HHS Public Access

Author manuscript

Sci Total Environ. Author manuscript; available in PMC 2025 February 25.

Published in final edited form as:

Sci Total Environ. 2024 February 25; 913: 169605. doi:10.1016/j.scitotenv.2023.169605.

Hypobaric hypoxia drives selection of altitude-associated adaptative alleles in the Himalayan population

Samantha Sharma[a,b,c], **Remya Koshy**[a], **Rahul Kumar**[d,e], **Ghulam Mohammad**[f], **Tashi Thinlas**[f], **Brian B. Graham**[d,e], **Qadar Pasha**[a,g,*]

[a]Genomics and Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Delhi 110007, India

[b]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

[c]Department of Medical and Molecular Genetics, Indiana University, Indianapolis 46202, IN, USA

[d]Department of Medicine, University of California San Francisco, San Francisco, CA, USA

[e]Lung Biology Center, Zuckerberg San Francisco General Hospital, San Francisco, CA, USA

[f]Department of Medicine, Sonam Norboo Memorial Hospital, Leh, Ladakh 194101, India

[g]Institute of Hypoxia Research, New Delhi 110067, India

Abstract

Genetic variants play a crucial role in shaping the adaptive phenotypes associated with high-altitude populations. Nevertheless, a comprehensive understanding of the specific impacts of different environments associated with increasing altitudes on the natural selection of these genetic variants remains undetermined. Hence, this study aimed to identify genetic markers responsible for high-altitude adaptation with specific reference to different altitudes, majorly focussing on an altitude elevation range of ~1500 m and a corresponding decrease of ≥5 % in ambient oxygen availability. We conducted a comprehensive genome-wide investigation (n = 192) followed by a validation study (n = 514) in low-altitude and three high-altitude populations (>2400 m) of Nubra village (NU) (3048 m), Sakti village (SKT) (3812 m), and Tso Moriri village (TK) (4522 m). Extensive genetic analysis identified 86 SNPs that showed significant associations with high-altitude adaptation. Frequency mapping of these SNPs revealed 38 adaptive alleles and specific haplotypes that exhibited a strong linear correlation with increasing altitude.

*Corresponding author at: Institute of Hypoxia Research, New Delhi 110067, India. pashamaq53@gmail.com (Q. Pasha).

CRediT authorship contribution statement

Samantha Sharma: Conceptualization and study design, Visualization, Methodology, Formal analysis, Validation, Data curation, Writing- Original draft preparation. **Remya Koshy**: Formal analysis, Writing- Original draft preparation. **Rahul Kumar**: Data analysis, Visualization, Writing - Review & Editing. **Ghulam Mohammad**: Clinical investigation and sample collection. **Tashi Thinlas**: Clinical investigation and sample collection. **Brian B Graham**: Data interpretation, Writing - Review & Editing. **Qadar Pasha**: Conceived the study, data curation, Supervision, Funding Acquisition, Writing - Review & Editing. All authors read and approved the final manuscript.

Declaration of competing interest

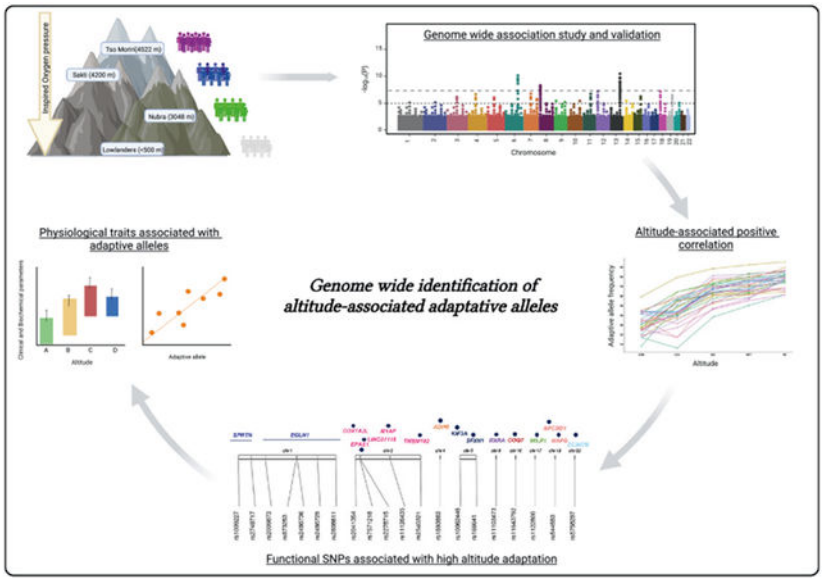
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169605.

Notably, these SNPs spanned crucial genes, such as ADH6 and NAPG along with the vastly studied genes like EGLN1 and EPAS1, involved in oxygen sensing, metabolism, and vascular homeostasis. Correlation analyses between these adaptive alleles and relevant clinical and biochemical markers provided evidence of their functional relevance in physiological adaptation to hypobaric hypoxia. High-altitude population showed a significant increase in plasma 8-isoPGF2α levels as compared to low-altitude population. Similar observation showcased increased blood pressure in NU as compared to TK ($P < 0.0001$). In silico analyses further confirmed that these alleles regulate gene expression of *EGLN1*, *EPAS1*, *COQ7*, *NAPG*, *ADH6*, *DUOXA1* etc. This study provides genetic insights into the effects of hypobaric-hypoxia on the clinico-physiological characteristics of natives living in increasing high-altitude regions. Overall, our findings highlight the synergistic relationship between environment and evolutionary processes, showcasing physiological implications of genetic variants in oxygen sensing and metabolic pathway genes in increasing high-altitude environments.

Graphical Abstract



Keywords

High-altitude population; Different altitudes; Genetic variants; Selection; Physiological conditions; Adaptation

1. Introduction

A high-altitude environment refers to regions located at significant elevations above sea level, typically exceeding 2400 m. In these areas, the atmospheric pressure is low, resulting in reduced oxygen availability, thinner air, colder temperatures, and potentially harsher weather conditions compared to lower-altitude regions. These attributes profoundly impact the morphological and physiological characteristics of high-altitude native populations (West et al., 2012; Bartsch and Gibbs, 2007). Despite the challenging conditions, over 140 million

people worldwide have established thriving human settlements in these regions, driven by the interplay of hypobaric hypoxia and evolutionary forces (Beall, 2006; Mishra et al., 2015a; Petrassi et al., 1985).

Over the years, genetic investigations have revealed that the high-altitude populations, such as Andeans, Tibetans, Ladakhis, and Ethiopians have adapted distinctly (Beall, 2007; Mishra et al., 2015b; Simonson, 2015). Among these, the inhabitation of Ladakhi populations living at varying altitudes dates back over 25,000 years (Beall, 2007). These highlanders have undergone significant physiological adaptations to cope with persistent hypobaric hypoxic conditions. This is further supported by the observation that an increase in altitude of ~500 m above 3500 m has a significant impact on the body's physiology and acclimatization, as demonstrated by expeditions to Mount Everest and other similar mountains (West, 1984). However, the specific influence of the altitude of residence and its environmental impact, particularly differences in available oxygen levels, on differential natural selection remains unclear.

Physiological adaptations have contributed to the enhanced adaptation of populations residing at high-altitude. Notably, individuals in these areas exhibit a reduced pulmonary vasoconstrictor response, and elevated haemoglobin and plasma volume, which provide crucial physiological advantages (Bartsch and Gibbs, 2007). Moreover, the Tibetan population demonstrates increased pelvic circulation, promoting efficient oxygen delivery and correlating with higher reproductive success in high-altitude conditions (Beall et al., 2010). Genetic and molecular factors play significant roles in high-altitude adaptation (Mallet et al., 2023; Bigham, 2016; Arciero et al., 2018; Palmo et al., 2022). Several epidemiological studies support the involvement of HIF pathway genes, Endothelial PAS Domain Protein 1 (*EPAS1*) and Egl-9 Family Hypoxia Inducible Factor 1 (*EGLN1*) in positive selection among high-altitude populations (Hu et al., 2017; Peng et al., 2017; Yi et al., 2010; del Peso et al., 2003; Simonson et al., 2010; Mishra et al., 2013; Lorenzo et al., 2014) with a significant impact on the physiological charateristics. Genetic polymorphisms in *EGLN1* have been correlated with elevated VO_2max under hypoxic conditions which are shown to correlate negatively with haemoglobin (Hb) concentration (Brutsaert et al., 2019; Simonson et al., 2015). Further, linkage of Protein Kinase AMP-Activated Catalytic Subunit Alpha 1 (*PRKAA1*) and Endothelin Receptor Type A (*EDNRA*) genetic variations has been associated with infant birth weight under high-altitude conditions (Bigham et al., 2014). However, the precise relationship between the accumulation of causal mutations and their potential mechanistic impact on the adaptation of these highland populations remains to be investigated.

We hypothesized that the genetic variability among residents of increasing altitudes in the Himalayas is associated with their adaptability to high-altitude environments. Specifically, we postulate that a correlation analysis with clinical and biochemical data, coupled with in silico analysis, will identify genetic variants of oxygen sensing and metabolic genes with potential physiological implications in diverse high-altitude environments for these populations/inhabitants of respective altitudes.

2. Material and methods

2.1. Study populations

We performed a cross-sectional study to investigate two well-characterized study groups. The first group, referred as Highlanders (HLS), consisted of healthy individuals who have been living for generations at various altitudes within the high-altitude region (3000–4500 m). Specifically, the HLS cohort included native population from Nubra village (NU) (3048 m), Sakti village (SKT) (3812 m), and Tso Moriri village (TK) (4522 m). The second group, referred as Lowlanders (LLS) encompassed healthy individuals of Indo-Aryan descent from northern India who permanently reside at low altitudes (<500 m). Importantly, this group did not experience altitude-related illnesses when visiting high-altitude areas. The samples for our study were collected through the general outpatient clinic from Sonam Norboo Memorial (SNM) hospital Leh (3500 m), Ladakh, India.

2.2. Sample inclusion and exclusion criteria

The participants belonging to both the HLS and LLS categories for this research underwent a screening process to confirm their absence of mountain-related disorders, as well as other conditions like influenza or the common cold. Moreover, they engaged in regular strenuous physical activities without experiencing high-altitude related disorders. As a means of confirming the absence of acute mountain sickness within the LLS group, the Lake Louise scoring system was employed to evaluate any linked symptoms (Roach et al., 2018). Additionally, individuals with a history of cardiopulmonary diseases were excluded.

