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Comparative Analysis of Lowlander Transcriptomes at Himalayas and Andes Reveals Differential Regulation of Erythropoiesis at Extreme Altitude

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ABSTRACT

Systematic human expeditions to very high (≥ 3500 meters) and extreme altitudes (≥ 5500 meters) have documented marked changes in human physiology. However, only a handful of studies have reported lowlander transcriptome alterations at extreme altitudes. In this study, we compared the lowlander transcriptomes available in the literature for Chinese mountaineers (n=4, 3 males and 1 female) in the Himalayas (Mount Xixabangma base camp, 5600 meters) and French mountaineers (n=8, all males) at Andes (La Rinconada, Peru, 5100 meters). We sought to find out significantly altered pathways, gene networks, and Transcription Factors (TFs) for each data set. We observed profound upregulation of *GATA1* in the Himalaya transcriptome data set (+ 1.38-fold) in comparison to the Andes data set (-1.36-fold). Core transcriptome analysis revealed that *GATA1* upregulated erythropoiesis genes like *KLF1*, *HBD*, *HBG*, *EPB42*, *ALAS2,* and *AHSP* in the Himalayan dataset in contrast to the Andean data set. We also observed contrasting expression profiles of *KLF1* in the Himalayas (+1.22-fold) and Andes (-1.15-fold) for lowlander populations and differential expression regulation of its downstream target genes like *AHSP, ALAS2, SLC4A1, EPB42, HBG2,* and *HBB.* We also observed upregulation of *SP1* (+ 2.46-fold) in the Himalayan transcriptome as compared to the Andean transcriptome which also regulates erythropoiesis genes along with *GATA1*. Our results indicate profound upregulation of erythropoiesis-promoting TFs and genes in Chinese mountaineers at extreme altitudes in contrast to French mountaineers at similar altitudes. Though our present analysis does not provide possible reasons for the observed differences in hypoxia-responsive erythropoiesis gene signatures, it certainly highlights ethnicity-dependent transcriptome level variations in lowlanders at extreme altitudes.

Keywords: High altitude, Extreme altitude, Hypobaric hypoxia, Transcriptome, Erythropoiesis

NOMENCLATURE

AHSP	Alpha haemoglobin Stabilising Protein
ALAS2	5'-Aminolevulinate Synthase 2
ARNT	Aryl Hydrocarbon Receptor Nuclear Translocator
DMTN	Dematin Actin Binding Protein
EGF	Epidermal Growth Factor
EPB42	Erythrocyte Membrane Protein Band 4.2
ERBB2	Human Epidermal Growth Factor Receptor 2
FGF2	Fibroblast Growth Factor 2
FLT1	Fms Related Receptor Tyrosine Kinase 1
GATA1	GATA Binding Protein 1
GP9	Glycoprotein IX Platelet
GUCY1B1	Guanylate Cyclase 1 Soluble Subunit Beta 1
HEMGN	Hemogen
ICAM4	Intercellular Adhesion Molecule 4
KEL	Kell Metallo-Endopeptidase
KLF1	Kruppel-like factor 1
LOX	Lysyl Oxidase
MAP2K2	Mitogen-Activated Protein Kinase Kinase 2
MDA	Malondialdehyde
MMP9	Matrix Metallopeptidase 9
PHD2/HIF2α/EPAS1	Endothelial PAS Domain Protein 1
PLCG2	Phospholipase C Gamma 2
PTK2B	Protein Tyrosine Kinase 2 Beta
RELB	RELB Proto-Oncogene, NF-KB Subunit
SLC4A1	*Solute Carrier* Family 4 Member 1
SP1	*Specificity* Protein 1 Transcription Factor
SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase
TFR2	Transferrin Receptor 2
TGFB1	Transforming Growth Factor Beta 1

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1. INTRODUCTION

The partial pressure of oxygen linearly decreases with increasing altitude. This decrease results in compromised levels of inspired oxygen in humans ascending to high altitudes (≥ 2500 meters) for professional, sports, pilgrimage, and leisure activities[1]. The primary physiological response to hypobaric hypoxia includes activation of the sympathetic nervous system, hyperventilation, increase in heart rate, and blood flow to counteract hypoxia and preserve sufficient tissue oxygenation. At the molecular level, the hypoxia response is centrally coordinated by components of the Hypoxia-Inducible Factors (HIFs) signaling pathway[2]. Two central TFs of this pathway *HIF1α* and *HIF2α*/*EPAS1*orchestrate at least 100 genes involved in processes such as cellular energy metabolism, angiogenesis, and erythropoiesis restoring oxygen homeostasis, stress signaling, and promoting adaptation to low oxygen availability[3,4].

