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High altitude genetic adaptation in Tibetans: No role of increased hemoglobin–oxygen affinity

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ABSTRACT

High altitude exerts selective evolutionary pressure primarily due to its hypoxic environment, resulting in multiple adaptive responses. High hemoglobin–oxygen affinity is postulated to be one such adaptive change, which has been reported in Sherpas of the Himalayas. Tibetans have lived on the Qinghai–Tibetan plateau for thousands of years and have developed unique phenotypes, such as protection from polycythemia which has been linked to *PDH2* mutation, resulting in the downregulation of the HIF pathway. In order to see if Tibetans also developed high hemoglobin–oxygen affinity as a part of their genetic adaptation, we conducted this study assessing hemoglobin–oxygen affinity and their fetal hemoglobin levels in Tibetan subjects from 3 different altitudes. We found normal hemoglobin–oxygen affinity in all subjects, fetal hemoglobin levels were normal in all except one and no hemoglobin variants in any of the subjects. We conclude that increased hemoglobin–oxygen affinity or increased fetal hemoglobin are not adaptive phenotypes of the Tibetan highlanders.

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Introduction

High-altitude hypoxia poses a great challenge to maintain adequate tissue oxygenation, and multiple physiologic responses occur to adapt to such an environment. Random mutations altering these responses undergo natural selection when they are beneficial, then the frequency of these mutations increases in subsequent generations. Thus, the Amerindians in the Andes show an increased Bohr effect compared to non-Amerindians at the same altitude [1]. In Sherpas of the Himalayas, a left-shifted oxygen dissociation curve has been reported which enables better pulmonary uptake of oxygen in hypoxia [2]. These adaptive changes to hypoxia ensure adequate oxygen delivery at the cellular levels, but the genetic basis of these adaptations is yet to be elucidated.

Native Tibetans have lived at an average of 3000–5000 m on the Tibetan Plateau for about 20,000 years [3], and have acquired genetic adaptations that have enabled them to thrive in this reduced oxygen environment. Most Tibetans thus have evolved distinctive phenotypes, such as reduced low birth weights, low prevalence of pulmonary hypertension and protection from polycythemia and other features of chronic mountain sickness [4–7,20]. All of these phenotypic traits are components of underlying genetic adaptation and some, such as protection from polycythemia, have been linked to a gain-of-function mutation of *PDH2* (encoded by *EGLN1* gene) resulting in the downregulation of the HIF pathway [6,7].

Increased hemoglobin–oxygen affinity has been described as an adaptive response to hypoxia in high-altitude Camelidae [8] similar to what has been reported in Sherpas [2]. However, Moore et al in 1992 reported no increased hemoglobin-oxygen affinity in Tibetans, but the P_{50} value was extrapolated from arterial and venous saturation values, and the number of subjects were small (n=5) [9], and the technique did not allow calculation of Hill coefficient. Other studies have reported that Tibetans have higher resting arterial oxygen saturations compared to non-Tibetans [10]. Higher arterial oxygen saturation was reported in Tibetan infants during the first four months of life, compared to Han Chinese counterparts [11]. A genetic basis for enhanced oxygen transport in Tibetans as a part of their high-altitude adaptation has been suggested, using a complex statistical analysis [12]. Further, recent genomic studies have reported that β-globin haplotypes for adult hemoglobin (β subunit encoded by *HBB* gene) and a γ2 subunit of fetal hemoglobin (HbF, γ2 subunit encoded by *HBG2* gene) have undergone genetic selection in Tibetans [13], suggesting that a hemoglobin variant and/or modified transition from HbF to adult hemoglobin may be beneficial factors of Tibetan high altitude adaptation.

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The role of hemoglobin–oxygen affinity in oxygen transport at high altitude is complex and cannot be based solely on arterial oxygen saturation. Arterial oxygen saturation is largely a reflection of alveolar function and does not indicate increased oxygen affinity, which is correctly measured by deriving its P_{50} value — the partial pressure of oxygen at which hemoglobin is 50% saturated with oxygen. In addition to high affinity hemoglobin mutants, several physiologic variables, including 2,3-diphosphoglycerate (2,3 DPG), temperature, and pH, can also increase hemoglobin–oxygen affinity and thus decrease P_{50} value. Further, an increased proportion of HbF, that has reduced interaction with 2,3 DPG, could also increase hemoglobin–oxygen affinity.

To test whether altered hemoglobin–oxygen affinity is a constituent of Tibetan high-altitude adaptation, we conducted a study assessing oxygen–hemoglobin affinity by direct and indirect measurement of P_{50} among Tibetans living at different altitudes.

