypes to investigate. That is, they can be applied without prior knowledge of the relevant loci or phenotypes. This may be counterintuitive to many scientists studying high-altitude adaptations who are intensely interested in the phenotypes involved in maintaining homeostasis under severe and chronic stress. Yet, the two strategies are complementary and ask related questions using different data and outcome measures.

The basic concept of genomic signals of natural selection involves interpreting patterns of similarity or differences in variation emerging after genotyping SNPs at (usually) hundreds of thousands or even more than a million loci throughout the entire genome of individuals in samples of one or more populations. "This approach is based on the idea that natural selection introduced a local perturbation in the patterns of neutral genetic variation surrounding an advantageous allele relative to regions where variation is shaped only by genetic drift" ([83] p. 198).

Analyzing signals of natural selection can involve (a) one of several measures of allele frequency differences between two or more samples, ideally including the ancestral and descendent populations or (b) identifying haplotypes, measuring haplotype frequencies, and haplotype homozygosity within or between samples. Haplotypes are combinations of polymorphisms along a stretch of DNA; in the case of SNPs, many regions of the genome have just a few combinations. For the purposes of analyzing natural selection, haplotypes are generally identified as groups of alleles on a chromosome that are inherited together as a block because "they are descended from a single ancestral chromosome" [83]. A high frequency of a distinctive haplotype is evidence of past positive natural selection for a mutation in that group of SNPs inherited together. The causal mutation could be in a measured SNP or an unmeasured one inherited in the same block. If the haplotype is universal in a sample then that is evidence that a selective sweep occurred.

Genome-wide signals have different strengths to detect selection depending on what happened. A variety of signals of natural selection were measured in the studies of Tibetans because there was no prior knowledge of what had happened. Relevant features to consider when designing and interpreting studies include whether the current frequency of the selected allele or haplotype is moderate (50–80 %) or high (more than 90 %), whether selection occurred on standing or on new variants, and the length of time an allele existed before selection and how long since selection began [83, 108, 109]. For example, some alleles with frequencies in the range of 50–80 % in Tibetan samples had frequencies of just 10–30 % in the HapMap Han sample [94]. The moderately high frequency in the Tibetan samples means that an approach relying solely on cross-population analysis of haplotypes might have been unsuccessful because there is less statistical power when allele frequencies are moderate. However, *EPAS1* alleles associated with lowered hemoglobin concentration among Tibetans had larger differences with allele frequencies of 80 % or more among Tibetans and 20 % or lower among the HapMap Han [89]. That is in the range where cross-population extended haplotype homozygosity measures have the most statistical power [108, 109]. The SNP analyses in that study were based on a custom array designed to genotype *EPAS1* as a candidate locus. Many of those SNPs are not included on commercial arrays.

With respect to the age of the allele, some of the variants found in high frequency among Tibetans are found globally which implies that they are very old. If so, then selection had a long time to act before the ancestral population migrated to Tibet and for recombination to occur that would remove some types of selection signals. Similarly, because at least some Tibetans have been at altitude for tens of millennia, there would have been time for haplotypes to decay due to recombination. Both historical features could compromise approaches based on haplotype length [29, 109]. For such reasons, most studies report the results of multiple signals of selection.

The effect of the high-frequency-in-Tibetans alleles on hemoglobin concentration varied among samples. The largest was a 1.7 g/dL decrease in hemoglobin concentration with each additional copy of the Tibetan haplotype. That was reported for a sample that included people who would have been excluded from a study of normal variation because of low values in the range of anemia and high values in the range of polycythemia [90]. The smallest effect, yet still appreciable at around one-half a standard deviation, was 0.8 g/dL lower hemoglobin with each additional copy of the Tibetan SNP allele. That was reported for a sample that had been extensively screened to include only normal, healthy individuals outside the normal range of variation [89]. Both findings among Tibetans contrast with those from a genome-wide analysis among more than 24,000 low altitude Europeans. It detected tiny associations with hemoglobin concentration ranging only as high as 0.06 g/dL [110] and did not identify the loci that emerged as influential among Tibetans. The contrast illustrates again information based on loci identified or excluded on the basis of associations at low altitude may not apply to high-altitude populations. Interestingly, the loci associated with hemoglobin concentration among Tibetans variation was not associated with percent of oxygen saturation of hemoglobin [90, 91].

Similar genomic analyses of an Andean sample identified *EGLN1*, *PRKAA1*, and *NOS2A* as possible candidates for having undergone selection although no tests of association with high-altitude phenotypes have been reported [92, 93]. The Andean highlanders from two ethnic groups were compared with highland Tibetans, lowland Mesoamericans, Europeans, and East Asians. The SNPs identified in *EGLN1* in the Andean samples were not the same as those identified in the Tibetan samples described above. Those analyses are consistent with the known pattern of different phenotypic patterns among highland Tibetan and Andean natives (Table 19.3).

A highland Amhara population from Ethiopia was compared with two unrelated lowland populations and four completely different loci were identified as candidates for having undergone selection—*CBARA1*, *VAV3*, *ARNT2*, and *THR*. Those results support a hypothesis of a different history of natural selection. Tests of association

with hemoglobin concentration were undertaken without finding significant results, perhaps because more than half of one of the low altitude samples had hemoglobin levels below the usual cutoff for anemia [96]. A different highland Amhara sample from Ethiopia was compared with an Amhara lowland sample. The samples were screened for potential confounding factors that could influence hemoglobin concentration. The study found no evidence that *EPAS1* or *EGLN1* variants associated with hemoglobin concentration or oxygen saturation of hemoglobin. Instead, using a genome-wide analysis, it detected a single variant at another locus not associated with a currently known gene that associated with hemoglobin concentration at both high and low altitudes [56]. These findings suggest the possibility of convergent evolution on the same phenotype of dampened hematological responses to high-altitude hypoxia using different genetic pathways.