The better availability of road, railway, and airport infrastructure at high altitude have resulted in the flow of many lowlander sojourners at high altitude. Lowlanders acclimatise to this hypobaric hypoxia by initiating physiological responses like increased heart rate and cerebral blood flow, hyperventilation, haemoconcentration, erythropoiesis, and increasing capillary density with different time courses. The failure of acclimatization process results in several high-altitude maladaptive responses including acute mountain sickness, high altitude induced pulmonary and cerebral edemas[5]. Despite this, a handful of lowlanders travel and stay at very high altitudes (≥ 4,500 meters) exhibiting successful human acclimatisation to low oxygen environments[6].

The application of omics technologies to understand high altitude adaptation and acclimatization has found widespread application in recent years[7]. In this regard, researchers have used transcriptome sequencing whole genome sequencing epigenetic modifications, and genome-wide SNP genotyping studies to identify putative genes and associated regulatory networks for both native highlanders and lowlanders at high altitude[8,9,10,11,12]. Along with a plethora of physiological alteration, incremental changes in altitude bring dynamic changes in the human transcriptional landscape and gene networks facilitating human acclimatisation and adaptation. It is noteworthy that very few research groups have studied lowlander transcriptome alterations at very high and extreme altitudes in the Himalayas and the Andes despite limitations like availability of volunteers and difficulties in sample collection, logistic difficulties in setting up a laboratory, processing, storage, and transport of collected samples to name a few[13,9]. However, a comparative analysis of human transcriptomes at extreme altitudes between Himalayas and Andes is missing in the literature.

In the present study, we searched the literature for all the available lowlander human transcriptomes at very high or extreme altitudes (≥ 5000 meters). Then we categorised the transcriptomes based on the geographical regions (Himalayas and Andes) of sample collection as well as collected ethnicity information for the studied volunteers. We identified the most significant gene networks, pathways, and TFs for both data sets. Using a comparative approach, we gained insight into the differential regulation of TFs and downstream target genes between Himalayan and Andean transcriptomes at extreme altitudes.

2. MATERIALS AND METHODS

2.1 Data Collection

A data collection methodology was designed to search research articles in databases like Scopus, PubMed, and Directory of open access journals. Research articles were filtered using keywords like acute and subacute hypoxia, extreme altitude, gene expression, gene sequencing, global gene expression, transcriptome sequencing, and NGS. Research articles reporting high throughput gene expression studies at extreme altitudes were filtered and considered for further analysis. The qualifying studies reporting blood mRNA sequencing at different geographic locations (Himalayas and Andes) were identified. One data set at La Rinconada, Peru (5100 meters), GEO (accession number GSE196728) and another data set at Mt. Xixabangma (5600 meters) Himalayan range (https://doi.org/10.1371/journal.pone.0031645.s009) was considered for further analysis. These studies individually report the global gene expression profile of mountaineers at an altitude of 5600 m (Mount Xixabangma Base Camp) and 5100 m in La Rinconada[13,9].

2.2 Himalayan Transcriptome

Blood from four mountaineers who participated in Mt. Xixabangma expedition includes two 55-year-old male climbers, one 44-year-old male climber, and one 33-year-old female climber. The participant subjects did not travel to an altitude above 3000 m in the past six months. The mode of induction at Mt. Xixabangma was through climbing and mountaineers completed the expedition in a total duration of 35 days. On day 0 the subjects started climbing for Lhasa (3650 m) and attained the altitude by day 1 which followed the blood collection. After day 5 at 3650 m, the subjects started climbing Mt. Xixabangma Base Camp and reached between days 17-18 which followed the blood collection, the subjects reached Mt. Xixabangma peak at day 24 followed by descent at an altitude of 4400 m in Dingri for sample collection at day 30. The subjects further descended at sea level (100 m) at Lhasa followed by a blood collection after 5 days of descent on day 35[13]. The physiological parameters like SPO_2, heart rate, and systolic and diastolic blood pressure were measured at all the sample collection points. (Supplementary file 1).