2.3. Ethical considerations

The study protocol received approval from the human ethical committees of both the CSIR-Institute of Genomics and Integrative Biology, Delhi and SNM hospital, Leh, Ladakh, India. Prior to their inclusion in the study, every participant gave their informed consent.

2.4. Clinical measurements

Upon admission, an array of measurements, including age, height, weight, body mass index (BMI), blood pressure (BP), and arterial oxygen saturation (SaO_2), were documented for both study groups. BP readings were taken while participants were in supine position, utilizing a calibrated mercury sphygmomanometer fitted with an appropriate adult cuff size. Systolic blood pressure (SBP) was determined at the initial audible Korotkoff sound, while diastolic blood pressure (DBP) marked at the cessation of the Korotkoff sound. This process was repeated thrice, and the average of the three independent measurements was employed as the SBP and DBP values. Mean arterial pressure (MAP) was computed based on these SBP and DBP values. Arterial oxygen saturation (SaO_2) was measured three times using a Finger-Pulse Oximeter 503 (Criticare Systems Inc., USA). Calculations for body mass index (BMI) were performed using recorded body weight and height, while subjects were dressed in light clothing and without footwear.

2.5. Blood sample collection

Ten milliliters of blood sample was collected from each subject in acid-citrate-dextrose as anticoagulant, to obtain both plasma and cells. Genomic DNA was extracted from peripheral blood leukocytes through an adapted salting-out technique, after which it was stored at −20 °C for subsequent analysis (Miller et al., 1988). Concentration and quality of DNA was assessed using nanodrop and agarose gel electrophoresis, respectively. The plasma was aliquoted and stored at −80 °C, until further use.

2.6. Genome-wide genotyping

Genome-wide genotyping was performed using the HumanOmni1M-Quad Beadchip of the Illumina genotyping platform (Illumina, Inc.) according to the manufacturer's guidelines in both the HLS group ($N = 96$) and LLS group ($N = 96$). This bead chip encompasses more than one million markers distributed across the entire genome, with an average inter-SNP distance of 2.4 kb. The fluorescence intensities of each bead were measured using an Illumina iScan machine, and the raw intensity data files were stored. The intensity signals were then converted into specific alleles for each sample. Genotype calls were assigned using the GenCall algorithm implemented in Illumina Genome Studio (version 2010.3; Illumina Inc.).

2.7. Validation of high-altitude associated SNPs

For the purpose of the validation study, SNPs associated with adaptations to high-altitude environments were genotyped using the Illumina GoldenGate assay (Illumina Inc., San Diego, CA), as per manufacturer guidelines. This validation study was conducted within an independent cohort encompassing both the HLS and LLS study populations. The HLS population consisted of individuals from diverse altitudes within the Ladakh region, including individuals from Nubra village (NU) (3048 m; $N = 142$), Sakti village (SKT) (3812 m; $N = 104$), and Tso Moriri village (TK) (4522 m; $N = 74$), in addition to the LLS group (<500 m; $N = 194$).

2.8. Quality control of genotype data

In the initial phase of quality control (QC), SNPs exhibiting intensity-only signals were excluded from the dataset. The remaining SNPs were evaluated for poorly defined clusters using both GenTrain and Cluster separation scores. SNPs with a GenTrain score >0.6 and cluster separation score >0.4 were excluded and the remaining SNPs underwent manual verification process to ensure the accuracy of genotype calls. Following this, subsequent QC analyses were conducted using PLINK v1.07 (Purcell et al., 2007). Stringent QC criteria were implemented separately for both SNP and sample filtering across both the study groups to ensure high-quality genotype data. Samples with a missing rate of surpassing 95 % and discrepancies in gender across all SNPs were removed from the dataset.

For the assessment of cryptic relatedness among samples, identity-by-descent (IBD) analysis was carried out using PLINK v1.07. To facilitate this analysis, the dataset was pruned by removing SNPs in linkage disequilibrium (LD). The pruning was conducted utilizing the indep-pairwise function of PLINK v1.07, employing a window size of 50 SNPs, a step size

of 5, and a linkage disequilibrium coefficient (r^2) of 0.2. Subsequently, samples displaying evidence of cryptic relatedness (pi_hat score > 0.1875) were removed from the dataset.

2.9. Population structure analysis

Principal Component Analysis (PCA) was conducted as a part of the analysis to assess population structure and detect any outliers. This was accomplished using a set of independent pruned SNPs. SMARTPCA, implemented in EIGENSOFT version 3.03, for this analysis. The identification and subsequent removal of outliers was carried out through the outlier removal option within SMARTPCA. In this analysis, a threshold of 6, along with 5 iterations along the first 10 principal components, was applied. A burn-in period of 10,000 and 10,000 iterations were employed for this procedure. Moreover, in comparison to the HapMap Phase III samples (Thorisson et al., 2005; International HapMap, C, 2003), we conducted PCA for our samples. This enabled a contextual understanding of our sample distribution relative to these established reference samples.

2.10. Association analyses

To identify SNPs associated with high-altitude adaptation, logistic regression analysis was performed under a log-additive model using PLINK v1.07. Specifically, this analysis was conducted using SNPs that had successfully passed the quality control criteria. A quantile-quantile plot was also constructed to investigate deviations between observed *P* values for association and the expected *P* values under the null hypothesis. Additionally, the genomic inflation factor, computed based on the average chi-square statistics, was calculated using PLINK v1.07. This factor offers insights into any inflation or deflation of test statistics due to underlying population stratification or other confounding factors.

2.11. Haplotype analysis

Haplotype analysis was performed for the study samples, with reference samples sourced from the 1000 Genomes Project (Genomes Project, C, et al., 2012; Delaneau et al., 2014). For this analysis, genotype data were phased, and subsequent haplotype calling was caried out using fastPHASE v.1.4.8 (Scheet and Stephens, 2006). This analysis encompassed a total of 10 starts/runs of the EM algorithm and 15 clusters to provide a robust assessment of the haplotype structure and distribution.

2.12. Estimation of 8-iso-prostaglandin F2α level

The quantification of plasma 8-isoPGF2α levels was conducted using an ELISA kit obtained from Enzo Life Sciences Inc., (Cat.#BML-AK150, Enzo Life Sciences, Inc., NY, USA) Farmingdale, New York, NY, USA according to manufacturer’s instructions.

2.13. In silico functional prediction of associated SNPs

To better understand the potential functional implications of the identified SNPs, we conducted in silico analyses using the Enrichr tool (Kuleshov et al., 2016). Enrichr facilitates pathway enrichment and gene ontology (GO) analyses based on various databases, including the Protein ANalysis THrough Evolutionary Relationships (PANTHER) pathway (Mi et al., 2017; Mi and Thomas, 2009), Kyoto Encyclopedia of Genes and Genomes (KEGG)

(Kanehisa et al., 2017; Kanehisa and Goto, 2000; Kanehisa et al., 2016), and the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (Huang da et al., 2009a; Huang da et al., 2009b).

Additionally, we harnessed Enrichr to identify SNPs that might potentially impact transcription factor binding sites (TFBS). This analysis allowed us to prioritize specific SNPs and gain novel insights into the associated regions and genes. Enrichr provides graphical and tabular TFBS hit representation with four scores: *P* value, q-value, rank score, and combined score (log(*P* value) * rank score) for comprehensive SNP impact assessment on TFBS.

2.14. Correlation analyses

The correlation analyses between clinical and biochemical parameters were performed using SPSS.ver.12. Similarly, the correlation analyses between genetic markers, clinical and biochemical parameters was performed using Plink v1.07. For assessing the overall associations among biomarkers and clinical parameters, a linear regression model was employed. The determination of *P* values, correlation coefficients (R), regression coefficients or estimated differences (β), and odds ratios (OR) was achieved using ANCOVA, followed by Bonferroni's correction test to control for multiple comparisons. Additionally, the correlation analyses between the alleles and clinical parameters were performed through linear regression, as implemented in PLINK v1.07.

3. Results

3.1. Demographic and clinical parameters

The demographic characteristics of the research participants involved in association analysis for both the discovery (GWAS) and validation phase of this study are outlined in Fig. 1A. Notable, significant differences were observed in age and gender between the two study groups ($P < 0.001$). To adequately address these differences during the genetic analysis, both age and gender were taken into consideration as covariates within the regression analysis.

Clinical characteristics of the study subjects of the discovery phase are outlined in Fig. 1B. Among the clinical characteristics analysed, it was observed that the average BMI was elevated in the HLS group as compared to the LLS group. Additionally, the assessment of SaO_2 level, indicative of hypoxemia, exhibited a significant decrease in the HLS group with respect to the LLS group (Fig. 1B). Nevertheless, there was no significant difference in the average MAP between the study groups (Fig. 1B).

Genome-wide association analysis revealed SNPs associated with high-altitude adaptation.

We conducted an extensive comprehensive genome-wide investigation aimed at elucidating the SNPs contributing to high-altitude adaptation across different altitudes. Fig. 2 presents a schematic representation that outlines the analysis approach for identifying SNPs associated with high-altitude adaptation.

Following stringent quality control measures on the genome-wide genotype data, 794,442 out of 1,134,514 SNPs that satisfied the quality criteria were employed for PCA within our study groups. When conducting PCA with the HapMap populations, we filtered and employed a shared set of SNPs (n = 751,316) present in both our study and HapMap populations. The primary eigenvector of the PCA effectively differentiated the African populations from the remaining HapMap and study populations, explaining 55.6 % of the genetic variation among these groups. Notably, in our context, the HLS individuals exhibited a distinct cluster, with the nearest neighbouring populations being the East Asians (EAS), specifically the Han Chinese in Beijing, China (CHB), Chinese in Metropolitan Denver, Colorado (CHD), followed by the LLS population (Fig. 3A).

We employed the Fixation index (Fst) score to assess the degree of genetic differentiation between our study groups and the HapMap populations. The result of the Fst analysis reaffirmed a minimal genetic divergence among HLS, LLS, and EAS populations (Fst ≤ 0.035), as depicted in Fig. 3B. Given that the EAS and HLS exhibited the closest genetic affinity, we opted to utilize the EAS population as our reference group for the purpose of investigating the SNPs associated with high-altitude adaptation.