In contrast to the genome-wide approaches, candidate gene approaches are hypothesis-driven and have been described succinctly as follows. "Such studies rely on intelligent reviews of all the available scientific literature and the proposal of a system thought to be of relevance (the *candidate system*)." From this, a key component is chosen (perhaps a rate-limiting enzyme) and its gene identified (the *candidate gene*). A polymorphic variant is then identified in the gene …. ([82] p. 125).

The HIFs and their target genes have been the focus of candidate analysis. The target genes are inducible by the family of transcription factors including HIF1 and HIF2 described above. Before publication of the evidence about *EPAS1*, attention focused on *HIF1A*.

A case–control study of Sherpas tested for an association of *HIF1A* and *VHL* variants with Acute Mountain Sickness symptoms at extremely high altitude. The VHL protein targets HIF1A and EPAS1 for ongoing degradation when oxygen levels are normal for low altitude. The study found no association, although it did acknowledge that it had low statistical power owing to the small sample size of 49 cases and 54 controls [111]. Another study reported a new sequence in the *HIF1A* gene among Sherpas as compared with Japanese and also reported other allele frequency differences at that locus [112]. In contrast, the *HIF1A* sequence of Andean highlanders did not differ from that of low-altitude controls [113]. A case–control study examining a number of genes involved in the response to hypoxia found no association with CMS in an Andean sample [114]. The Sherpa results suggested tentatively the possibility of a new adaptive mutation while the Andean did not. However, none of the studies of genome-wide variation identified *HIF1A* variants as likely to have undergone selection at altitude in either population. An explanatory hypothesis reasons that because HIF1 is expressed in all tissues, it may have little latitude for variation whereas HIF2 is expressed in fewer tissues and could have more focused effects [98].

With respect to variance in the response to HIF, the candidate gene first examined among high-altitude populations was *ACE*. It has a polymorphism associated with athletic ability and in some cases with successful adaptation to acute exposure to high altitude [115]. The variants are called the insertion (I, associated with better endurance and with metabolic efficiency) and deletion (D, associated with power and strength) alleles. At high altitude, I allele genotypes were associated with lower pulmonary artery pressure—better functional adaptation—in a sample of lowlanders acutely exposed [116]. However, they were associated with increased risk of pulmonary hypertension in a sample of Kyrgyz highlanders [117] and were not associated with pulmonary artery pressure in a sample of Amhara highlanders [65]. The contrasts may illustrate another example of the differences in adaptive success between lifelong high-altitude natives and acclimatized newcomers summarized in Table 19.2. Alternatively, they could indicate that ACE I/D polymorphism may be closely linked to another locus that is the true influence on phenotypes.

In contrast to the Kyrgyz and similar to the acutely exposed highlanders, Andean residents from high and low altitude with the ACE II genotype had some benefits. They had higher oxygen saturations of hemoglobin at rest and during exercise at 4,300 m [118]. ACE genotype accounted for ~4 % of the variance in oxygen saturation phenotype. That study quantified the proportion of Native American Ancestry for each participant in

order to establish genetic homogeneity of the two samples and increase confidence that the I allele or a linked locus accounted for the difference in oxygen saturation. The *ACE* locus was not identified by the genome-wide studies as having been subject to natural selection at high altitude.

Other candidate genes such as some involved in pulmonary vascular tone have been analyzed including endothelin-1 (*EDN1*) and *NOS3* (also known as *eNOS*). Their proteins code for the vasoconstrictor endothelin and for an enzyme catalyzing the synthesis of the vasodilator nitric oxide, respectively. For example, two polymorphisms of *NOS3* were analyzed to test the hypothesis that alleles associated with higher rates of nitric oxide synthesis at low altitude would be associated with higher rates at high altitude and with less vasoconstriction among high-altitude Sherpas. One polymorphism at the *NOS3* locus was associated with higher levels of nitric oxide metabolites and one with lower levels while neither was associated with blood pressure as might be expected from a vasodilator [119]. This may be another illustration that candidate gene—phenotype associations identified in low-altitude Europeans samples may not be replicated in high-altitude, non-European samples. The *NOS2* locus rather than the *NOS3* locus was identified as a possible candidate for selection in the Andean population [92].

However, factors in addition to—or perhaps instead of—population differences can contribute to the outcome of genome-wide or candidate-gene analyses. One is the selection of appropriate controls that are likely to provide the clearest evidence of what happened. For example, the Yi population is more closely related to the Tibetans than the HapMap Han sample and may be a better low-altitude comparison sample [95]. Some studies address that issue with multiple control populations.

Another crucial factor influencing the detection of population differences is statistical power. Power to detect differences is lower with small sample sizes or small effects or when doing multiple statistical tests. The latter is particularly relevant for genomic strategies testing hundreds of thousands of hypotheses, one for each SNP, for example. The Bonferroni adjustment is a widely used method of multiple-testing correction that can illustrate this point. If a significance threshold of 0.05 is adopted and 20 separate tests of significance are performed, then the Bonferroni adjustment calls for dividing 0.05 by 20 for a p-value of 0.0025 as the appropriate significance threshold. If one million SNPS were tested for association with a single phenotype, then the 0.05 would be divided by 1,000,000 for a p-value of 0.000000005 as the appropriate significance threshold [120]. Going forward, it is very important to collect adequately sized samples.