2.3 Andean Transcriptome

The Andes data set comprises of 8 healthy, male French mountaineers with mean age 35.3 ±10.8, mean height 177 ±4.8 cm, weight 67.5 ±2.8 kg and mean BMI 21.56 ±0.78. Blood samples were collected at an interval of 4h throughout 24h from every subject, at

3 different altitudes. First set of blood samples were collected at 3800 m after 7 days of acclimatisation. Later, the volunteers acclimatised for a period of 7 days at 5,100 m (La Rinconada, Peru) and second round of blood sample collection was done. After the descent to sea level, blood samples were collected after 4 months at sea level. During the three entire sampling durations, participants had a standardized lifestyle[9].

2.4 Data Analysis

We analysed gene expression data using Ingenuity Pathway Analysis (IPA, QIAGEN). IPA is a web-based database that utilises gene expression log ratios/p-value of expression data. An expression log ratio dataset filter (cut off ±1-fold, species and biofluid) was applied to study datasets for IPA core analysis. The results of the core analysis like dataset summary, top canonical pathways, upstream regulators, and dataset comparisons were identified for both data sets. We majorly focussed on the cluster of genes regulated by activated master regulators in study datasets. Further, we filtered the genes that negatively regulated in response to the master regulator, and the genes that positively regulated, and checked the predicted regulation (activation/inhibition) in the database and the pattern of clustered genes of the dataset. Using a pathway filter (Z-score >2.5 and -log p-value 2.5), key pathways associated with the dataset were shortlisted. Pathways with significant z-score values were then mapped to trace the associated molecular clusters and biological significance was derived from the available literature.

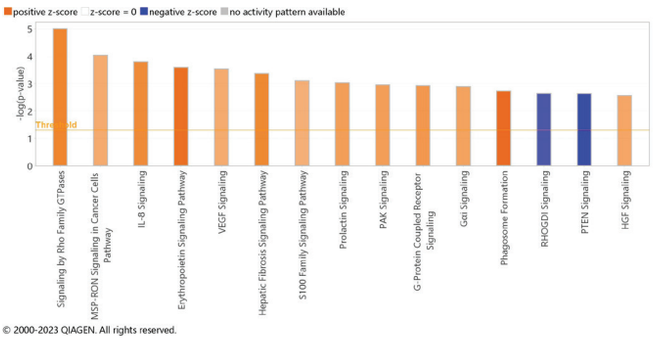
3. RESULTS

3.1 Molecular Pathways and Mediators Identified for Mt. Xixabangma, Himalaya

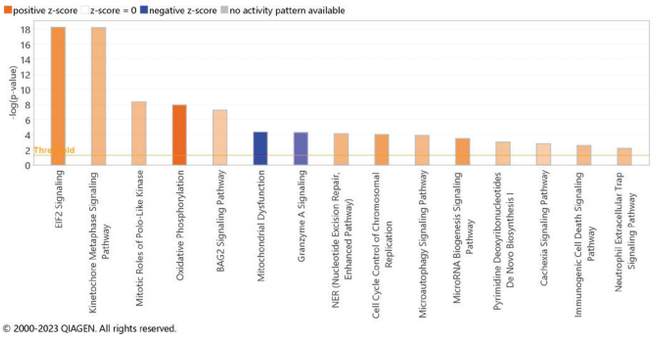
The dataset summary analysis of Mt. Xixabangma blood transcriptome revealed molecular responses like VEGF signaling, development of vasculature, Erythropoietin mediated gene signaling, Estrogen receptor signaling, and angiogenesis anchored by genes like *HIF1A*, *EPAS1*, *ARNT*, *EGF*, *FGF2*, *ETS1*, *ETS2*, *KLF6*, *SRC*, *STAT3,* and *IL6*.The top canonical pathways for this data set include signaling by Rho family GTPase, IL-8 signaling, erythropoietin signaling pathway, VEGF signaling, and S100 family signaling pathway (Figure 1A). We observed upregulation of major erythroid transcription regulators *KLF1* (1.22-fold), *GATA1* (1.38-fold), and *SP1* (2.46-fold) with several downstream target genes (Table 1). We also observed the role of TFs and associated genes for cytokine expression. In corroboration, we observed upregulation of *IL8RB* (1.5-fold) which regulates systemic inflammation.