For the analysis of high-altitude adaptation, we performed association analysis including adjustment for age, gender, and potential population stratification. The result of the association analysis revealed 1070 SNPs exhibiting significant difference in allele frequency between the HLS and lowland HapMap CHB population (P < 1.0E-08), suggesting their role in high-altitude adaptation (Fig. 3C). Based on the association result and LD, 456 SNPs were prioritized for their association with high-altitude adaptation. These selected SNPs exhibited a significant level (P ≤ 1.0E−07), as outlined in Table S1. Spanning diverse genes and genomic locations across the entire genome, these SNPs underscore their potential pivotal role in high-altitude adaptation.

Furthermore, pathway analysis unveiled notable enrichments in pathways associated with fatty acid degradation, cell adhesion molecules, and glycolysis in high-altitude adaptation (Fig. 3D and Table S2).

Distinct genetic clustering within the HLS population implies an evolution guided by selection pressure.

According to the GWAS data, it was clear that the HLS represented a genetically distinct population. Our subsequent goal was to investigate the relationship between the high-altitude environment and genetic variability. To conduct this analysis, we reclassified the HLS based on their residential altitude, and found that individuals from various high-altitude regions formed separate genetic clusters (Fig. 4A). Specifically, these populations included residents of Nubra (NU, 3048 m), Sakti (SKT, 3812 m), and Tso Moriri (TK, 4522 m) regions of Ladakh (as shown in the inset of Fig. 4A). These individuals demonstrated strong adaptation to incremental altitude changes within the Ladakh region of Himalayas, spanning an altitude difference of ~700 m.

Subsequently, we explored the ancestry of the study populations by estimating global admixture proportions. In line with the outcome of PCA analysis, the admixture analysis

confirmed that the HLS is an admixed population, sharing similarities in genetic background with both LLS (49.8 %) and EAS (50.6 %) groups. This shared genetic background was accompanied by noticeable genetic clustering associated with different altitudes within these populations (Figs. 4B and S1). Notably, from both genetic and geographical standpoints, the TK population exhibited the closest genetic affinity to the EAS population (62 %), while the NU population demonstrated its closest genetic association with the LLS population (59 %).

To gain insights into the environmental influence of the altitudes inhabited by these populations, we assessed the levels of hypobaric hypoxia within these groups. Interestingly, as shown in Fig. 4C, the ascending altitudes of the HLS group coincided with a significant reduction of ≥5 % in ambient oxygen availability and ≥50 mmHg barometric pressure. In summary, this data indicates that environmental factors exert selection pressures, driving comprehensive genetic alterations that aid in high-altitude adaptation.

Hypobaric hypoxia drives altitude-associated positive selection of adaptation alleles.

To further comprehend the correlation between adaptation alleles and altitude we performed a validation study using Illumina GoldenGate assay. For validation, 456 significant markers associated with high-altitude adaptation were genotyped across three distinct study groups: NU, SKT, and TK, along with the reference lowland study populations, LLS, and HapMap CHB. As part of the validation phase, we additionally incorporated SNPs previously reported in literature, along with our own data for high-altitude adaptation, making comparisons with both the CHB and the LLS group. Notably, a total of 302, 371, and 289 markers demonstrated significant associations with high-altitude adaptation in NU, SKT, and TK, respectively, in contrast to CHB and LLS reference groups (Fig. 5A). Tables 1-3 enlist the top 20 markers associated with high-altitude adaptation in NU, SKT and TK, respectively. Additionally, we deciphered this data to reveal the markers enriched in the TK population, which represents the highest-altitude residents in our study. This allowed us to comprehend the impact of extreme hypobaric hypoxia. Among these markers, we identified 34 SNPs that displayed significant associations exclusively within the TK population, in comparison to the LLS and CHB populations (Table 4). This unveiling shed light on potential genes implicated in oxidative stress response, such as Dual Oxidase Maturation Factor 1 (*DUOXA1*) (Zhou et al., 2013).

In relation to markers associated with high-altitude adaptation, we observed a variable trend in the frequency of adaptive alleles across the three high-altitude populations. This observation led us to investigate a potential correlation between the allele frequency of these adaptive SNPs with increasing altitude. To explore this correlation, we conducted an analysis of adaptive allele frequency with respect to altitude. In this analysis, exclusive focus was placed on SNPs that were significantly enriched in the HLS compared to the low altitude populations (LLS and CHB). To ensure robustness, stringent quality control measures were employed on the significant high-altitude associating SNPs. The aim was to exclude SNPs with contradictory association pattern, specifically the SNPs that followed opposite association trend, particularly those displaying opposing trends in association – SNPs with an odds ratio >1 in comparison to either LLS or CHB but <1 in other comparison. (Fig. 5A).

Such contradictory SNPs were omitted from the analysis (Fig. 5A), leaving only variants exhibiting consistent high-altitude association trends for further investigation.

This comprehensive analysis unveiled a total of 86 SNPs with genetic aligning to high-altitude adaptation ($0.05 > P > 6.8E{-}48$, Table S3). Detailed information about their genomic locations, genes and association *P* values are presented in Table S4. We perused the correlations by mapping the frequency of these 86 SNPs against the respective altitude of residence (Fig. S2). Of consequence, among the 86 SNPs, 38 SNPs displayed a linear correlation with increasing altitude, suggesting an evolution process driven by selection pressure (Fig. 5B and C). A significant proportion of these SNPs were located on chromosomes 1 and 2, with a notable concentration in genes such as EGLN1 (rs973253 A/G, rs2486736 A/G, rs480902 G/A, rs2486729 A/G, and rs2808611 A/G), EPAS1 (rs2121266 C/A, rs4953353 A/C, and rs7571218 A/G) and SprT-Like N-Terminal Domain (SPRTN) (rs1009227 and rs2749717), exhibiting a prominent presence of SNPs following this pattern. Both *EPAS1* and *EGLN1* are integral to the complex network of genes and pathways that help individuals acclimate and adapt to high-altitude environments. *SPRTN* serves as a nuclear metalloprotease involved in DNA repair processes, where human mutations have been linked to genomic instability (Brutsaert et al., 2019). Variations in these genes can impact their function, potentially influencing an individual's ability to adapt to altitude-related challenges. The presence of multiple SNPs associated within these genes underscores their significance in high-altitude adaptation.

Multi-allele association of EGLN1 and EPAS1 SNPs correlate with distinctive altitude-associated linear amplification of haplotypes.

To comprehensively understand high-altitude adaptation, we adopted a haplotype-based approach. For haplotype analysis, we used the GWAS data to include SNPs flanking the 38 significant SNPs. By examining haplotypes, our goal was to capture the combined influence of multiple alleles. Interestingly, our analysis revealed an enrichment of altitude-associated haplotypes that carried variants such as rs2009873, rs973253, rs480902, rs2486729, rs2808611 within EGLN1 and rs4953353 and rs7571218 within EPAS1 genes in the HLS population (Fig. 5D). These genes are recognized for their pivotal roles in responding to hypoxia, making them vital markers of adaptation. The genetic characterization of these SNPs and their neighbouring loci concerning altitude and diminishing oxygen levels yields significant insights into the mechanisms of natural adaptation. This exploration opens new avenues for further investigation into the intricate dynamics of high-altitude adaptation.

In silico analyses unveiled the functional roles of significant SNPs in modulating gene expression.

To assess the potential functional impact of the identified significant SNPs on gene expression, we leveraged data from the Genotype-Tissue Expression (GTEx) project (Consortium, G.T, 2013). Through this analysis, we identified 20 SNPs distributed across 16 genes that demonstrated pertinent expression quantitative trait loci (eQTLs) in various tissues, including lung, muscle, blood, and liver (Fig. S3). The precise genomic positions, linked genes, and corresponding associated *P* values are detailed in Fig. 6A. Additionally,

Table 5 provides a comprehensive breakdown of the adaptive alleles associated with these SNPs. This comprehensive analysis sheds light on the potential functional implications of these SNPs in regulating gene expression across different tissues.

To uncover the impacted signalling pathways by these SNPs, we conducted pathway analysis. Notably, there was enrichment in pathways related to glycolysis and the HIF-1α transcription network (Table S5). Reactome pathways associated with the 20 genes indicated significant associations, highlighting cryoprotection by Heme Oxygenase 1 (HMOX1), oxygen dependent proline hydroxylation of Hypoxia Inducible Factor 1 Subunit Alpha (HIF-1α) and cellular responses to hypoxia as prominent pathways (Fig. 6B). Additionally, an alternative tool exhibited strong networking within these signalling systems (Fig. S4a), reinforcing their relevance in high-altitude adaptation. For deeper insights into regulatory mechanisms, Enrichr was employed to curate transcription factor's target networks from the CHEA (Fig. S4b), and TRANSFAC and JASPAR transcription factor databases (Fig. S4c). Among the other notable signalling systems, Runt-domain transcription factors family (RUNX), Transcription Factor 4 (TCF4), Jun Proto-Oncogene AP-1 Transcription Factor Subunit (JUN), Myogenic Differentiation 1 (MYOD1) and Nuclear Receptor Subfamily 2 Group F Member 1 (NR2F1) transcription factors depicted strong networking in context to high-altitude adaptation. Several reports indicate the involvement these transcription factors in mediating the physiological responses to hypoxic conditions (Alfranca et al., 2002; Lee et al., 2017; Abu-Jamous et al., 2017; Beaudry et al., 2016). For example, HIF-1α stabilization is reported to suppress TCF-4, inducing quiescence (Kaidi et al., 2007). Similarly, the NR2F1 gene triggers the phosphatidylinositol-3-kinase (PI3K)/Akt and the mammalian target of rapamycin (mTOR) signalling, enhancing HIF-1α expression and protein stability (Liu et al., 2022; Agani and Jiang, 2013). Overall, our analysis revealed crucial genes for highlighting the intricate nature of high-altitude adaptation, which is governed by a diverse array of genes and physiological pathways.

SaO_2 and plasma 8-isoPGF2α emerge as significant clinical and biochemical markers modulated by alleles associated with high-altitude adaptation.