Mitochondrial genotypes have been considered as well although with inconsistent results. One study concluded there was little evidence that mitochondrial mutations might contribute to successful adaptation among Tibetans, while another hypothesized a contribution by one haplotype over-represented among Tibetans as compared with Han Chinese could contribute [121, 122]. Neither reported phenotypes. A study of Andean women giving birth at >3,800 m collected information on mitochondrial DNA haplotypes and reproductive histories. Sixty-five percent of the sample had mt haplotype B (42–80 % of Andean highland populations have this haplotype [123]) and the rest had other New World haplotypes. Compared to the reproductive histories of women with mt haplotype B, the women with non-B haplotypes had more than three times the risk of losing offspring between conception and 1 month post-partum, partly due to ten times higher risk of neonatal mortality [124]. This could reflect very strong selection among Andean women.

Potential Confounding Factors

The classic and genomic strategies using phenotypes rely on accurate reflection of the response to hypoxia. Many studies carefully defined and controlled for a number of other influences such as smoking or health status, yet still there was uncertainty about the potential role of unknown confounding factors. Ironically some of the most relevant discoveries for discovering natural selection at high altitude may have come from recent gains in knowledge about potential confounding factors that influence expression of oxygen

homeostasis-associated loci or high-altitude phenotypes. These could foil attempts to associate genotypes with phenotypes or even efforts to distinguish differences between high and low altitude. For example, a long-standing puzzle was the wide range of variation in mean hemoglobin concentration of samples of Andean highlanders. Samples from mining communities in the Central Peruvian Highlands were long known to have very high hemoglobin concentrations. High cobalt levels were detected in the ground water in one mining community. Cobalt stabilizes HIF1A; that could in turn induce transcription of target genes including *EPO*, perhaps partly explaining the very high mean values of some samples. Men with high cobalt levels in that community had higher EPO levels and higher hemoglobin concentration [52].

While cobalt can stabilize, iron is required to degrade HIF1A. Thus iron depletion or supplementation can exacerbate or dampen high-altitude pulmonary hypertension [54] although it is not known whether naturally occurring variation in iron levels influences variation in pulmonary blood pressure in natural populations.

Intergenerational influences on newborn phenotypes and perinatal origins of adult high-altitude phenotypes may further complicate efforts to link heritable variation to phenotype variation and survival to test the natural selection hypothesis. The relationship of birth weight to infant survival is appreciated as an example of balancing selection because newborns of very high or low birth weight have higher mortality [125]. Maternal effects on birth weight were demonstrated in a study of women of European ancestry delivering babies at 3,100 m in Colorado. Women born and raised at that altitude tended to give birth to infants of lower weight than migrant women [126], a suggestive sign of intergenerational epigenetic effects. Paternal effects on birth weight were demonstrated in a study of babies born to Andean, European or mixed parents that found that having an Andean father raised the birth weight slightly but significantly. The authors suggested that genomic imprinting—"modification of gene expression through the addition of molecules … to specific genes affecting intrauterine growth, based upon parental origin" ([127] p. 596) may be the mechanism.

Furthermore, perinatal events may influence adult phenotype. Retrospective data on a sample of young men with excessive erythrocytosis at 3,700 m in Bolivia indicated that they had had particularly low birth weights and all but one had experienced perinatal hypoxia [128]. Andean highlanders whose mothers had had pre-eclampsia had higher pulmonary artery pressures as teenagers than those who mothers had had normal pregnancies [50]. Similarly, Europeans who experienced hypoxia during the first week of life at low altitude had a larger pulmonary vascular response to acute high-altitude hypoxia as adults [129]. Unusual hypoxia perinatally may be over-represented among high-altitude residents with relatively unsuccessful adaptations. Thus, other environmental factors and individual life-history events may contribute to phenotype variance and confound the search for natural selection at high altitude.

Summary

In summary, indigenous high-altitude populations comprise an informative case study of evolution because they seem likely to have experienced natural selection favoring adaptations to the unavoidable, severe stress of hypobaric hypoxia. Beginning in the 1960s, scientists used classic strategies to detect natural selection in such populations [20]. A large body of evidence of distinctive phenotypes at high altitude, along with altitude gradients, cross-population and cross-species comparisons suggested the action of natural selection.

Recently, purely genomic strategies have been developed along with others that integrate genomic with phenotypic information. Recently findings from such studies confirmed that natural selection has occurred, thus supporting and expanding the large body of earlier evidence. So far, the genomic evidence is strong for one trait in one population—hemoglobin concentration among Tibetans. It is more indirect for Andean and East African populations and to a large extent rests on the failure to replicate the Tibetan findings. Detecting positive information on the genetic and phenotypic bases for the long-term success of these two populations is an important goal.

Future work integrating genomic and classical strategies will expand the phenotype–genome associations with the aim of building thoroughly integrated cases of natural selection linking phenotypic variation to heritable variation to survival at high altitude. That work will help explain the extent to which Andean, Tibetan, and East African differences in phenotypes as due to longer Tibetan residence [6], to chance difference in the natural experiments of colonization [130] or other reason to be discovered.

The key remaining gap in understanding the process of natural selection at high altitude is associating phenotypic with genomic variation and then determining that the "variation be it ever so slight, would also have a better chance….of survival" ([1] p. 49).

In summary, *Natural selection is not easy to detect* ([20] p. 97), however progress is under way.

Appendix: Global Population Estimates

The global population estimates were obtained by Ann Holstein, Manager of GIS Systems and Numeric Data Services at the Kelvin Smith Library, Case Western Reserve University using ESRI's ArcGIS Desktop 9.3 Geographic Information System (GIS) software. The following data sets were merged.