3.2 Molecular Responses and Mediators Identified for La Rinconada, Peru

The blood transcriptome summary analysis of French lowlanders at La Rinconada revealed molecular responses like EIF2 signaling, senescence of cells,



(A)



(B)

Figure 1. Top 15 canonical pathways identified for low landers at extreme altitude. (A) Chinese mountaineers at Mt. Xixabangma (5,600 meters), (B) French mountaineers at La Rinconanda (5,100 meters). The summary filters for IPA analysis were: Activation/Inhibition Z-score ±2.5, -log P-value +3 and above, fold change ±1.

and proliferation of fibroblast cell lines anchored by genes like *VEGFA*, *ERBB2*, *E2F3,* and *FOXM1*. The top canonical pathways for this dataset include EIF2 signaling, Kinetochore metaphase signaling pathway, mitotic roles of Polo-like kinases, oxidative phosphorylation, and mitochondrial dysfunction (Figure 1B). Interestingly, we observed downregulation of erythroid transcription regulators *KLF1*(-1.15-fold),*GATA1*(-1.36-fold), and no change for *SP1* in French lowlanders at 5100 meters in contrast to Chinese lowlanders at similar altitudes. More importantly, we also observed the downregulation of erythropoiesis-related genes regulated by these TFs (Table 1). Our IPA-based analysis also revealed upregulation of antioxidant (*SOD1,* + 2.6-fold) and anti-inflammatory genes *IL10* (+1.77-fold), *IL4* (+2.63-fold) as well as downregulation of inflammatory genes like *TNF* (-1.2 fold), *TGFB1* (-1.52 fold) and *IL1B* (-1.4 fold) for French mountaineers. These results indicate differential antioxidant and anti-inflammatory responses between both the studied groups.

3.3 Differential Regulation of Erythropoiesis-related TFs and Genes

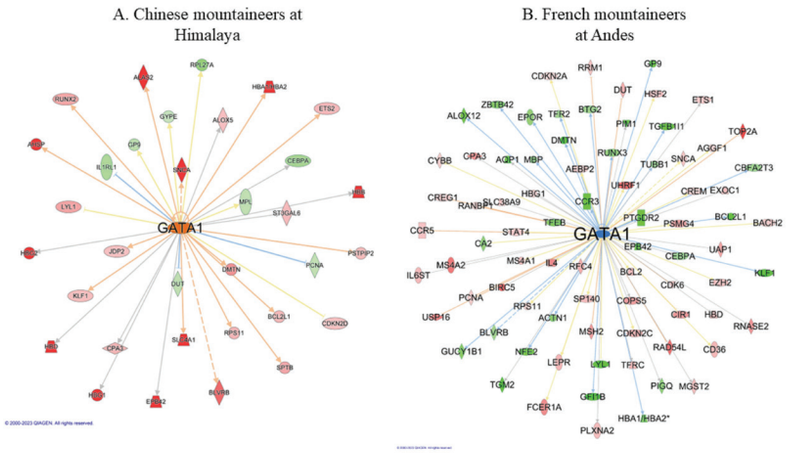
One of the contrasting observations between the analysed data sets was the differential expression of erythropoiesis-associated TFs *GATA1*, *KLF1,* and *SP1*. For the Mt. Xixabangma transcriptome data set, we observed

Table 1. Erythropoiesis-associated transcription factors and gene mRNA fold changes identified for Chinese mountaineers at Mount Xixabangma (5600 meters), Himalaya and French mountaineers at La Rinconada (5100 meters), Peru.

Sr. No.	Gene Name	Mt. Xixabangma	La Rinconada	References
		Fold change	Fold change	
1.	*GATA1*	1.380	-1.369	(Manella, *et al.*, 2022); (Villafuerte, *et al.*, 2022); (F. Liu, *et al.*, 2023); (Azad, *et al.*, 2016); (Tumburu and Thein 2017); (Li, *et al.*, 2011)
2.	*SP1*	2.466	0.009	(Chen, *et al.*, 2012); (Chanana, *et al.*, 2020); (Xu, *et al.*, 2014)
3.	*KLF1*	1.22	-1.15	(Manella, *et al.*, 2022); (Tumburu and Thein 2017); (Azad, *et al.*, 2023)
4.	*SENP1*	NA	0.356	(Cole, *et al.*, 2014); (Villafuerte 2015); (Villafuerte, *et al.*, 2022)
5.	*HBD*	4	0.858	(Storz 2016)
6.	*HBB*	4	-0.401	-
7.	*HBG1*	4	0.542	(Ghukasyan 2022); (Parikh 2021)
8.	*HBG2*	4	0.447	-
9.	*HBM*	4	-0.593	-
10.	*EPB42*	4	-0.866	(Feng, *et al.*, 2023)
11.	*STAT5B*	1.459	-0.137	-
12.	*RELB*	1.358	-0.498	-
13.	*MAP2K2*	1.288	-0.499	-
14.	*ARNT*	1.914	-0.114	-
15.	*BCL2L2*	1.341	-1.090	-
16.	*ALAS2*	4	-0.384	(Gaur, Saini, Ray, Kishore, *et al.*, 2020); (Feng, *et al.*, 2023)
17.	*AHSP*	4	0.349	(Lai, *et al.*, 2006)