We next proceeded with the evaluation of potential clinical and biological markers, including BP, SaO_2, and 8-isoPGF2α, a stress biomarker in HLS. Furthermore, we examined the correlations between these clinical and stress markers and their associations with altitude-associated SNPs. BMI was higher among HLS but did not demonstrate significant differences between HA populations (Fig. 7a i). However, a noteworthy finding was the significant altitude-associated deficit in SaO_2 ($P < 0.0001$) (Fig. 7a ii) suggesting that the physiological stress among HLS is exacerbated by the decreased percentage of environmental oxygen (Fig. 7a ii). Hypobaric hypoxia, a consequence of high altitude, has been reported to elevate BP. Consistent with previous findings from our laboratory and the present study, elevated BP emerged as one of the physiological traits of HLS. Specifically, NU and SKT exhibited an elevation of approximately 10.0 mmHg in SBP compared to LLS ($P < 0.0001$), suggesting an inverse correlation between altitude and BP (Fig. 7a iii-v). To assess the impact of altitude on the biological system, we measured the circulating levels of 8-isoPGF2α. Data showed HLS individuals exhibited elevated levels of 8-isoPGF2α compared to LLS, coupled with an altitude-associated incremental pattern (Fig. 7a vi).

Correlation analyses revealed an inverse correlation between SaO_2 and SBP (Fig 7b i), DBP (Fig. 7b ii), and mean arterial pressure (MAP) (Fig 7b iii), as well as a linear correlation between SBP and DBP (Fig 7b iv), implying the positive association and dependence of body's physiological parameters with high altitude.

In addition, we conducted extensive correlation analyses to validate the in silico functional observations and assess the impact of these expression quantitative trait loci (eQTLs) on the clinical and biomarker parameters in our study subjects (Fig. 7c). Notably, several significant genes and their associated SNPs emerged from our analysis, including *SPRTN* (rs1009227, rs2749717), *EGLN1* (rs2009873, rs973253, rs2486736, rs480902, rs2486729, rs2808611), *EPAS1* (rs7571218), Transmembrane Protein 182 (*TMEM182*) (rs2540321), *COQ7* (rs11643792), and the metabolism-associated gene *ADH6* (rs1893883). Furthermore, we found that the adaptive alleles of ADH6 (rs1893883), Coenzyme Q7, Hydroxylase (*COQ7*) (rs11643792), and NSF Attachment Protein Gamma (*NAPG*) (rs544553) exhibited correlations with decreased SBP, providing further evidence of their functional role in stabilizing blood pressure under hypobaric hypoxia. Furthermore, *SPRTN* and *EGNL1* SNPs (rs1009227, rs2749717, rs2009873, rs973253, rs2486736, rs480902, rs2486729 and rs2808611) exhibited correlations with plasma 8-isoPGF2α. These findings strengthen the link between the identified SNPs and their influence on BP regulation in high-altitude adaptation.

4. Discussion

The present study aimed to identify the role of hypobaric hypoxia in modulating the genetic and physiological hallmarks of high-altitude adaptation. Through a comprehensive genome-wide association analysis, we identified alleles and haplotypes that exhibit a correlation with increasing altitude, alongside clinical and biochemical markers. Notably, these genetic variants encompassed key genes such as EGLN1, EPAS1, SPRTN, ADH6, and DUOXA1 related to oxygen sensing, metabolism, and mitochondrial homeostasis. In silico analyses further confirmed the functional relevance of these SNPs in orchestrating the body's physiological response to high-altitude conditions.

Our population genetic analyses identified HLS as an admixed population with genetic similarities to LLS and CHB. We aimed to assess the relationship between the high-altitude environment and genetic variability. To explore this, we revisited our population stratification data obtained from the GWAS. The analysis highlighted discernible genetic clustering that corresponds to different altitudes, underscoring the distinctive genetic response to varying environmental conditions. Interestingly, we observed altitude-associated genetic clustering within the HLS populations inhabiting diverse altitudes, particularly NU, SKT, and TK. To comprehend the impact of extreme hypobaric hypoxia, we identified 34 SNPs that displayed significant associations exclusively within the TK population, in comparison to the LLS and CHB populations, and provided a valuable perspective on possible genes, especially DUOXA1 that has known association with the body's response to high-altitude associated oxidative stress (Zhou et al., 2013). Further, the association analysis identified 86 common SNPs between NU, SKT, and TK, depicting a significant association with high-altitude adaptation in comparison to the LLS and CHB populations.

The correlation analysis of these SNPs revealed that 38 out of 86 SNPs displayed a strong positive correlation of adaptive alleles with increasing altitude. The analysis provided a significant proportion of SNPs presence on hypoxia-associated genes such as EGLN1, EPAS1, and SPRTN. Furthermore, the haplotype analysis suggested a strong enrichment of altitude-associated haplotypes in EGLN1 and EPAS1 genes. A recent study suggests that progressive exposure to increasing altitude can impact the epigenome, specifically EPAS1, which in turn could play a significant role in the process of altitude acclimatization (Childebayeva et al., 2019; Childebayeva et al., 2021). Our analysis corroborates with the recent findings providing insights into high-altitude adaptation and corresponds to a significant drop of over 5 % ambient oxygen with increasing altitude, highlighting the impact of environmental factors on genetic selection (Beall, 2006; Beall, 2007; Bigham, 2016; Horscroft et al., 2017).

Genes like EGLN1, EPAS1, SPRTN, ADH6, and DUOXA1 emerged with the most substantial evidence of selection with high-altitude associated metabolism, and oxidative stress in populations around the world (Yi et al., 2010; Simonson et al., 2010; Mishra et al., 2013; Huerta-Sanchez et al., 2013; Peng et al., 2011). In hypoxic conditions, the enzyme EGLN1 is responsible for ubiquitinating HIF-1α, leading to increased transcription of hypoxia-inducible genes (del Peso et al., 2003; Huerta-Sanchez et al., 2013). Similarly, EPAS1 variants play a role in stabilizing HIF2α, the master transcriptional regulator for hypoxia adaptation (Peng et al., 2017). Additionally, *EPAS1* knockout study has predicted a vital role of EPAS1 genetic variants in regulating the haemoglobin levels and various physiological traits in Tibetan population (Hu et al., 2017; Peng et al., 2017; Yi et al., 2010). Other notable SNPs of significance in relation to hypobaric hypoxia involve genes like *KIF3A* and *NAPG*, involved in the elevation of platelet cytosolic Calcium and platelet exocytosis, respectively (Temiyasathit et al., 2012; Polgar and Reed, 1999). Other genes like *COQ7* and *COX7A2L* are involved in oxidoreductase activity along with *LINC01118*, which strongly associates with haemoglobin and hypoxic diseases (Lapuente-Brun et al., 2013; Astle et al., 2016).

The eQTLs analysis further confirmed the results identified through the various analyses. Notably, the *EGLN1* SNPs (rs2009873, rs973253, rs2486736, rs2486729, rs2808611), and *EPAS1* SNP rs7571218, emerged as significant eQTLs in lung and muscle, and whole blood. Adding to metabolic insights of the high-altitude adaptation, rs1893883 emerged as a significant eQTL for various genes of alcohol dehydrogenase pathway, like *AHD1C*, *ADH4*, *ADH5*, and *ADH6*, as previously reported to be involved in high-altitude adaptation (Huerta-Sanchez et al., 2013). Nonetheless, the results suggests that these SNPs may act as functional switches to control the gene expression in regulating the body's response towards high-altitude adaptation. Further, pathway and transcription analyses revealed the enrichment of genes downstream of transcription factors like RUNX and JUN. These transcription factors along with a multitude of gene expression changes are known to govern cellular responses and signalling pathways associated with hypoxia (Alfranca et al., 2002; Lee et al., 2017; Kumar et al., 2023). Whereas, other transcription factors like MYOD1 and VDR regulate myogenesis and diverse metabolic pathways in hypoxic and other environmental conditions (Abu-Jamous et al., 2017; Beaudry et al., 2016; Singh and Arora, 2022). Overall, this data highlights a robust synergistic interplay of the genes

identified in this study in maintaining physiological stability in the context of hypoxic conditions at high altitudes.

Lastly, clinical and biochemical parameters further supported the possible role of these SNPs in influencing physiological responses at high-altitude. The SaO_2 showed a decreased oxygen saturation with Nubra, Sakti and Tso Moriri having approximately 90 %, 85 % and 83 % SaO_2, respectively. This decline in SaO_2 triggers the necessary response for preserving cellular oxygen homeostasis and, consequently, the adaptive mechanisms (Alfranca et al., 2002). Notably, blood pressure values displayed a noteworthy trend, indicating a decrease with increasing altitude, implying a negative relationship between altitude and blood pressure levels.

Over the years, oxidative stress has been predominantly linked with increased blood pressure in various experimental models of hypertension. Our analyses also revealed elevated levels of 8-isoPGF2α in HLS, advocating adaptation to environmentally induced oxidative stress. These observations imply that the vascular system possibly slows down to stabilise with altitude. Our study presents the crucial cross-talk between the fundamental parameters, SaO_2 and BP, illustrating that the HLS have remodelled their physiological system such as the metabolic, vascular, and oxygen-signalling, safeguarding the crucial steps towards high-altitude adaptation; the very phenomenon strongly regulated by the environment-evolutionary synergism.

Finally, the outcome of the correlation analysis of alleles with clinical and biochemical parameters suggests that alleles that significantly affect SaO_2, BP, and 8-isoPGF2α levels are likely a consequence of natural selection. We observe that the significant SNPs of oxygen-sensing genes and their flanking regions correlate with elevated SaO_2. This data suggests a reinforcing evidence, indicating that multigenic interactions contribute to adaptability through consistent physiological modifications over successive generations, ultimately supporting life at high altitudes.

The present study explores the environment-induced genetic adaptation, influenced by a ~ 5 % reduction in environmental oxygen with increasing altitude. We present novel functional adaptive alleles that linearly correlate with the altitude of residence, underscoring the significance of various attributes in comprehending high-altitude adaptation. This data could potentially influence our comprehension of human disorders characterized by hypoxia. While previous studies have highlighted EGLN1 and EPAS1 as key genes for high-altitude adaptation, our research broadens this perspective by considering the impact of varying degrees of hypobaric hypoxia on the adaptation process. Importantly, within high-altitude populations, alleles associated with altitude and spanning hypoxia-sensing and metabolism genes exhibit positive correlations with SaO_2 and negative correlations with 8-isoPGF2α.