Materials and methods

A cohort case study on 15 ethnic Tibetans living at 3 different locations in the Tibetan plateau in China, India and the USA at altitudes ranging from 1300 m, 1730–2300 m and 4320 m was conducted with the approval of local IRBs in USA, China, and India. Written informed consents (in Tibetan and English) were obtained from each volunteer after detailed explanation in Tibetan or English as preferred by each volunteer. All studied subjects were healthy ethnic Tibetans ranging from 30 to 75 years (see Table 1). Four non-Tibetan volunteers constituted the control arm. The additional 29 Tibetan, 25 Aymara, and 5 Caucasian Bolivian high altitude residents participated in an evaluation of fetal hemoglobin (HbF).

Blood sample collection

A 5 ml ACD tube of venous blood was obtained from the antecubital vein in each subject, and a small aliquot was used for venous blood gas and complete blood count analysis. High pressure liquid chromatography (HPLC) was done for evaluation of hemoglobin variants and HbF.

Measurement of P_{50}

The hemoglobin–oxygen dissociation and P_{50} are optimally derived by hemoximeter measurements of the percent saturation of hemoglobin at various partial pressures of oxygen. The resultant curve has a sigmoid shape due to the cooperative binding of oxygen to the four globins in the hemoglobin tetramer, enumerated as Hill coefficient "n". If a hemoximeter is not available, the P_{50} can be estimated from the venous blood gas using the measured PO_2, oxygen percent saturation and pH using a formula described by Lichtman and colleagues [14]; however, the Hill coefficient "n" cannot be derived by this method.

For the subjects from Salt Lake City, USA the peripheral blood was evaluated by *Hemox Analyzer* (TCS Scientific Corporation, New Hope, PA) for obtaining P_{50} values and "n" Hill coefficients for hemoglobin–oxygen binding. The normal range for P_{50} by *Hemox Analyzer* is 22–28 mm Hg.

On subjects from Huashixia in Qinghai, China, venous blood gasses were done on *Nova pHOx* (Nova Biomedical, Waltham, MA), and in Srinagar, India, on *GEM Premier 3000* (Instrumentation Laboratory, Lexington, MA). P_{50} was derived using the formula below [14]. The normal range for P_{50} was 22.6–29.4 mm Hg.

$$P_{50}\ \text{std} = \text{anti}\log\frac{\log\left(\frac{1}{k}\right)}{n};\ \text{where}\quad \frac{1}{k} = \left[\text{anti}\log\left(n\ \log\ PO_{2(7.4)}\right)\right]\cdot\frac{100-SO_2}{SO_2}.$$

A Hill constant "n" for hemoglobin A of 2.7 was used. The PO_2 in venous blood at 37 °C was converted to PO_2 at pH 7.4 with the formula:

$$\log\ PO_{2(7.4)} = \log\ PO_2 - [0.5(7.40 - \text{pH})]$$

where pH is measured from the antecubital venous blood [14].

Results and discussion

The P_{50} measured by *Hemox Analyzer* on Tibetan subjects from Salt Lake City, USA, and calculated by the formula described by Lichtman et al. [14] on all subjects from differing altitudes were all within normal range, regardless of their arterial oxygenation measured by a finger pulse oximeter (see Table 2). No hemoglobin variants were detected by HPLC in any subjects. HbF was less than 1% in all 19 subjects, but one subject's HbF was 2.1%. HPLC was also done on the additional 29 Tibetan (Huashixia 4320 m), 25 Aymara and 5 Caucasian Bolivian residents of La Paz (3800 m) and Tiwanaku (4100 m) and also revealed less than 1% HbF (mean HbF 0.45%, SD 0.14).

The evolutionary selection of a β-globin haplotype (*HBB*) has been reported in Tibetans [13] and also in high altitude deer mice [15]. However, when we analyzed the P_{50} of the 15 native Tibetan highlanders in differing altitudes, they were all within normal limits, and no mutant hemoglobins were found by HPLC in any of them including the additional 29 Tibetan subjects from high altitude. Thus, our data rule out that this selected *HBB* Tibetan haplotype is associated with the presence of a β-globin mutant.