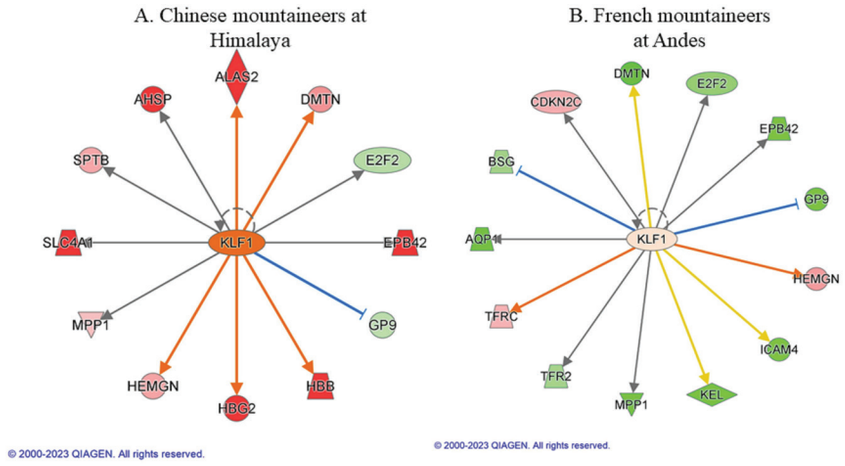
upregulation of *GATA1* in Chinese mountaineers at 5600 meters (Figure 2A). Network analysis revealed that *GATA1*can activate 32 downstream genes (26 upregulated and 8 downregulated respectively) majorly associated with erythropoiesis. Conversely, we observed downregulation of *GATA1* (-1.36-fold) and its downstream target genes *KLF1, LYL1, GUCY1B1, EPB4,*and *ALAS2* for French lowlanders at La Rinconada. For this cluster, we observed 51 up and 31 down-regulated genes in comparison to their sea-level gene expression profile (Figure 2B).

Similarly, we observed higher expression of *KLF1* (+1.22-fold), in Chinese mountaineers (Figure 3A). For this network, we observed positive regulation of 11 downstream genes *ALAS2* (+4-fold), *AHSP* (+4-fold), *DMTN* (+2.07-fold), *SPTB* (+1.66-fold), *SLC4A1* (+4-fold), *MPP1* (+1.18-fold), *HEMGN* (+1.72-fold), *HBG2* (+4-fold), *HBB* (+4-fold) and *EPB42* (+4-fold) respectively. We observed 1.15-fold downregulation of *KLF1* in the French lowlanders as compared to their sea-level gene expression profile. For the *KLF1* network at La



(A) **(B)**

Figure 2. The GATA1 transcription factor network. (A) In Chinese mountaineers at Himalayas. In this network containsGATA1 (+1.38-fold) and 32 downstream target genes out of which 26 were upregulated and 8 were downregulated, (B) In French mountaineers at Andes. This network contains GATA1 (-1.36-fold) and 81 downstream target genes out of which 31 were upregulated and 50 were downregulated.