4.1. Limitations of the study

Limitations of our study include the complexity of gene-environment interactions and establishing causality with potential confounding factors and challenges in distinguishing between adaptation and random genetic drift. Additionally, ethnic diversity, and variations in altitude-related parameters can impact the generalizability of findings. Here, we identified

the correlation between the genes and their associated SNPs with physiological parameters providing confounding evidences of natural selection among populations residing in high altitude areas. However, the biological functions of associated genes towards the body's physiological adaptation to hypobaric hypoxia need further exploration for functional relevance of the associated SNPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We appreciate the encouragement of the Director, CSIR-Institute of Genomics and Integrative Biology, Delhi, the cooperation of medical staff at SNM hospital, Leh, Ladakh, and the participation of the volunteers in the study.

Funding

This work was supported by grants from the Council of Scientific & Industrial Research (CSIR), India, under projects SIP0006 (QP) and BSC0123 (QP). SS availed senior research fellowship from CSIR, India. RK was supported by United Therapeutics Jenesis Innovative Research Award and BBG was supported by NIH grants R01HL135872 and P01HL152961.

Data availability

Data will be made available on request.

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HIGHLIGHTS

- Study sought key genetic markers for altitude adaptation with increasing elevation.
- 86 SNPs validated from GWAS showed association with increasing altitude adaptation.
- Strong linear correlation of 38 SNPs with increasing altitude was observed.
- Variants of key genes like EGLN1, EPAS1 & ADH6 regulate high-altitude adaptation.
- Functional genetic variants impact physiological adaptation at varying altitudes.

A

Clinical characteristics		LLS	HLS	*P* value LLS vs HLS
Discovery phase				
Age (years)		26.4 ± 7.5	38.8 ± 10.9	<0.001
Gender	Males (n, %)	96 (100%)	80 (83.3%)	<0.001
	Females (n, %)	0 (0.0%)	16 (16.7%)	
Validation phase				
Age (years)		25.4 ± 6.1	41.2 ± 11.5	<0.001
Gender	Males (n, %)	186 (95.9%)	121 (37.8%)	<0.001
	Females (n, %)	8 (4.1%)	199 (62.2%)	

B

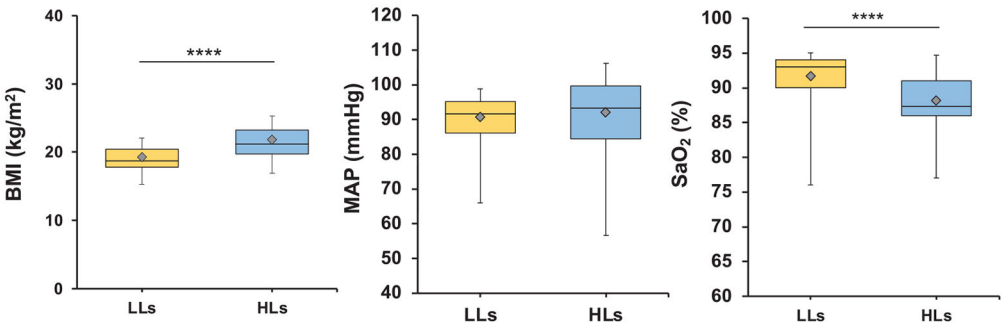


Fig. 1.

Demographic and clinical characteristics of the study groups. A) The table depicts the demographic characteristics of the study groups. The data are presented as mean ± SD. (B) The box plots depicts the clinical characteristics of the study groups. The X-axis represents the study groups and Y-axis represents the clinical parameter, Body mass index (BMI, kg/m^2), Mean arterial pressure (MAP, mmHg), and Arterial oxygen saturation (SaO_2,%). The diamond in each boxplot represents the mean value for the study group. A *P* value ≤0.05 was considered statistically significant. n, number of samples; SD, standard deviation; LLS, Lowlanders; HLs, Highlanders. ****, P value <0.0001.

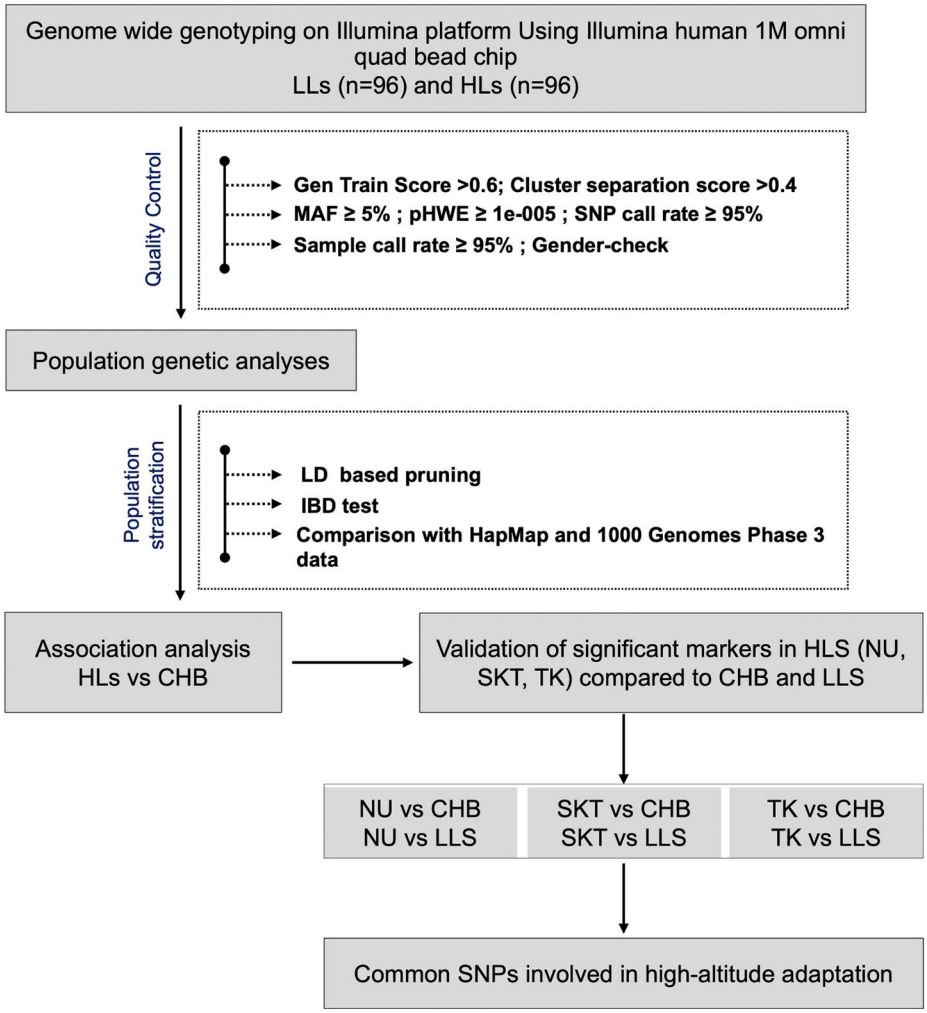


Fig. 2.

Schematic representation outlining the analysis strategy for identifying genetic variants associated with high-altitude adaptation.

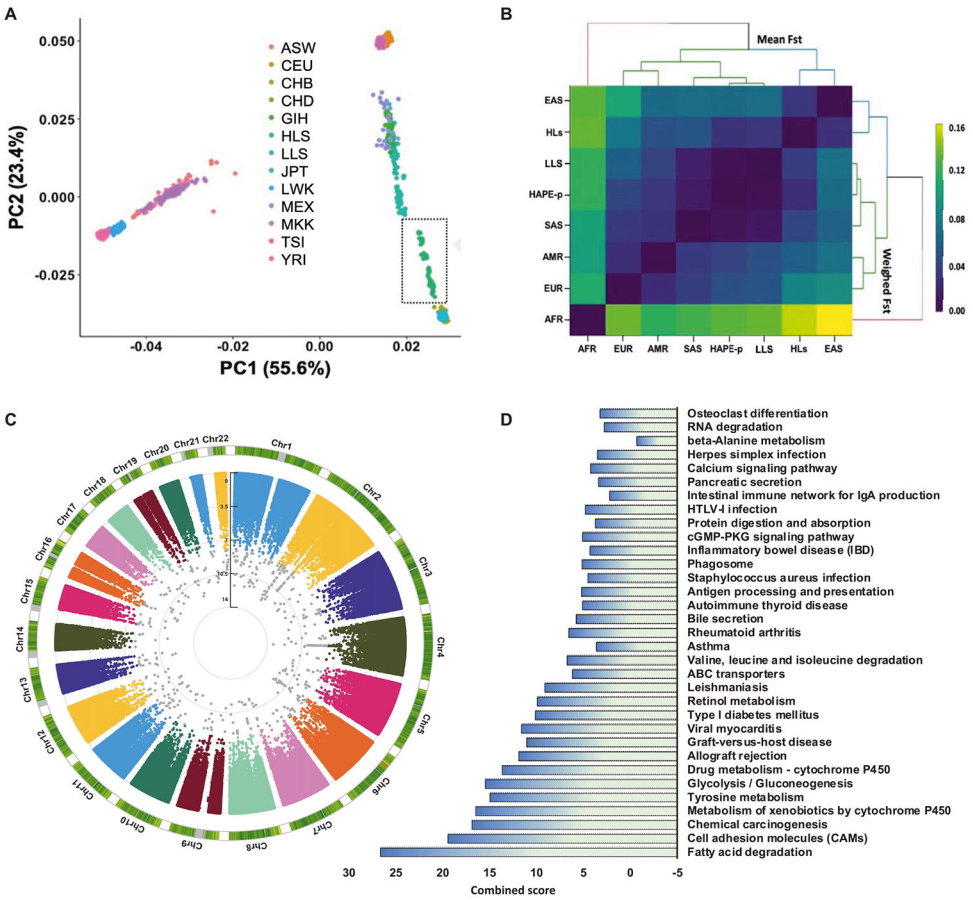


Fig. 3.

Genome-wide analysis to identify genetic variants associating with high-altitude adaptation. A) Principal component analysis plot of the study populations with HapMap populations. Points correspond to individuals, and colors indicate population assignments. The first and second principal components are plotted on the x- and y-axes, respectively. (B) Pairwise mean and weighed Fixation index (Fst) within all the study populations and 1000 genome phase III populations. (C) Circos plot for the genome-wide association analysis of SNPs of high-altitude adaptation between HLS and HapMap Han Chinese population. In this plot, the axis going inwards represents the −log10 *P* value. (D) Pathway analysis of significant genes associating with high-altitude adaptation. ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; HLS, Highlanders, LLS, Lowlanders, HAPE-p, High-altitude pulmonary edema patients, JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California; MKK, Maasai in Kinyawa, Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria West Africa. AFR, Africa; EUR, European; AMR, American; SAS, South Asian; EAS, East Asian.