1. Global Digital Elevation Model (GTOPO30), developed by USGS EROS Data Center in 1996, represents gridded 30 arc seconds (+- 1 km) elevation for the world (available at http://www1.gsi.go.jp/geowww/globalmap-gsi/gtopo30/gtopo30.html).
2. Gridded population of the world, version 3 (GPWV3) centroids, were acquired from SEDAC—Socioeconomic Data and Applications Center (available at http://sedac.ciesin.columbia.edu/). Each centroid includes a population value attribute for P05A, the UN-adjusted population estimates for 2005.

The spatial analysis methods were as follows. Contour lines were derived from the Global Digital Elevation Model at 500 m intervals. Line data were converted to polygons (areas), where the area located between two contour lines was defined and calculated by the software. Population centroids spatially located within polygons identified as over 2,500 m elevation were retained. The sum of the P05A field of centroids for each country having populations living above 2,500 m was calculated. These population values are listed in the table below.

Global population at Elevations above 2,500 m		
		2005 Population Estimate
AFRICA		**12,849,539**
	Congo	740,595
	Ethiopia	10,874,518
	Kenya	616,229
	Lesotho	123,225
	Rwanda	182,515
	Tanzania	268,912
	Uganda	43,545
ASIA		**31,869,915**
	Afghanistan	3,234,170
	Bhutan	609,298
	China	16,018,246
	India	7,428,994
	Kyrgyz Republic	608,636
	Mongolia	96,718
	Myanmar	4,436
	Nepal	1,397,479
	Pakistan	1,408,409
	Russia	21,761
	Tajikistan	872,514
	Uzbekistan	169,254
NORTH AMERICA		**9,516,326**
	Greenland	537
	Guatemala	1,098,961
	Mexico	8,028,898
	United States	387,930
SOUTH AMERICA		**29,143,068**
	Argentina	417,503
	Bolivia	5,071,377
	Chile	289,843
	Colombia	10,426,067
	Ecuador	4,120,668
	Peru	8,456,051
	Venezuela	361,559
TOTAL		**83,378,848**

References

1. Darwin C, Wallace A. On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. In: Proceedings of the Linnaean society. 1858. p. 45–62.
2. Monge C. Acclimatization in the Andes. Reissued 1948 edition ed. Baltimore: The Johns Hopkins Press; 1978.
3. Hurtado A. Studies at high altitude: blood observations on the Indian natives of the Peruvian Andes. Am J Physiol. 1932;100:487–505.
4. Hurtado A. Respiratory adaptation in the Indian Natives of the Peruvian Andes. Studies at high altitude. Am J Phys Anthropol. 1932;17(2):137–65.
5. Baker PT. Human adaptation to high altitude. Science. 1969;163:1149–56.
6. Niermeyer S, Zamudio S, Moore LG. The people. In: Hornbein TF, Schoene RB, editors. High altitude an exploration of human adaptation. New York, NY: Marcel Dekker, Inc.; 2001. p. 43–100.
7. Hurtado A. Animals in high altitudes: resident man. In: Dill DB, editor. Handbook of physiology section 4: adaptation to the environment. Washington, DC: American Physiological Society; 1964. p. 843–59.
8. Beall CM, Laskowski D, Strohl KP, Soria R, Villena M, Vargas E, et al. Pulmonary nitric oxide in mountain dwellers. Nature. 2001;414:411–2.
9. Aldenderfer M. Peopling the Tibetan plateau: insights from archaeology. High Alt Med Biol. 2011; 12(2):141–7.
10. Aldenderfer MS. Moving Up in the World; archaeologists seek to understand how and when people came to occupy the Andean and Tibetan plateaus. Am Sci. 2003;91:542–9.
11. Fehren-Schmitz L, Warnberg O, Reindel M, Seidenberg V, Tomasto-Cagigao E, Isla-Cuadrado J, et al. Diachronic investigations of mitochondrial and Y-chromosomal genetic markers in pre-Columbian Andean highlanders from South Peru. Ann Hum Genet. 2011;75(2):266–83.
12. Weinstein KJ. Thoracic skeletal morphology and high-altitude hypoxia in Andean prehistory. Am J Phys Anthropol. 2007;134(1):36–49.
13. Pleurdeau D. Human technical behavior in the African middle stone age: the Lithic Assemblange of Porc-Epic Cave (Dire Dawa, Ethiopia). Afr Archaeol Rev. 2006;22(4):177–97.
14. Bersaglieri T, Sabeti PC, Patterson N, Vanderploeg T, Schaffner SF, Drake JA, et al. Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet. 2004;74(6):1111.
15. Itan Y, Powell A, Beaumont MA, Burger J, Thomas MG. The origins of lactase persistence in Europe. PLoS Comput Biol. 2009;5(8):e1000491.
16. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, et al. Convergent adaptations of human lactase persistence in Africa and Europe. Nat Genet. 2007;39(1):31–40.
17. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992;20(6):1433.
18. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet. 1992; 51(1):197.
19. Weedon MN, Lango H, Lindgren CM, Wallace C, Evans DM, Mangino M, et al. Genome-wide association analysis identifies 20 loci that influence adult height. Nat Genet. 2008;40(5):575.
20. Endler JA. Natural selection in the wild. In: May RM, editor. Princeton, NJ: Princeton University Press; 1986.
21. Soria R, Julian CG, Vargas E, et al. Graduated effects of high-altitude hypoxia and highland ancestry on birth size. Pediatr Res. 2013 (in press).
22. Cosio G. Caracteristicas Hematicas y Cardiopulmonares Del Minero Andino. Bol Off Sanit Panama. 1972;72(June):547–57.
23. Villafuerte FC, Cardenas R, Monge CC. Optimal hemoglobin concentration and high altitude: a theoretical approach for Andean men at rest. J Appl Physiol. 2004;96(5):1581–8.
24. Ferrell RE, Bertin T, Barton SA, Rothhammer F, Schull WJ. The multinational Andean genetic and health program. IX. Gene frequencies and rate variants of 20 serum proteins and erythrocyte enzymes in the Aymara of Chile. Am J Hum Genet. 1980; 32:92–102.
25. Ferrell RE, Bertin T, Schull WJ. An electrophoretic study of glycolytic enzymes in a human population living at high altitude: the Aymara of Northern Chile and Western Bolivia. Hum Genet. 1981;56:397–9.
26. Chakraborty R, Clench J, Ferrell RE, Barton SA, Schull WJ. Genetic components of variation of red cell glycolytic intermediates at two altitudes among the South American Aymara. Ann Hum Biol. 1983;10(2):174–84.
27. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399–408.
28. Aggarwal S, Negi S, Jha P, Singh PK, Stobdan T, Pasha MA, et al. EGLN1 involvement in high-altitude adaptation revealed through genetic analysis of extreme constitution types defined in Ayurveda. Proc Natl Acad Sci. 2010;107(44):18961–6.
29. Xu S, Li S, Yang Y, Tan J, Lou H, Jin W, et al. A genome-wide search for signals of high-altitude adaptation in Tibetans. Mol Biol Evol. 2011;28(2):1003–11.
30. Schödel J, Oikonomopoulos S, Ragoussis J, Pugh CW, Ratcliffe PJ, Mole DR. High-resolution genome-wide mapping of HIF-binding sites by ChIP-seq. Blood. 2011;117(23):e207–17.
31. Harrison GA. Human adaptability with reference to the IBP proposals for high altitude research. In:

Baker PT, Weiner JS, editors. The biology of human adaptability. Oxford: Clarendon; 1966. p. 509–19.
32. Brutsaert TD, Soria R, Caceres E, Spielvogel H, Haas JD. Effect of developmental and ancestral high altitude exposure on chest morphology and pulmonary function in Andean and European/North American Natives. Am J Hum Biol. 1999;11:385–95.
33. Duplain H, Sartori C, Lepori M, Egli M, Allemann Y, Nicod P, et al. Exhaled nitric oxide in high-altitude pulmonary edema. Am J Crit Care Med. 2000;162:221–4.
34. Busch T, Bärtsch P, Pappert D, Grunig E, Hildebrandt W, Elser H, et al. Hypoxia decreases exhaled nitric oxide in mountaineers susceptible to high-altitude pulmonary edema. Am J Respir Crit Care Med. 2001;163:368–73.
35. Brown DE, Beall CM, Strohl KP, Mills PS. Exhaled nitric oxide decreases upon acute exposure to high-altitude hypoxia. Am J Hum Biol. 2006;18(2): 196–202.
36. Janocha AJ, Koch CD, Tiso M, Ponchia A, Doctor A, Gibbons L, et al. Nitric oxide during altitude acclimatization. N Engl J Med. 2011;365(20):1942–4.
37. Beall CM, Laskowski D, Erzurum SC. Nitric oxide in adaptation to altitude. Free Radic Biol Med. 2012;52(7):1123–34.
38. Donnelly J, Cowan DC, Yeoman DJ, et al. Exhaled nitric oxide and pulmonary artery pressures during graded ascent to high altitude. Respir Physiol Neurobiol. 2011;177:213–217.
39. Julian CG, Wilson MJ, Moore LG. Evolutionary adaptation to high altitude: a view from in utero. Am J Hum Biol. 2009;21(5):614–22.
40. Brutsaert T, Parra E, Shriver M, Gamboa A, Rivera-Chira M, Leon-Velarde F. Ancestry explains the blunted ventilatory response to sustained hypoxia and lower exercise ventilation of Quechua altitude natives. Am J Physiol Regul Integr Comp Physiol. 2005;289:R225–34.
41. Brutsaert TD, Parra EJ, Shriver MD, Gamboa A, Palacios JA, Rivera M, et al. Spanish genetic admixture is associated with larger V(O2) max decrement from sea level to 4338 m in Peruvian Quechua. J Appl Physiol. 2003;95(2):519–28.
42. Brutsaert TD, Parra E, Shriver M, Gamboa A, Palacios JA, Rivera M, et al. Effects of birthplace and individual genetic admixture on lung volume and exercise phenotypes of Peruvian Quechua. Am J Phys Anthropol. 2004;123(4):390–8.
43. Harvey PH, Purvis A. Comparative methods for explaining adaptations. Nature. 1991;351(6328): 619–24.
44. Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, Bryan NS, et al. Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. Proc Natl Acad Sci. 2007;104(45):17593.
45. Hoit BD, Dalton ND, Erzurum SC, Laskowski D, Strohl KP, Beall CM. Nitric oxide and cardiopulmonary hemodynamics in Tibetan highlanders. J Appl Physiol. 2006;99:1796–801.
46. Jansen GF, Basnyat B. Brain blood flow in Andean and Himalayan high-altitude populations: evidence of different traits for the same environmental constraint. J Cereb Blood Flow Metab. 2011;31(2):706–14.
47. Beall CM, Strohl K, Blangero J, Williams-Blangero S, Brittenham GM, Goldstein MC. Quantitative genetic analysis of arterial oxygen saturation in Tibetan highlanders. Hum Biol. 1997;69(5):597–604.
48. Beall CM. Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci. 2007;104 Suppl 1:8655.
49. Schwab M, Jayet PY, Stuber T, Salinas CE, Bloch J, Spielvogel H, et al. Pulmonary-artery pressure and exhaled nitric oxide in Bolivian and Caucasian high altitude dwellers. High Alt Med Biol. 2008; 9(4):295.
50. Jayet PY, Rimoldi SF, Stuber T, Salmon CS, Hutter D, Rexhaj E, et al. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. Circulation. 2010;122(5):488–94.
51. Morin Y, Têtu A, Mercier G. Cobalt cardiomyopathy: clinical aspects. Br Heart J. 1971;33(Suppl):175–8.
52. Jefferson JA, Escudero E, Hurtado ME, Pando J, Tapia R, Swenson ER, et al. Excessive erythrocytosis, chronic mountain sickness, and serum cobalt levels. Lancet. 2002;359:407–8.
53. Tufts DA, Haas JD, Beard JL, Spielvogel H. Distribution of hemoglobin and functional consequences of anemia in adult males at high altitude. Am J Clin Nutr. 1985;42:1–11.
54. Smith TG, Talbot NP, Privat C, Rivera-Ch M, Nickol AH, Ratcliffe PJ, et al. Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials. JAMA. 2009;302(13):1444.
55. Harrison GA, Kuchemann CF, Moore MAS, Boyce AJ, Baju T, Mourant AE, et al. The effects of altitudinal variation in Ethiopian populations. Philos Trans R Soc Lond B Biol Sci. 1969;256(805):147–882.
56. Alkorta-Aranburu G, Beall CM, Witonsky DB, Gebremedhin A, Pritchard JK, Di Rienzo A. The genetic architecture of adaptations to high altitude in Ethiopia. PLoS Genet. 2012;8(12):e1003110.
57. Teshome D, Telahun T, Solomon D, Abdulhamid I. A study on birth weight in a teaching-referral hospital, Gondar, Ethiopia. Cent Afr J Med. 2006;52(1–2): 8–11.
58. Nekatibeb G, G/Mariam A. Analysis of birth weight in Metu Karl hospital: South West Ethiopia. Ethiop Med J. 2007;45(2):195–202.
59. Feleke Y, Enquoselassie F. Maternal age, parity and gestational age on the size of the newborn in Addis Ababa. East Afr Med J. 1999;76(8):468–71.
60. Madebo T. A two year retrospective study of birth weight in Sidamo Regional Hospital. Ethiop Med J. 1994;32(4):255–60.
61. Sheferaw T. Some factors associated with birth weight in Jima, southwestern Ethiopia. Ethiop Med J. 1990;28(4):183–90.