Based on the published data of an increase in hemoglobin–oxygen affinity in high altitude animals as well as in Sherpas [1,2], an increase of HbF could account for potential benefits to Tibetan high-altitude adaptation and, indeed, a selection of *HBG2* haplotypes (encoding for a γ-globin subunit of HbF) was also reported in Tibetans [13]. Further, promoter variants of the *HBG2* locus have been reported in native Chilean Andes highlanders, causing delayed transition from HbF to adult hemoglobin [16]; however, we are not aware of any previous studies of dysregulated expression of HbF in Tibetans. As there are reports of increased HbF in individuals exposed to hypoxia [17], we also quantitated HbF in 29 additional Tibetan subjects living at 4320 m, 25 Aymara and 5 Caucasian Andean residents and all of them had normal HbF. Our data are consistent with a recent report of normal HbF in subjects with Chuvash polycythemia who have aberrant hypoxia sensing due to an underlying von Hippel–Lindau gene mutation that leads to augmented hypoxia sensing at low altitudes [18].

Thus, our study demonstrates that neither high hemoglobin–oxygen affinity, nor significant changes of 2,3 DPG levels (as assessed by normal P_{50}), nor elevated HbF are features of the adaptive phenotype of Tibetan highlanders. However we could not rule out that promoter variants of *HBB* or *HBG2* haplotypes may cause delayed transition to adult hemoglobin in Tibetan children.

The small number of the sample size is the major limitation in our study, mainly due to the logistic and technical issues related to access to the remote areas in India and China.

Adaptation to a hypoxic environment involves a series of complex but integrated physiologic responses that are all aimed at ensuring a

Table 1
Demographics.

	Salt Lake City, UT, USA	Srinagar, Jammu & Kashmir, India	Huashixia, Qinghai Province, PR China
Altitude	1320 m	1730 m	4320 m
Subjects	5	5	9
Tibetans/non-Tibetans	5/0	5/0	5[a]/4
Male	2	2	8
Female	3	3	1
Age range	40–77	37–70	23–66

[a] Additional 29 Tibetans from Huashixia, China and 25 Aymaras and 5 European Caucasians from La Paz and Tiwanaku (4100 m) were also quantitated for fetal hemoglobin (HbF).

Table 2
P_{50} and hemoglobin results.

Location (altitude)	Subject ID	P_{50} (mm Hg)	Hill Co-off (n)	HbF %	HbA2 %	Hb (g/dl)
Salt Lake City, USA (1320 m)	SL-1	25.16	2.83	2.1	2.3	13.5
	SL-2	22.50	2.89	0.2	2.6	13.7
	SL-3	24.06	2.87	0.2	2.5	13.7
	SL-4	24.28	2.83	0.3	2.8	12.0
	SL-5	22.35	2.82	0.3	2.4	13.3
Srinagar, India (1730 m)	SR-1	26.38	–	0.5	2.3	15.3
	SR-2	25.95	–	0.6	2.1	12.5
	SR-3	26.55	–	0.6	2.2	11.8
	SR-4	23.68	–	0.7	1.8	14.7
	SR-5	22.72	–	0.8	2.1	11.9
Huashixia, China (4320 m)	HX-1	25.99	–	0.6	2.3	19.3
	HX-2	25.75	–	0.5	2.7	16.3
	HX-3	25.75	–	0.3	2.5	13.8
	HX-4	25.74	–	0.7	2.4	15.2
	HX-5	25.80	–	0.3	2.4	15.2
Non-Tibetan controls at Huashixia, China (4320 m)	CNTRL-1	26.01	–	0.7	1.5	16.3
	CNTRL-2	26.00	–	0.5	2.2	17.8
	CNTRL-3	25.74	–	0.4	2.6	16.7
	CNTRL-4	25.42	–	0.3	2.6	15.3

SL: Salt Lake City; SR: Srinagar; HX: Huashixia; CNTRL: controls; ND: not determined.

goal of adequate oxygenation at the cellular level. Although high hemoglobin–oxygen affinity is postulated to be at an advantage at high altitudes [19], it is plausible that other physiologic adaptive mechanisms on the cellular level ensuring adequate oxygenation may offset the need for high hemoglobin–oxygen affinity. Clearly, further in-depth studies of the complex and unique phenotype of Tibetans' high-attitude adaptation are needed.

Conflict of interest disclosure

All the authors declare no competing financial interests.

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Authorship

JTP designed the study. TT, TF, RA, FRL, PK, JTP, and GRL recruited the subjects. PK, RA, TF and GRL assisted with recruitment of volunteers in India, Bolivia, and China and managed local data acquisition and IRB approvals. DH performed hemoximeter and HPLC analyses and with TT and JTP analyzed the data. TT and JTP drafted and wrote the manuscript and assured IRB compliance.