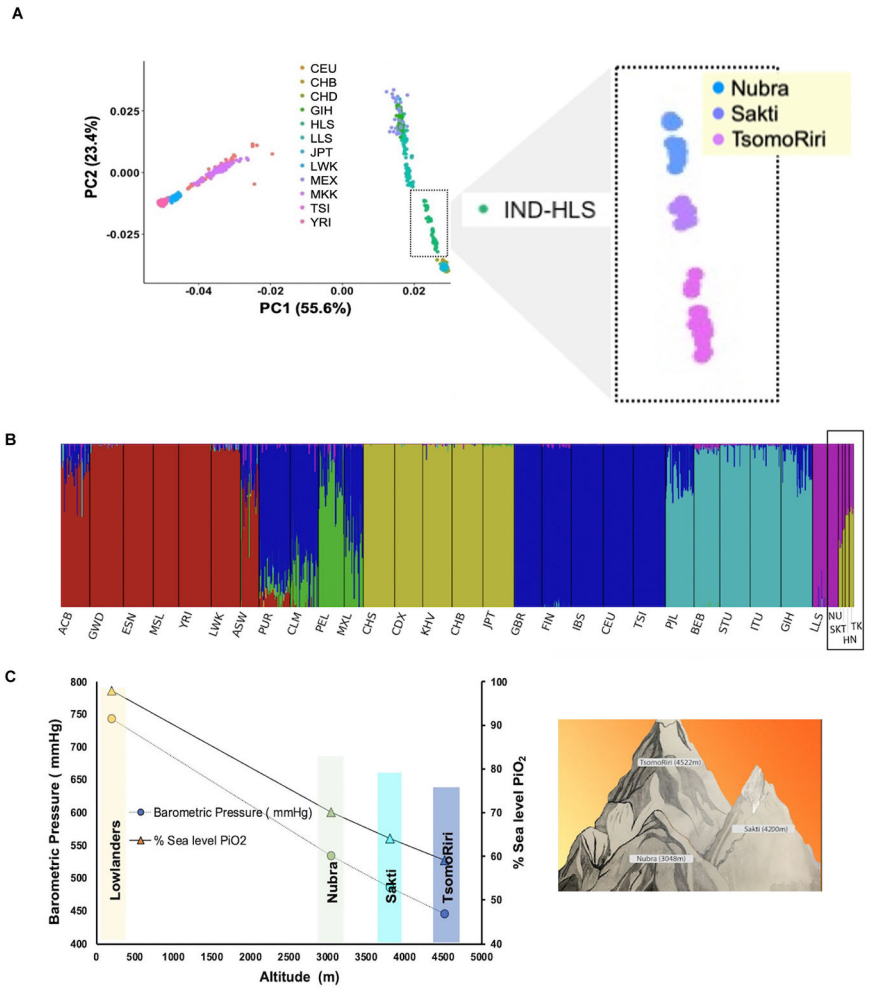
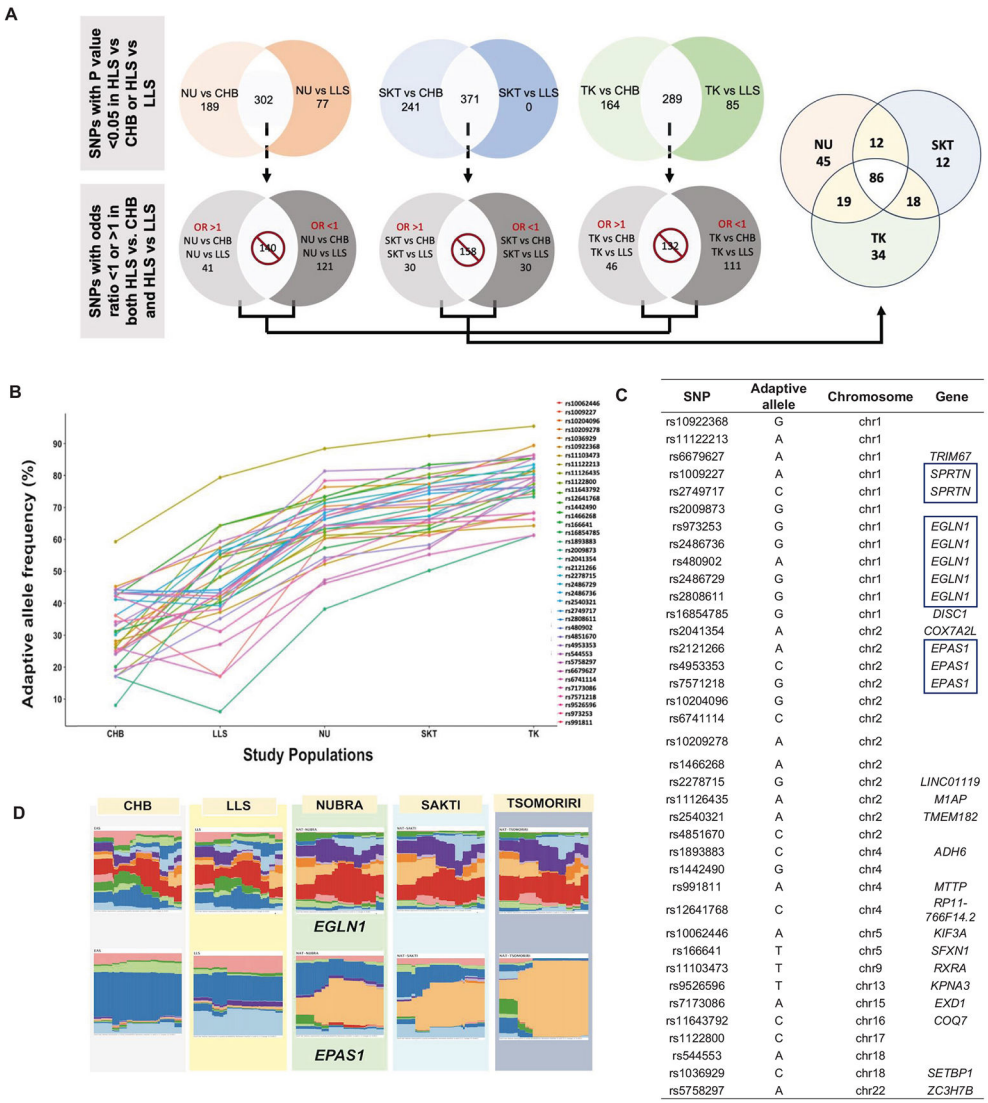


Fig. 4.

Genome-wide analysis identifies altitude-associated genetic clustering within the HLS population. A) Principal component analysis plot of the study populations with HapMap populations. Points correspond to individuals, and colors indicate population assignments. The first and second principal components are plotted on the x- and y-axes, respectively. The inset presents the clustering of different populations of high-altitude. (B) Genetic admixture analysis plot represents the genetic admixture analysis of study populations with 1000 Genomes Phase 3 populations for K = 6, where K is the number of presumed ancestral populations. (C) The graph represents the relationship between the altitude of residence, the barometric pressure and partial pressure of inspired oxygen for the study subjects. The X-axis represents the altitude of residence (m). The primary Y-axis represents the barometric pressure (mmHg) and the secondary Y-axis represents the percentage of sea level partial pressure of oxygen (PiO2) in ambient air for the study populations. ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; HLS, Highlanders, LLS, Lowlanders, HAPE-p, High-altitude pulmonary edema patients, JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California;

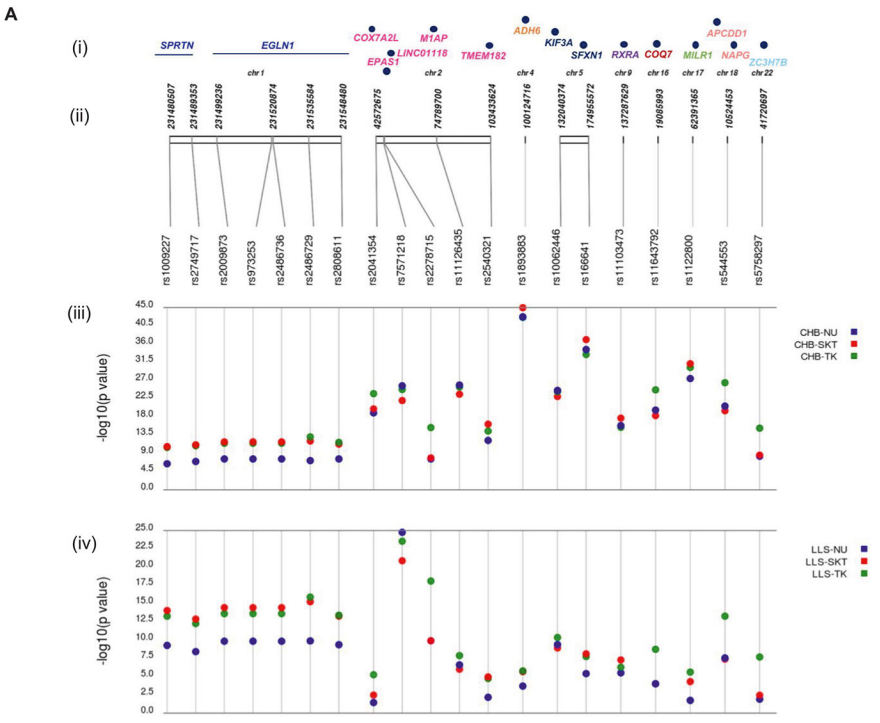
MKK, Maasai in Kinyawa, Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria West Africa. AFR, Africa; EUR, European; AMR, American; SAS, South Asian; EAS, East Asian.