(A) **(B)**

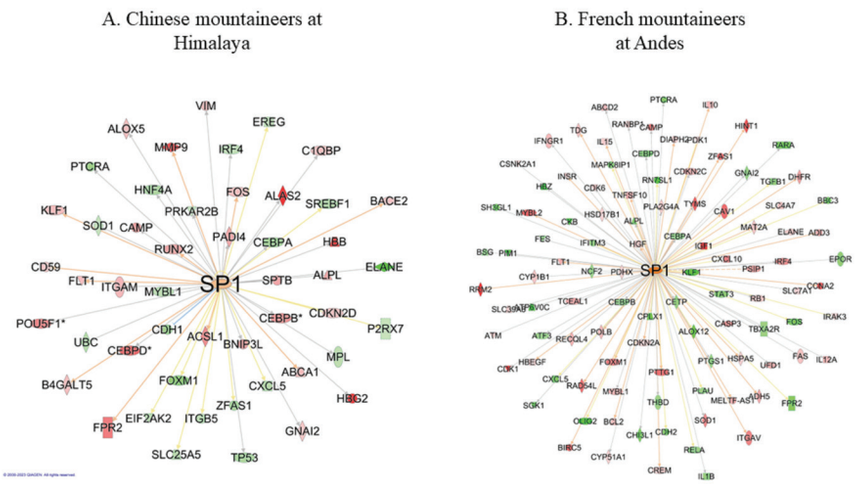
Figure 3. The KLF1 transcription factor network. (A) In Chinese mountaineer at Himalayas. This network contains KLF1 (+1.22-fold) and 12 downstream target genes out of which 11 were upregulated and 2 were downregulated, (B) In French mountaineers at Andes. This network contains KLF1 (-1.15-fold) and 13 downstream target genes out of which 3 were upregulated and 10 were downregulated.

Rinconada, we observed downregulation of 13 target genes including *DMTN* (-1.10-fold), *E2F2* (-0.62-fold), *EPB42* (-0.86-fold), *GP9* (-0.85-fold), *ICAM4* (-0.83-fold), *KEL* (-0.87-fold), *MPP1* (-0.90-fold), *TFR2* (-0.50-fold), *AQP1* (-0.95-fold) and *BSG* (-0.52-fold); (Figure 3B).

We also observed differential expression of *SP1* that regulates *EPAS1* expression and maintains oxygen homeostasis by erythropoietin-mediated pathway. We observed a positive regulation of *SP1*Chinese mountaineers (+2.44-fold) and overlapping downstream targets with *KLF1* and *GATA1*. Analysis of *SP1* downstream genes in the Mt. Xixabangma-associated network revealed upregulation of *KLF1*, *HBB*, *HBG2*, *FLT1*, *ALAS2*, *MMP9,* and *VIM* (Figure 4). In contrast, we observed negligible upregulation of *SP1* (+0.009-fold) for French lowlanders at La Rinconada along with downregulation of *KLF1, EPOR, HB,* and *STAT3*.

4. DISCUSSION

In healthy humans, RBCs are generated from a complex, multistep process called erythropoiesis that produces 2 million RBCs every second[14]. Erythropoiesis starts with multipotent HSCs in the bone marrow and undergoes a maturation process over14-18 days to produce mature RBC. Erythropoiesis is regulated by multiple sets of genes and core transcriptional network regulators like *GATA1*, *TAL1*/*SCL*, *LMO*, and *LDB1*[15]. Remarkably, erythropoiesis is the primary response to hypoxic stress particularly at high altitudes. It is well established that during hypoxia, *HIF1α*-mediated transcription of



(A) **(B)**

Figure 4. The SP1 transcription factor network. (A) In Chinese mountaineers at Himalaya. This network contains SP1 (+2.44-fold) and 50 downstream target genes out of which 30 were upregulated and 20 were downregulated, (B) In French mountaineers at Andes. This network contains SP1(+0.009-fold) and 104 downstream genes out of which 54 were upregulated and 39 ere downregulated.

the *EPO* gene stimulates erythropoiesis[16] to augment oxygen delivery. Hypoxia pathway proteins particularly regulated by the *PHD2/HIF2α (EPAS1)* axis play direct and indirect roles during the process of erythropoiesis. Several SNPs in the *HIF2α* gene have been reported for Tibetans and are associated with only a slight increase in hemoglobin concentrations[17,18]. Our IPA-based dataset summary analysis identified *EPAS1* and angiogenesis for Chinese mountaineers at extreme altitude, unlike French mountaineers at similar altitude. In corroboration, we also identified positive regulation of erythropoiesis-promoting pathways like erythropoietin signaling pathway and VEGF signaling pathway for Chinese mountaineers.