References

[1] G. Morpurgo, P. Battaglia, L. Bernini, A.M. Paolucci, G. Modiano, Higher Bohr effect in Indian natives of Peruvian highlands as compared with Europeans, Nature 227 (5256) (1970) 387–388.
[2] G. Morpurgo, P. Arese, A. Bosia, et al., Sherpas living permanently at high altitude: a new pattern of adaptation, Proc. Natl. Acad. Sci. U. S. A. 73 (3) (1976) 747–751.
[3] H. Hu, T.S. Simonson, G. Glusman, et al., Insights on the evolutionary history of Tibetans from whole-genome sequence data, [abstract] Annual Meeting of the American Society of Human Genetics, American Society of Human Genetics, Boston, MA, October 25, 2013, [Abstract # 2050F].
[4] C.M. Beall, Two routes to functional adaptation: Tibetan and Andean high-altitude natives, Proc. Natl. Acad. Sci. U. S. A. 104 (Suppl. 1) (2007) 8655–8660.
[5] P. Wagner, T. Simonson, G. Wei, et al., High altitude physiology: lessons from Tibet, Sensing Technologies for Global Health, Military Medicine, and Environmental Monitoring III, Proc SPIE, 8723, May 29, 2013, http://dx.doi.org/10.1117/12.2020038, (87230S, Accessed November 30, 2013).
[6] T.S. Simonson, Y. Yang, C.D. Huff, et al., Genetic evidence for high-altitude adaptation in Tibet, Science 329 (5987) (2010) 72–75.
[7] F.R. Lorenzo, T.S. Simonson, Y. Yang, R. Ge, J.T. Prchal, A novel PHD2 mutation associated with Tibetan genetic adaptation to high altitude hypoxia, [abstract] Blood 116 (21) (2010) (Abstract # 2602 and under review in Nature Genetics, 2013).
[8] C. Reynafarje, J. Faura, D. Villavicencio, et al., Oxygen transport of hemoglobin in high-altitude animals (Camelidae), J. Appl. Physiol. 38 (5) (1975) 806–810.
[9] L.G. Moore, L. Curran-Everett, T.S. Droma, et al., Are Tibetans better adapted? Intl. J. Sports Med. 13 (1992) S86–S89.
[10] T. Wu, B. Kayser, High altitude adaptation in Tibetans, High Alt. Med. Biol. 7 (3) (2006) 193–208.
[11] S. Niermeyer, P. Yang, Shanmina, Drolkar, J. Zhuang, L.G. Moore, Arterial oxygen saturation in Tibetan and Han infants born in Lhasa, Tibet, N. Engl. J. Med. 333 (19) (1995) 1248–1252.
[12] C.M. Beall, J. Blangero, S. Williams-Blangero, M.C. Goldstein, Major gene for percent of oxygen saturation of arterial hemoglobin in Tibetan highlanders, Am. J. Phys. Anthropol. 95 (3) (1994) 271–276.
[13] X. Yi, Y. Liang, E. Huerta-Sanchez, et al., Sequencing of 50 human exomes reveals adaptation to high altitude, Science 329 (5987) (2010) 75–78.
[14] M.A. Lichtman, M.S. Murphy, J.W. Adamson, Detection of mutant hemoglobins with altered affinity for oxygen. A simplified technique, Ann. Intern. Med. 84 (5) (1976) 517–520.
[15] J.F. Storz, H. Moriyama, Mechanisms of hemoglobin adaptation to high altitude hypoxia, High Alt. Med. Biol. 9 (2) (2008) 148–157.
[16] I. Rottgardt, F. Rothhammer, M. Dittmar, Native highland and lowland populations differ in γ-globin gene promoter polymorphisms related to altered fetal hemoglobin levels and delayed fetal to adult globin switch after birth, Anthropol. Sci. 118 (1) (2010) 41–48.
[17] A. Risso, D. Fabbro, G. Damante, G. Antonutto, Expression of fetal hemoglobin in adult humans exposed to high altitude hypoxia, Blood Cells Mol. Dis. 48 (3) (2012) 147–153.
[18] J. Salomon-Andonie, G. Miasnikova, A. Serqueeva, L.A. Polyakova, X. Niu, S. Nekhai, V.R. Gordeuk, Effect of congenital upregulation of hypoxia inducible factors on percentage of fetal hemoglobin in the blood, [letter to the editor] Blood 122 (17) (2013) 3088–3089.
[19] R.P. Hebbel, J.W. Eaton, R.S. Kronenberg, E.D. Zanjani, L.G. Moore, E.M. Berger, Human Llamas: adaptation to altitude in subjects with high hemoglobin oxygen affinity, J. Clin. Invest. 62 (3) (1978) 593–600.
[20] L.G. Moore, Human genetic adaptation to high altitude, High Alt. Med. Biol. 2 (2) (2001) 257–279.