62. Green-Abate C. Changes in birthweight distribution from 1973 to 1982 in Addis Ababa. Bull World Health Organ. 1986;64(5):711–4.
63. Andersen GS, Girma T, Wells JC, Kæstel P, Michaelsen KF, Friis H. Fat and fat-free mass at birth: air displacement plethysmography measurements on 350 Ethiopian newborns. Pediatr Res. 2011;70(5):501–6.
64. Moore LG, Charles SM, Julian CG. Humans at high altitude: hypoxia and fetal growth [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, Non-P.H.S. Review]. Respir Physiol Neurobiol. 2011;178(1):181–90.
65. Hoit BD, Dalton ND, Gebremedhin A, Janocha A, Zimmerman PA, Zimmerman AM, et al. Elevated pulmonary artery pressure among Amhara highlanders in Ethiopia. Am J Hum Biol. 2011;23(2):168–76.
66. Claydon VE, Gulli G, Slessarev M, Appenzeller O, Zenebe G, Gebremedhin A, et al. Cerebrovascular responses to hypoxia and hypocapnia in Ethiopian high altitude dwellers. Stroke. 2008;39(2):336–42.
67. Beall CM, Decker MJ, Brittenham GM, Kushner I, Gebremedhin A, Strohl KP. An Ethiopian pattern of human adaptation to high-altitude hypoxia. Proc Natl Acad Sci U S A. 2002;99(26):17215–8.
68. Mekbeb T, Ketsela K. Pre-eclampsia/eclampsia at Yekatit 12 Hospital, Addis Ababa, Ethiopia (1987–1989). East Afr Med J. 1991;68(11):893–9.
69. Gong J, Savitz DA, Stein CR, Engel SM. Maternal ethnicity and pre-eclampsia in New York City, 1995–2003. Paediatr Perinat Epidemiol. 2012; 26(1):45–52.
70. López-Jaramillo P, García RG, López M. Preventing pregnancy-induced hypertension: are there regional differences for this global problem? J Hypertens. 2005;23(6):1121–9.
71. Beall CM, Song K, Elston RC, Goldstein MC. Higher offspring survival among Tibetan women with high oxygen saturation genotypes residing at 4,000 m. Proc Natl Acad Sci U S A. 2004;101(39):14300–4.
72. Buderer MC, Page N. Hemopoiesis in the pig-tailed monkey, *Macaca nemestrina* during chronic altitude exposure. Am J Physiol. 1972;223(2):346–52.
73. Yu L, Wang X, Ting N, Zhang Y. Mitogenomic analysis of Chinese snub-nosed monkeys: evidence of positive selection in NADH dehydrogenase genes in high-altitude adaptation. Mitochondrion. 2011; 11(3):497–503.
74. Storz JF, Sabatino SJ, Hoffmann FG, Gering EJ, Moriyama H, Ferrand N, et al. The molecular basis of high-altitude adaptation in deer mice. PLoS Genet. 2007;3(3):e45.
75. Ramirez JM, Folkow LP, Blix AS. Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. Annu Rev Physiol. 2007;69:113–43.
76. Winslow RM, Monge CC, Statham NJ, Gibson CG, Charache S, Whittembury J, et al. Variability of oxygen affinity of blood: human subjects native to high altitude. J Appl Physiol. 1981;51(6):1411–6.
77. McCracken KG, Bulgarella M, Johnson KP, Kuhner MK, Trucco J, Valqui TH, et al. Gene flow in the face of countervailing selection: adaptation to high-altitude hypoxia in the betaA hemoglobin subunit of yellow-billed pintails in the Andes. Mol Biol Evol. 2009;26(4):815.
78. Storz JF, Moriyama H. Mechanisms of hemoglobin adaptation to high altitude hypoxia. High Alt Med Biol. 2008;9(2):148–57.
79. Storz JF, Scott GR, Cheviron ZA. Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. J Exp Biol. 2010;213(Pt 24):4125–36.
80. Monge C, Leon-Velarde F. Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiol Rev. 1991;71(4):1135–71.
81. Beall CM. Detecting natural selection in high-altitude human populations. Respir Physiol Neurobiol. 2007;158(2–3):161–71.
82. Grocott M, Montgomery H. Genetophysiology: using genetic strategies to explore hypoxic adaptation. High Alt Med Biol. 2008;9(2):123–9.
83. Hancock AM, Di Rienzo A. Detecting the genetic signature of natural selection in human populations: models, methods, and data. Annu Rev Anthropol. 2008;37:197–217.
84. Sabeti PC, Schaffner SF, Fry B, Lohmueller J, Varilly P, Shamovsky O, et al. Positive natural selection in the human lineage. Science. 2006;312(5780): 1614–20.
85. Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, et al. Genome-wide detection and characterization of positive selection in human populations. Nature. 2007;449(7164):913–8.
86. The International HapMap Consortium. A haplotype map of the human genome. Nature. 2005;437(7063): 1299–320.
87. Harris EE, Meyer D. The molecular signature of selection underlying human adaptations. Yearb Phys Anthropol. 2006;49:89–130.
88. Bamshad M, Wooding SP. Signatures of natural selection in the human genome. Nat Rev Genet. 2003;4(2):99.
89. Beall CM, Cavalleri GL, Deng L, Elston RC, Gao Y, Knight J, et al. Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. Proc Natl Acad Sci. 2010;107(25):11459–64.
90. Simonson TS, Yang Y, Huff CD, Yun H, Qin G, Witherspoon DJ, et al. Genetic evidence for high-altitude adaptation in Tibet. Science. 2010; 329(5987):72–5.
91. Yi X, Liang Y, Huerta-Sanchez E, Jin X, Cuo ZX, Pool JE, et al. Sequencing of 50 human exomes reveals adaptation to high altitude. Science. 2010; 329(5987):75–8.
92. Bigham A, Bauchet M, Pinto D, Mao X, Akey JM, Mei R, et al. Identifying signatures of natural selection