SNP	Adaptive allele	Chromosome	Gene
rs10922368	G	chr1	
rs11122213	A	chr1	
rs6679627	A	chr1	*TRIM67*
rs1009227	A	chr1	*SPRTN*
rs2749717	C	chr1	*SPRTN*
rs2009873	G	chr1	
rs973253	G	chr1	*EGLN1*
rs2486736	G	chr1	*EGLN1*
rs480902	A	chr1	*EGLN1*
rs2486729	G	chr1	*EGLN1*
rs2808611	G	chr1	*EGLN1*
rs16854785	G	chr1	*DISC1*
rs2041354	A	chr2	*COX7A2L*
rs2121266	A	chr2	*EPAS1*
rs4953353	C	chr2	*EPAS1*
rs7571218	G	chr2	*EPAS1*
rs10204096	G	chr2	
rs6741114	C	chr2	
rs10209278	A	chr2	
rs1466268	A	chr2	
rs2278715	G	chr2	*LINC01119*
rs11126435	A	chr2	*M1AP*
rs2540321	A	chr2	*TMEM182*
rs4851670	C	chr2	
rs1893883	C	chr4	*ADH6*
rs1442490	G	chr4	
rs991811	A	chr4	*MTTP*
rs12641768	C	chr4	*RP11-766F14.2*
rs10062446	A	chr5	*KIF3A*
rs166641	T	chr5	*SFXN1*
rs11103473	T	chr9	*RXRA*
rs9526596	T	chr13	*KPNA3*
rs7173086	A	chr15	*EXD1*
rs11643792	C	chr16	*COQ7*
rs1122800	C	chr17	
rs544553	A	chr18	
rs1036929	C	chr18	*SETBP1*
rs5758297	A	chr22	*ZC3H7B*

Fig. 5.

(A) Venn diagram depicting the criteria and number of SNPs associating with high-altitude adaptation (B) the altitude-associated allele frequency for 38 significant variants. The x-axis represents the study populations, and the y-axis represents the altitude-associated adaptive allele frequency. The table on the right side depict the SNPs with their adaptive allele and chromosome location. C) Details of the 38 SNPs displayed depicting a linear correlation with increasing altitude. (D) The altitude-associated haplotype differentiation ($P < 0.05$) for Prolyl Hydroxylase Domain-Containing Protein 2 (EGLN1) and Endothelial PAS Domain Protein 1 (EPAS1) within the study populations. ASW, African ancestry in Southwest USA; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; CHD, Chinese in Metropolitan Denver, Colorado; GIH, Gujarati Indians in Houston, Texas; HLS, Highlanders, LLS, Lowlanders, JPT, Japanese in Tokyo, Japan; LWK, Luhya in Webuye, Kenya; MEX, Mexican ancestry in Los Angeles, California; MKK, Maasai in Kinyawa, Kenya; TSI, Tuscan in Italy; YRI, Yoruban in Ibadan, Nigeria West Africa, NU, Nubra; SKT, Sakti; TK, Tso Moriri.



B

Index	Name	P-value	Odds Ratio	Combined score
1	Cytoprotection By HMOX1 R-HSA-9707564	0.0009334	51.76	361.14
2	Oxygen-dependent Proline Hydroxylation Of Hypoxia-inducible Factor Alpha R-HSA-1234176	0.001175	45.90	309.68
3	Cellular Response To Hypoxia R-HSA-1234174	0.001525	40.07	259.85
4	COPI-dependent Golgi-to-ER Retrograde Traffic R-HSA-6811434	0.001739	37.42	237.79
5	Cellular Responses To Stress R-HSA-2262752	0.002166	8.94	54.87
6	Cellular Responses To Stimuli R-HSA-8953897	0.002324	8.77	53.17
7	Phase I - Functionalization Of Compounds R-HSA-211945	0.003064	27.85	161.17
8	Golgi-to-ER Retrograde Transport R-HSA-8856688	0.003543	25.81	145.64
9	PTK6 Expression R-HSA-8849473	0.003994	333.00	1839.15
10	NR1H2 And NR1H3 Regulate Gene Expression Linked To Gluconeogenesis R-HSA-9632974	0.003994	333.00	1839.15

Fig. 6.

A) Schematic presentation of twenty variants depicting significant altitude-wise association. (i) The top row represents the gene names for loci spanning the significant variants. (ii) Next row depicts the variants plotted on respective chromosomes and their genomic coordinates. (iii) The first scatter plot represents the variants on x-axis and the −log10(*P* value) for association analysis between the HapMap Han Chinese in Beijing, China (CHB) and the high- altitude study populations Nubra (NU), Sakti (SKT) and Tso Moriri (TK) on the *y*-axis. (iii) Similarly, the second scatter plot represents on *y*-axis the −log10(*P* value) for association analysis between the Indian lowlanders (LLS) and the high-altitude study populations Nubra (NU), Sakti (SKT) and Tso Moriri (TK). (B) Reactome pathways significantly enriched for genes spanning the 20 variants associated with altitude-wise association.

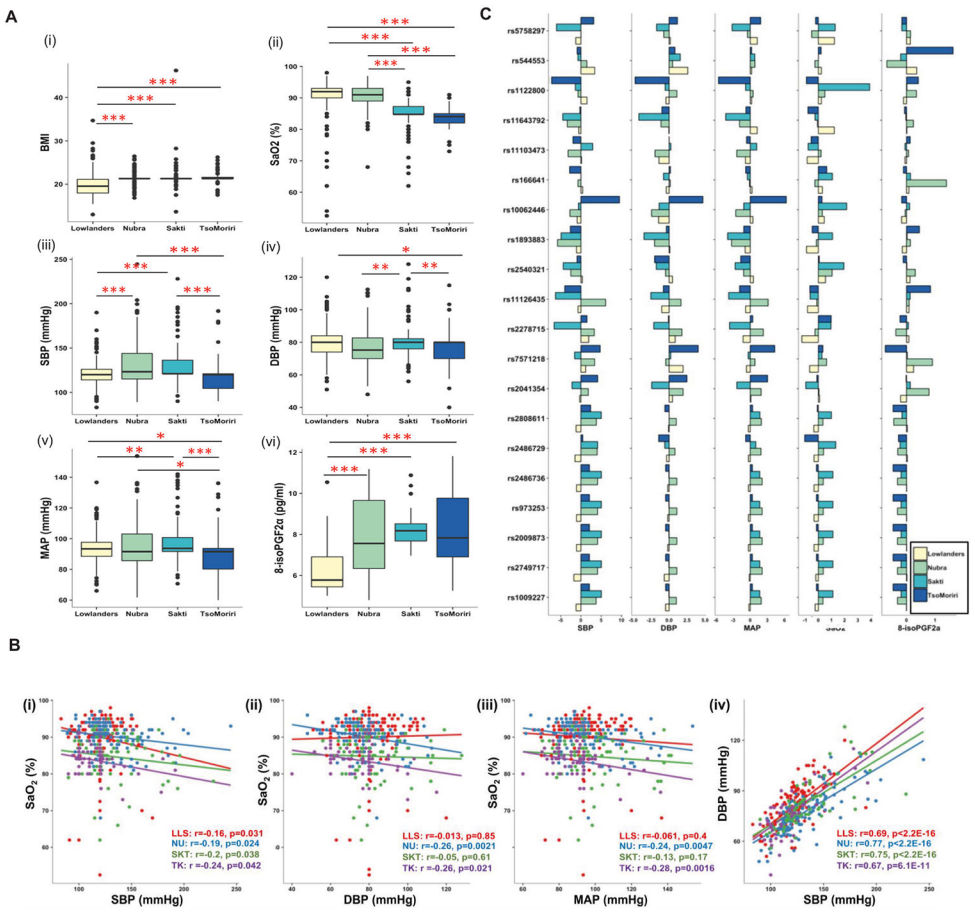


Fig. 7.

Clinical characteristics and biomarker level and their correlation analyses with the adaptive alleles of variants associating with high-altitude adaptation.

a) Clinical characteristics and biomarker levels and the correlation analyses. The x-axis represents the study populations, i.e., Lowlanders and highlanders from Nubra, Sakti and Tso Moriri and the y-axis represents the levels of clinical and biomarker characteristics of the study populations, i) BMI, body mass index (kg/m^2); ii) SaO_2, arterial oxygen saturation (%); iii) SBP, systolic blood pressure (mmHg); iv) DBP, diastolic blood pressure (mmHg); v) MAP, mean arterial pressure (mmHg), and vi) 8-isoPGF2α, 8-isoprostaglandin F2α (pg/ml).

b) Correlation analyses between clinical parameters in the study populations. The x- and y-axis represent the clinical characteristics in the study populations, i) SaO_2 versus SBP; ii) SaO_2 (%) DBP (mmHg); iii) SaO_2 (%) versus MAP, and iv) SBP (mmHg) versus DBP (mmHg). Each point represents the individuals of the study populations, i.e., Indian Lowlanders (LLS) and highlanders from Nubra (NU), Sakti (SKT) and Tso Moriri (TK). r, Pearson correlation coefficient; p, *P*-value.

c) Correlation analyses between the adaptive alleles and clinical characteristic. The x-axis represents the clinical characteristics, i.e., SBP (mmHg), DBP, (mmHg); MAP (mmHg); SaO_2, (%) and the biomarker, i.e., 8-isoPGF2α (pg/ml) in the study populations, i.e., Lowlanders and highlanders from Nubra, Sakti and Tso Moriri and the y-axis represents

the variants associating with high-altitude adaptation. The grey area highlight the most significant genetic loci/variants.

Table 1

List of 20 most significant SNPs associating with high-altitude adaptation in Nubra population.

CHR	SNP	Allele	OR	P	Gene
4	rs1893883	C	23.69	1.99E-21	*ADH6*
2	rs13021399	T	21.72	1.45E-20	*SULT1C4* | *GCC2*
1	rs4839472	A	11.25	1.75E-20	*VANGL1*
2	rs1469964	A	35.24	1.95E-20	*GCC2* | *LIMS1*
3	rs2840143	G	0.07	1.98E-20	*FBXO40*
13	rs9572995	C	0.05	2.14E-20	*DACH1* | *C13orf37*
21	rs2837952	G	12.93	2.83E-20	*DSCAM* | *C21orf130*
8	rs10103808	T	12.94	2.85E-20	*FAM135B*
9	rs6477021	G	0.09	3.39E-20	*KIAA2026*
4	rs3805322	G	0.03	3.48E-20	*ADH4*
1	rs10918077	G	12.54	6.26E-20	PBX1
3	rs7430671	G	16.88	6.51E-20	*HTR3D* | *HTR3C*
5	rs10053810	G	8.69	6.54E-20	*PIGL*
19	rs2285669	G	13.06	9.81E-20	*ZNF530* | *ZNF134*
14	rs746630	G	0.07	1.35E-19	*SEL1L* | *FLRT2*
2	rs1025542	G	12.81	1.75E-19	*LOC391359* | *FAM59B*
17	rs2277662	C	0.07	2.38E-19	*ZNHIT3*
18	rs11874696	G	11.58	2.51E-19	*C18orf55* | *CYB5A*
16	rs4780453	T	10.25	2.56E-19	*SNX29*
2	rs2353193	C	11.56	3.46E-19	*FASTKD1* | *PPIG*

Chr, chromosome; SNP, single nucleotide polymorphism; *P* value was calculated under a log-additive model using PLINK v1.07.