Our present study identified RBC-specific genes like *ALAS2, AHSP,* and *EPB42* positively associated with the Chinese mountaineer dataset. We observed significant upregulation of *ALAS2* in Chinese mountaineers and negative regulation in French lowlanders (Table 1). *ALAS2* is a mitochondrial enzyme, and it catalyses the condensation of glycine with succinyl-CoA to form δ-aminolevulinic acid, the first-rate limiting step in the mammalian heme biosynthesis pathway. Under hypoxia conditions, *HIF1* directly upregulates human erythroid-specific *ALAS2* mRNA levels increasing cellular heme content[19]. Feng, *et al*., have reported higher expression levels of *ALAS2* with high altitude polycythemia phenotype in Tibetans. We also observed higher levels of *AHSP* mRNA levels in Chinese mountaineers at extreme altitude. *AHSP* is an abundant, erythroid-specific chaperone for αHb protein which stabilises free αHb and HbA assembly in the absence of available β subunit. Zhoa, *et al*., have reported higher levels of *AHSP* mRNA and higher hemoglobin levels in iron-overloaded Tibetans[20]. EPB42 is an ATP-binding erythrocyte membraneprotein that regulates the association of protein 3 with ankyrin and maintains the structural integrity of the red cell membrane (Table 1)[21]. Our analysis revealed a higher transcript abundance of *EPB42* in Chinese mountaineers as compared to French mountaineers at extreme altitude. These results suggest that compensatory heme biosynthesis and erythropoiesis are major pathways facilitating Chinese mountaineers' acclimatisation to extreme altitude. On the contrary, such pathways are not observed for French mountaineers suggesting differential regulation of erythropoiesis within lowlanders at extreme altitude. Our analysis also identified higher transcript levels of several globin genes including *HBA1*, *HBB, HBG1, HBG2,* and *HBM* in Chinese mountaineers. (Table 1). These findings suggest a positive globin gene expression regulation in Chinese mountaineers while such positive stimulation of globin genes was absent in French mountaineers at similar altitude. For French mountaineers, we observed a moderate upregulation of *HBG1* and *HBG2.*

We also looked for the molecular pathways that directly or indirectly promote erythropoiesis, vasculogenesis, and hemoglobin genes at extreme altitude. We observed over-presentation of the positive regulators of EPO signaling pathway genes in Chinese mountaineers like *GATA1, STAT5B, RELB, MAP2K2, HBA1/HAB2, HBD, HBB, HBG1, ARNT, BCL2L1,* and *SOS*. Erythropoietic stress such as hypoxia stimulates a dramatic increase in *EPO* transcription in the kidney is mediated via hypoxia-inducible factor HIF2α[22]. Subsequently, EPO augments RBC production by binding and activating a cell surface high-affinity receptor (EPOR) expressed in immature erythroid cells. Along with the HIF family, the GATA TFs, key regulators of hematopoiesis such as *GATA1* and *GATA2* also contribute to EPO gene regulation[23]. We also observed over the presentation of VEGF signaling pathway genes like *FLT1, MAP2K2, PLCG2, PTK2B, SH2D2A,*and *SOS2* in the Chinese dataset[24]. VEGF plays a key role in regulating angiogenesis and vasculogenesis during development and physiological homeostasis[25]. Hypoxia selectively upregulates *VEGF* gene expression via the *HIF1α* pathway, and it is one of a network of genes involved in angiogenesis, erythropoiesis, and glycolysis, augmenting the blood's oxygen-carrying capacity[26,27]. Our current observation of positive regulation of EPO and VEGF pathways and downstream target genes in Chinese mountaineers indicates enhanced erythropoiesis and angiogenesis as an extreme altitude survival strategy. In contrast, we observed a negative regulation of the erythroid process and associated gene interactome (Table 1) for French mountaineers further corroborating the ethnicity-dependent extreme altitude response of low landers.

We further analysed the erythropoiesis-associated TFs in both data sets. We found *GATA1*(+1.4-fold) as a key TF in the Chinese mountaineer dataset (Table 1; Figure 2). *GATA1* the "master" TF in erythropoiesis, recognises conserved GATA motifs found in the regulatory regions of virtually all erythroid-expressed genes including globins, heme biosynthetic enzymes, membrane proteins, and red blood cell TFs[28]. It is noteworthy that *GATA1* plays a key role in the positive regulation of erythropoiesis at high altitude[29]. We also observed higher expression levels of GATA1 responsive genes in Chinese extreme altitude mountaineers like *HBA*, *HBB*, *HBG1*, *HBG2*, *HBD*, *EPB42*, *BCL2L1*, *ALAS2,* and *AHSP*. A handful of studies have reported that hypoxia-induced increased transcription of HIFs (*HIF1α* and *HI21α/EPAS1*) and *GATA1*, post-transcriptional modifications, and specific genetic variants regulate hemoglobin levels at high altitude conferring adaptive advantage. Although these adaptive phenotypes are reportedly true for high-altitude native populations, our present observations of differential transcript abundance of *GATA1* and its target genes in Chinese and French mountaineers raise the possibility of positive selection *GATA1* variants for hypoxia-induced erythropoiesis in lowlanders as well[17,30,31].