in Tibetan and Andean populations using dense genome scan data. PLoS Genet. 2010;6(9):e1001116.
93. Bigham AW, Mao X, Mei R, Brutsaert T, Wilson MJ, Julian CG, et al. Identifying positive selection candidate loci for high-altitude adaptation in Andean populations. Hum Genomics. 2009;4(2):79–90.
94. Peng Y, Yang Z, Zhang H, Cui C, Qi X, Luo X, Tao X, Wu T, et al. Genetic variations in Tibetan populations and high altitude adaptation at the Himalayas. Mol Biol Evol. 2011;28:1075–81.
95. Wang B, Zhang YB, Zhang F, Lin H, Wang X, Wan N, et al. On the origin of Tibetans and their genetic basis in adapting high-altitude environments. PLoS One. 2011;6(2):e17002.
96. Scheinfeldt LB, Soi S, Thompson S, Ranciaro A, Meskel DW, Beggs W, et al. Genetic adaptation to high altitude in the Ethiopian highlands. Genome Biol. 2012;13:R1.
97. Storz JF. Evolution. Genes for high altitudes. Science. 2010;329(5987):40–1.
98. MacInnis MJ, Rupert JL. 'ome on the Range: altitude adaptation, positive selection, and Himalayan genomics. High Alt Med Biol. 2011;12(2):133–9.
99. Rupert J. Will blood tell? Three recent articles demonstrate genetic selection in Tibetans. High Alt Med Biol. 2010;11(4):307–8.
100. Scheinfeldt LB, Tishkoff SA. Living the high life: high-altitude adaptation. Genome Biol. 2010;11(9):133–5.
101. Wilson MJ, Julian CG, Roach RC. Genomic analysis of high altitude adaptation: innovations and implications. Curr Sports Med Rep. 2011;10(2):59–61.
102. Cheviron ZA, Brumfield RT. Genomic insights into adaptation to high-altitude environments. Heredity. 2012;108:354–61.
103. van Patot MC, Gassmann M. Hypoxia: adapting to high altitude by mutating EPAS-1, the gene encoding HIF-2α. High Alt Med Biol. 2011;12(2):157–67.
104. Webster KA. Evolution of the coordinate regulation of glycolytic enzyme genes by hypoxia. J Exp Biol. 2003;206(Pt 17):2911–22.
105. Semenza GL. Hydroxylation of HIF-1: oxygen sensing at the molecular level. Physiology. 2004;19: 176–82.
106. Loenarz C, Coleman ML, Boleininger A, Schierwater B, Holland PW, Ratcliffe PJ, et al. The hypoxia-inducible transcription factor pathway regulates oxygen sensing in the simplest animal, Trichoplax adhaerens. EMBO Rep. 2011;12(1):63–70.
107. Brakefield PM. Evo-devo and accounting for Darwin's endless forms. Philos Trans Biol Sci. 2011;366(1574):2069–75.
108. Voight BF, Kudaravalli S, Wen X, Pritchard JK. A map of recent positive selection in the human genome. PLoS Biol. 2006;4(3):e72.
109. Pickrell JK, Coop G, Novembre J, Kudaravalli S, Li JZ, Absher D, et al. Signals of recent positive selection in a worldwide sample of human populations. Genome Res. 2009;19(5):826–37.
110. Ganesh SK, Zakai NA, van Rooij FJ, Soranzo N, Smith AV, Nalls MA, et al. Multiple loci influence erythrocyte phenotypes in the CHARGE Consortium. Nat Genet. 2009;41(11):1191–8.
111. Droma Y, Hanaoka M, Basnyat B, Arjyal A, Neupane P, Pandit A, et al. Adaptation to high altitude in Sherpas: association with the insertion/deletion polymorphism in the Angiotensin-converting enzyme gene. Wilderness Environ Med. 2008; 19(1):22–9.
112. Suzuki K, Kizaki T, Hitomi Y, Nukita M, Kimoto K, Miyazawa N, et al. Genetic variation in hypoxia-inducible factor 1alpha and its possible association with high altitude adaptation in Sherpas. Med Hypotheses. 2003;61(3):385–9.
113. Hochachka P, Rupert J. Fine tuning the HIF-1 'global' O2 sensor for hypobaric hypoxia in Andean high-altitude natives. BioEssays. 2003;25(5):515–9.
114. Mejía OM, Prchal JT, León-Velarde F, Hurtado A, Stockton DW. Genetic association analysis of chronic mountain sickness in an Andean high-altitude population. Haematologica. 2005;90(1):13.
115. Puthucheary Z, Skipworth JR, Rawal J, Loosemore M, Van Someren K, Montgomery HE. The ACE gene and human performance: 12 years on. Sports Med. 2011;41(6):433–48.
116. Kumar R, Qadar Pasha M, Khan A, Gupta V, Grover S, Norboo T, et al. Association of high-altitude systemic hypertension with the deletion allele of the angiotensin-converting enzyme (ACE) gene. Int J Biometeorol. 2003;48(1):10–4.
117. Morrell NW, Sarybaev AS, Alikhan A, Mirrakhimov MM, Aldashev AA. ACE genotype and risk of high altitude pulmonary hypertension in Kyrghyz highlanders. Lancet. 1999;353(March 6):814.
118. Bigham AW, Kiyamu M, León-Velarde F, Parra EJ, Rivera-Ch M, Shriver MD, et al. Angiotensin-converting enzyme genotype and arterial oxygen saturation at high altitude in Peruvian Quechua. High Alt Med Biol. 2008;9(2):167–78.
119. Droma Y, Hanaoka M, Basnyat B, Ariyal A, Neupane P, Pandit A, et al. Genetic contribution of the endothelial nitric oxide synthase gene to high altitude adaptation in Sherpas. High Alt Med Biol. 2006; 7(3):209–20.
120. Carroll KJ. Back to basics: explaining sample size in outcome trials, are statisticians doing a thorough job? Pharm Stat. 2009;8(4):333–45.
121. Luo Y, Gao W, Liu F, Gao Y. Mitochondrial nt3010G-nt3970C haplotype is implicated in high-altitude adaptation of Tibetans. Mitochondrial DNA. 2011;22(5–6):181–90.
122. Torroni A, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, et al. Mitochondrial DNA analysis in Tibet: implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol. 1994;93:189–99.
123. Merriwether DA, Ferrell RE. The four founding lineage hypothesis for the new world: a critical reevaluation. Mol Phylogenet Evol. 1996;5(1):241–6.
124. Myres JE, Malan M, Shumway JB, Rowe MJ, Amon E, Woodward SR. Haplogroup-associated differences