Table 2

List of 20 most significant SNPs associating with high-altitude adaptation in Sakti population.

CHR	SNP	Allele	OR	P	Gene
2	rs260711	T	55.01	3.42E-18	*EDAR*
2	rs3827760	A	21.57	4.23E-18	*EDAR*
4	rs1893883	C	27.37	5.65E-18	*ADH6*
8	rs17801727	T	10.38	5.87E-18	*LOC100287835* | *NKAIN3*
16	rs4783124	G	0.02	7.67E-18	*KIAA0513*
1	rs4839472	A	12.26	8.61E-18	*VANGL1*
8	rs10103808	T	13.53	1.01E-17	*FAM135B*
13	rs9572995	C	0.07	1.56E-17	*DACH1* | *C13orf37*
25	rs5939175	C	11.72	2.10E-17	*GYG2* | *ARSD*
2	rs13021399	T	18.56	2.41E-17	*SULT1C4* | *GCC2*
4	rs13123383	G	11.56	2.65E-17	*LOC100129410* | *AMTN*
11	rs3812748	C	0.05	2.73E-17	*MICAL2*
2	rs1003972	C	12.83	2.82E-17	*LOC100289094*
20	rs966567	G	0.06	2.91E-17	*CDH22*
5	rs166641	T	13.88	4.82E-17	*LEPREL1*
13	rs4773279	T	10.47	5.42E-17	*ANKRD10* | *ARHGEF7*
11	rs658641	C	11.42	6.11E-17	*PKNOX2*
8	rs1471009	C	0.10	6.30E-17	*C8orf86* | *RNF5P1*
1	rs11803575	T	12.11	1.30E-16	*FUCA1*
1	rs959460	A	16.66	1.42E-16	*GBP6*

Chr, chromosome; SNP, single nucleotide polymorphism; *P* value was calculated under a log-additive model using PLINK v1.07.

Table 3

List of 20 most significant SNPs associating with high-altitude adaptation in Tso Moriri population.

CHR	SNP	Allele	OR	P	Gene
4	rs1893883	C	16.40	7.08E-16	*ADH6*
20	rs966567	G	0.03	1.96E-15	*CDH22*
8	rs17801727	T	9.77	2.07E-15	*LOC100287835* | *NKAIN3*
6	rs986522	C	14.02	4.69E-15	*COL11A2*
2	rs4953353	T	0.06	7.17E-15	*EPAS1*
12	rs10772079	T	0.06	7.19E-15	*CLEC2D* | *CLECL1*
5	rs166641	T	15.94	9.30E-15	*LEPREL1*
1	rs959460	A	23.94	1.05E-14	*GBP6*
2	rs7571218	A	0.07	1.15E-14	*EPAS1*
3	rs1022109	G	10.49	1.65E-14	*FAM19A1*
18	rs544553	A	8.57	1.68E-14	*APCDD1* | *NAPG*
16	rs254353	A	15.62	1.71E-14	*LOC283867* | *CDH5*
6	rs1610585	G	0.09	1.90E-14	*ZFP57* | *HLA*-F
6	rs1611356	G	0.09	1.90E-14	*ZFP57* | HLA-F
17	rs1122800	C	22.54	2.03E-14	*TEX2* | *PECAM1*
16	rs4783124	G	0.09	2.25E-14	*KIAA0513*
11	rs658641	C	15.38	2.35E-14	*PKNOX2*
1	rs939592	T	10.36	2.37E-14	*TNRC4*
18	rs1787004	A	25.37	2.48E-14	*CEP192* | *C18orf1*
18	rs1036929	C	12.56	2.50E-14	*SETBP1*

Chr, chromosome; SNP, single nucleotide polymorphism; *P* value was calculated under a log-additive model using PLINK v1.07.

Table 4

List of 34 significant SNPs associating with high-altitude adaptation exclusively in Tso Moriri population.

CHR	SNP	Allele	HLS vs LLS		HLS vs CHB		Gene
			OR	P value	OR	*P* value	
19	rs2546019 C/G	G	0.6	1.41E-02	0.1	7.85E-13	*ZNF30* | *ZNF792*
17	rs2304905 A/G	A	0.2	2.44E-02	0.1	8.74E-04	*SCIMP* | *ZNF594-DT*
4	rs10021082 T/C	G	0.6	1.43E-02	0.2	7.63E-12	*C4orf17*
15	rs16962914 C/T	G	2.2	2.29E-02	12.7	7.95E-05	
4	rs10856838 T/A	A	0.6	4.12E-02	0.1	3.11E-13	*TLR10*
11	rs12797276 T/C	A	0.2	4.67E-03	0.2	5.68E-03	
11	rs7943887 C/G	G	0.6	5.99E-03	0.2	3.01E-10	*DSCAML1* | *FXYD2*
2	rs7565800 C/T	G	0.3	2.46E-03	0.1	4.54E-08	*SOCS5* | *LOC388948*
5	rs10056236 A/G	A	2.7	6.20E-03	13.3	6.10E-05	*SSBP2*
6	rs9389645 G/T	C	0.4	1.07E-03	0.5	3.42E-02	
20	rs6013143 T/C	A	14.4	1.27E-10	2.6	1.84E-03	
5	rs12187784 T/A	T	1.6	1.86E-02	4.2	3.79E-09	*OR2C1*
9	rs17793718 A/G	A	10.6	6.75E-12	2.0	6.35E-03	*PTPN3*
17	rs1872235 A/T	T	0.6	8.81E-03	0.2	1.20E-12	*WSCD1* | *AIPL1*
1	rs12132154 A/C	A	1.9	5.46E-03	14.3	7.49E-12	*LMO4* | *PKN2*
12	rs11112149 A/G	A	2.0	9.66E-04	1.8	1.01E-02	*CHST11*
3	rs9881117 G/A	G	1.5	3.92E-02	10.1	4.52E-13	*ROPN1*
8	rs7813132 G/C	C	0.6	4.08E-02	0.2	2.05E-11	*NCALD*
1	rs12133262 T/A	A	0.6	7.52E-03	0.1	1.45E-13	*LOC100288070* | *TNR*
18	rs1426879 C/G	G	0.6	1.58E-02	0.1	3.08E-12	*CHST9*
1	rs12084102 G/A	G	14.9	2.19E-07	2.9	4.22E-03	*COP1*
1	rs10864695 G/A	G	8.6	5.16E-14	2.1	6.02E-04	*DISC1* | *TSNAX-DISC1*
12	rs7316156 C/A	C	20.0	1.19E-07	1.9	4.88E-02	*LINC2955*
5	rs12521751 A/G	A	15.3	1.50E-16	1.8	1.34E-02	*LINC2112* | *LINC2221*
17	rs8076098 T/C	A	3.2	3.84E-06	2.2	1.78E-03	
1	rs939592 T/A	A	0.6	1.53E-02	0.1	2.37E-14	*TNRC4*
15	rs2470667 A/G	A	7.8	5.76E-10	1.9	1.05E-02	*DUOXA1*

CHR	SNP	Allele	HLS vs LLS		HLS vs CHB		Gene
			OR	P value	OR	P value	
1	rs2473241 C/T	A	0.6	2.87E-02	0.2	1.82E-08	*WNT4*| *ZBTB40*
2	rs12611491 G/A	G	63.2	1.21E-13	2.1	5.80E-03	*CEP68*
11	rs11228490 C/G	C	2.6	2.34E-05	6.6	1.64E-12	*TPCN2*| *MYEOV*
19	rs16992116 T/C	A	5.5	5.57E-07	1.7	4.48E-02	*ATCAY*
1	rs1034462 C/T	G	1.8	2.01E-02	7.6	7.91E-08	*CACYBP*| *MRPS14*
20	rs709023 A/T	T	0.5	1.56E-02	0.1	4.93E-14	*SMOX*| *ADRA1D*
12	rs16926318 G/A	G	16.1	4.04E-13	1.7	1.30E-02	

Chr, chromosome; SNP, single nucleotide polymorphism; *P* value was calculated under a log-additive model using PLINK v1.07.

Table 5

Genetic information of the 20 SNPs depicting linear amplification of alleles for high-altitude adaptation.

SNP	Nearest gene	A-Allele	B-Allele	Adaptive allele	Chromosome	Predicted function
rs1009227	*SPRTN*	G	A	A	chr1	intronic
rs2749717	*SPRTN*	A	C	C	chr1	3′ utr
rs2009873	*EGLN1–*	A	G	G	chr1	3′ downstream
rs973253	*EGLN1*	A	G	G	chr1	intronic
rs2486736	*EGLN1*	A	G	G	chr1	intronic
rs2486729	*EGLN1*	A	G	G	chr1	intronic
rs2808611	*EGLN1*	A	G	G	chr1	intronic
rs2041354	*COX7A2L*	T	A	A	chr2	intronic
rs7571218	*EPAS1*	A	G	G	chr2	intronic
rs2278715	*LINC1118*	A	G	G	chr2	non-coding
rs11126435	*M1AP*	T	A	A	chr2	intronic
rs2540321	*TMEM182*	T	A	A	chr2	3′ utr
rs1893883	*ADH6*	G	C	C	chr4	far gene
rs10062446	*KIF3A*	A	T	A	chr5	far gene
rs166641	*SFXN1*	A	T	T	chr5	3utr
rs11103473	*RXRA*	T	A	T	chr9	intronic
rs11643792	*COQ7*	G	C	C	chr16	intronic
rs1122800	*MILR1*	G	C	C	chr17	far gene
rs544553	*NAPG*	T	A	A	chr18	5′ upstream
rs5758297	*ZC3H7B*	G	A	A	chr22	intronic

Chr, chromosome; SNP, single nucleotide polymorphism.