We observed higher *KLF1* transcript levels in Chinese mountaineers as compared to French mountaineers at extreme altitude (Table 1; Figure 3). *KLF1* is a zinc finger transcription regulator for erythropoiesis and controls all aspects of HSCs towards erythroid lineage[32]. Erythroid expression of *KLF1* is mediated by *GATA1* binding to its promoter site. Both *KLF1* and *GATA1* act together

to regulate the expression of many erythroid genes and have considerably similar downstream gene interactome and regulate in response to lncRNA at high altitudes[33].

SP1 is a C2H2-type zinc finger-containing DNA-binding protein family of TFs and is a HIF1 α responsive gene[34]. ROS produced under severe hypoxia conditions activate the translation of SP1 protein via an IRES in the 5′-untranslated region of *SP1* mRNA. It is noteworthy that *SP1* regulates *EPAS1* in Tibetans by binding to the 40-bp insertion fragment at the −742 indel site and activating the transcription of *EPAS1* gene. This *SP1*-mediated *EPAS1* activation reportedly important for the development of amnion, fetalgrowth, and high altitude adaptation in Tibetans by regulating *LOX* expression[35]. We observed *SP1*-mediated positive regulation of more than 70 genes in the Chinese mountaineer transcriptome (Figure 4). Our functional enrichment analysis clustered these genes into cellular processes like heme biosynthesis (*ALAS2*), erythrocyte membrane stability (*SPTB*) erythropoiesis (*HBB* and *HBG2*), and mobilisation of hematopoietic cells. More importantly, we observed an overlapping of genes of erythropoiesis (*KLF1*, *ALAS2*, *HBG1,* and *HBB*) between *SP1* gene network and *GATA1* gene network (Figure 4). Another striking feature of *SP1* gene network of French mountaineers is the upregulation of antioxidant and anti-inflammatory genes as compared to Chinese mountaineers. Studies have shown that acute and chronic exposure to high altitude induces oxidative stress, causing alterations to molecular pathways and cellular macromolecules. High altitude pathologies are associated with oxidative stress as evidenced by an increase in the MDA levels with concomitant decreases in superoxide dismutase (SOD and GPx) antioxidant activity[36]. Hypoxia exposure also triggers inflammatory cytokines mediated through macrophage and neutrophil activation which may be detrimental for high altitude acclimatisation. In this regard, we observed increased mRNA levels of *SOD1* and anti-inflammatory cytokines (*IL4* and *IL10*) in French mountaineers at extreme altitude. On the other hand, we could not detect such molecular signatures for Chinese mountaineers at similar altitude. These results further provide evidence for the existence of ethnicity-specific extreme altitude acclimatisation signals among low landers[37,8].

5. CONCLUSION

Our research uncovered two important conclusions. First, there exists an ethnicity-dependent lowlander response to extreme altitude. We observed a "stress erythropoiesis" response in Chinese mountaineers at extreme altitude. This response was mediated by hypoxia-responsive*HIF1α* and *HIF2α*/*EPAS1* and erythropoiesis TFs (*GATA1* and *KLF1*) along with their downstream target genes to increase RBC maturation. In contrast, we observed a lower transcript abundance of erythropoiesis-associated TFs in French mountaineers at extreme altitude. Given the fact that the prevalence of excessive RBC formation and chronic mountain sickness is lower in the Himalayas as compared to the Andes, we speculate that certain genetic variants of *EPAS1* and *GATA1* might be contributing to observed differences in erythropoiesis in lowlanders like high-altitude natives. Second, we observed marked differences in anti-inflammatory and antioxidant transcript signatures between Chinese and French mountaineers at extreme altitude. The better antioxidant and anti-inflammatory response in French mountaineers further substantiates the ethnicity-dependent molecular response of lowlanders to extreme altitude. Though the precise reasons for such observations are beyond the scope of this study, further molecular elucidations may add new insights into human extreme altitude acclimatisation.

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