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Tibetan Enriched *PKLR* Variant Is Beneficial to High Altitude Adaption By Improving Oxygen Delivery

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Abstract

Background

Tibetans have been living at altitudes over 3500 m for ~20,000 years and have developed unique beneficial evolutionary genetic adaptions (PMID:28448578). Our previous study identified selected genetic haplotypes in two genes, EPAS1 (encoding hypoxia-inducible factor 2-alpha [HIF2-a], a transcription factor that mediates the hypoxic response), and EGLN1 (encoding prolyl hydroxylase 2 [PHD2], a principal negative regulator of HIF stability (PMID:25129147). The presence of these two haplotypes correlates with lower hemoglobin levels in Tibetans compared to other highlanders. However, the entire diverse complex of molecular mechanisms of high altitude adaptation is still largely unknown and our study showed that neither EPAS1 nor EGLN1 variants fully explain the mechanism of protection from polycythemia in Tibetans in high altitude (PMID:28233034).

We found an enriched haplotype in the PKLR gene (encoding pyruvate kinase [PK] expressed only in liver and red blood cells). The PK enzyme is in the terminal portion of the glycolytic pathway, and its decreased activity leads to accumulation of proximal glycolytic intermediates, including 2,3-diphosphoglycerate (DPG) which shifts the hemoglobin dissociation curve to right (high p50) and increases oxygen release to tissues from a unit of hemoglobin. We hypothesized that Tibetan enriched PKLR variants might improve oxygen delivery to tissues and help explain the protection from polycythemia at high altitude.

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Genomic analyses revealed that this PKLR haplotype is enriched in Tibetans but is not unique to Tibetans. It has the highest frequency in Tibetans (89%), with a lower prevalence in Chinese and Mongolians (~77%), Kyrgyz (~60 %), Aymara (~44 %), and Colombians (~30 %) and a much lower frequency in Caucasians (11%), perhaps explaining the heterogeneity of responses to hypoxia within and among these populations. Our study of reticulocyte RNA showed that transcript levels of PKLR progressively decrease with increasing altitude in controls and even more in Tibetans with the Tibetan evolutionary selected PKLR haplotype. Tibetans with the PKLR haplotype (heterozygotes and homozygotes) have lower PKLR transcript levels than wild type Tibetans. Because of the paucity of wildtype PKLR haplotype in Tibetans and the challenges of acquiring Tibetan samples in high altitude in China, we collected samples from 125 m (Cheboksary, Chuvashia); 800 m (Bishkek, Kyrgyzstan) and 2640 m (Bogota, Colombia). PK activity, PKLR transcript levels, and ATP decreased at 2640 m compared to 800 m, while p50 increased at 2640 m. PKLR transcript levels correlated with PK activity and ATP and inversely correlated with p50. PK activities also correlated with PKLR transcript levels and ATP and inversely correlated with p50. At 2640 m, PK activity was lower and p50 levels were higher in those with the enriched PKLR haplotype. These results demonstrate that increasing altitude decreases PK activity, resulting in increasing p50 providing a molecular basis for the previously reported improvement of oxygen delivery at high altitude (PMID:17394415).

To study the roles of HIFs in the regulation of PKLR gene expression, we also collected samples from Chuvash polycythemia (CP) homozygotes and Chuvash controls. CP homozygotes have a mutation in the VHL gene, a negative regulator of HIFs, that results in augmented HIF levels. CP homozygotes had lower PKLR mRNA in reticulocytes, PK activity, and PKR protein levels in red blood cells compared to controls, while their 2,3 DPG levels were higher. These data confirm that PKLR expression levels are negatively regulated by HIFs.

Our findings demonstrate that individuals in high altitudes have lower PKLR transcript levels and PK activity, resulting in high 2,3DPG and p50 which shifts the hemoglobin dissociation curve to right. This decreases affinity of hemoglobin for oxygen, which improves tissue oxygen delivery and as such is another mechanism in Tibetans that protects from high altitude polycythemia. We also demonstrate that HIFs negatively regulate PKLR expression, leading to better oxygen release from hemoglobin at high altitude.

Disclosures: Kosinski:Agios: Employment, Equity Ownership. Kung:Agios: Employment, Equity Ownership. van Wijk:RR Mechatronics: Research Funding; Agios Pharmaceuticals: Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding.

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