in neonatal death and incidence of low birth weight at elevation: a preliminary assessment. Am J Obstet Gynecol. 2000;182(6):1599–605.
125. Haldane JB. Natural selection in man. Acta Genet Stat Med. 1956;6(3):321.
126. Weinstein RS, Haas JD. Early stress and later reproductive performance under conditions of malnutrition and high altitude hypoxia. Med Anthrop. 1977;1(1):25–54.
127. Bennett A, Sain SR, Vargas E, Moore LG. Evidence that parent-of-origin affects birth-weight reductions at high altitude. Am J Hum Biol. 2008;20(5):592.
128. Moore LG, Niermeyer S, Vargas E. Does chronic mountain sickness (CMS) have perinatal origins? Respir Physiol Neurobiol. 2007;158(2–3):180.
129. Sartori C, Allemann Y, Trueb L, Delabays A, Nicod P, Scherrer U. Augmented vasoreactivity in adult life associated with perinatal vascular insult. Lancet. 1999;353(June 26):2205–7.
130. Beall CM. Adaptations to altitude: a current assessment. Annu Rev Anthropol. 2001;30:423–46.
131. Keyes LE, Armaza JF, Niermeyer S, Vargas E, Young DA, Moore LG. Intrauterine growth restriction, preeclampsia, and intrauterine mortality at high altitude in Bolivia. Pediatr Res. 2003;54(1):20–5.
132. Zamudio S. High-altitude hypoxia and preeclampsia. Front Biosci. 2007;12:2967.
133. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. Circulation. 2007; 115(9):1132.
134. Wu T. High altitude heart disease in children in Tibet. High Alt Med Biol. 2002;3(3):323–5.
135. Leon-Velarde F, Monge CC, Arregui A, Stanley C. Increased prevalence of excessive erythrocytosis with age in healthy high altitude miners. In: Sutton JR, Coates G, Remmers JE, editors. Hypoxia: the adaptations. Toronto: B.C. Decker Inc; 1990. p. 280.
136. Gonzales GF, Steenland K, Tapia V. Maternal hemoglobin level and fetal outcome at low and high altitudes. Am J Physiol Regul Integr Comp Physiol. 2009;297(5):R1477–85.