Original Article

Hypoxic Signature of High Altitude Acclimatization: A Gene Expression Study

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Abstract

Indian Air Force and Army Aviation Corps routinely undertake flight to high altitude region which presents an environment of hypoxia and cold. On arrival at altitude, a number of physiological changes occur which ultimately enables the body to function optimally in low oxygen environment through process of acclimatization. An integral part of the human cellular response to hypoxia is changes in gene expression. Profiles of gene expression patterns define the complex biological processes associated with both health and disease *in vivo*. Microarrays can identify changes in gene expression that can be used as biomarkers of environmental and/or any other stress related exposure and can provide information on mechanisms of various biological processes. In the present investigation, gene expression c2hanges were analysed in sea level residents who were air inducted to high altitude to identify gene transcripts of altitude exposure and thereby understand the mechanism of acclimatization. Gene expression profiling was done by Atlas Powerscript labeling system, California, on Atlas Glass Microarrays. About 89 gene transcripts showed a change in gene expression after acute induction to altitude and the transcripts were protein coding type. Seventy three gene transcripts had a decreased expression and about fifteen transcripts were upregulated under the high altitude hypoxic stress. The pathways found to be affected were antigen processing and presentation (hsa04612), h_ctlPathway: CTL mediated immune response against target cells, GnRH signaling pathway (hsa04912), vascular smooth muscle contraction (hsa04270), ubiquitin mediated proteolysis (hsa04120), regulation of actin cytoskeleton (hsa04810), calcium signaling pathway (hsa04020), neuroactive ligand-receptor interaction (hsa04080) and cytokine-cytokine receptor interaction (hsa04060). Findings of the study indicate high altitude hypoxia has more down regulatory effect on transcript expression in peripheral blood cells and the hypoxic signature of high altitude exposure is evidenced.

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Key words: high altitude hypoxia, acclimatization, gene expression.

Introduction

High altitude region presents an environment of hypoxia and cold. Indian Air Force and Army Aviation Corps routinely undertake flight to high altitude and engage in different operations wherein exposure to the harsh environment is inescapable. On arrival at altitude, a number of physiological changes occur through process of acclimatization which ultimately enables the body to function optimally in low oxygen environment. These physiological responses are complex and involve a range of mechanisms occurring within minutes of oxygen sensing resetting a cascade of biosynthetic and physiological events within the cellular milieu [1].

During the initial phase of ascent to HA, most sojourners experience symptoms of acute mountain sickness (AMS) characterized by headache, nausea, vomiting, giddiness, anorexia leading to hypophagia, sleep disturbance and adverse psychological effects (secondary), muscular weakness and depression [2].

High altitude pulmonary edema (HAPE) is a severe form of altitude sickness that generally occurs within 6 to 48 hours of ascent beyond a

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height of 2500 to 4000 m. Genetic predisposition and individual susceptibility in cases of HAPE has been postulated [3]. Mechanism of high altitude acclimatization and/or maladaptation still remains unclear. Gene expression responses of circulating leukocytes can potentially provide an early warning of threat they discover and have the potential to be used diagnostically for direct sampling of sites of infection or other disease processes. The present investigation aimed at studying the gene expression profile in individuals who were inducted to high altitude to identify changes related to high altitude exposure and understand the mechanism of acclimatization.

Material and Methods

24 male low landers (weight-63.7±6 kg, age-27.7±6 years) were included in the study who were studied at sea level (Chandigarh at 0700h before breakfast) and thereafter at high altitude (Leh, Jammu and Kashmir, AMSL 3650 m). Samples were also collected from HAPE patients (n=6) admitted in the hospital at Leh and age matched control subjects who did not develop HAPE (n=4). Verbal information on the experimental protocol and procedures were given to the subjects after which the subjects gave their informed, written consent to participate. The study conformed to Institute Ethical guidelines. Lake Luoise score was determined for each subject for assessment of AMS and seven volunteers who developed AMS were excluded. Samples were treated anonymously throughout the analysis. Blood samples were directly collected through a scalp vein set (Beckton Dickinson) in PAXgene Blood RNA tubes containing a stabilizing fluid (PreAnalytix, Qiagen).

RNA isolation, preparation of labeled cDNA and microarray hybridization

Total cellular RNA was isolated using PAXgene Blood RNA kit (PreAnalytix, Qiagen) along with on-column DNase digestion as per manufacturer's recommendation. Samples were quantified by absorbance measurement at 260 nm and integrity was analysed by native gel electrophoresis. Total RNA (~5-7 µg) was used as templates in reverse transcription reactions for first strand complimentary DNA synthesis in presence of oligo (dT)15-18 primer and 2-aminoallyl-dUTP (Atlas Powerscript labelly system; BD Biosciences Clontech, Palo Alto,California) following which they were labeled by N-hydroxysuccinimide-derivatized Cy3 (Amersham Pharmaci Ltd., Piscataway, N.J.) (Samples of sea level) and N-hydroxysuccinimide-derivatized Cy5 dyes (same samples at high altitude) respectively following the protocol of manufacturer (BD Biosciences Clontech). Samples of HAPE and controls were labeled with Cy3 and Universal Refererence RNA (URR, Statagene) was labeled with Cy5). 650 pg of synthetic lambda Q gene RNA containing an engineered poly(A) tail was spiked into each cDNA synthesis reaction mixture (Atlas Powerscript labeling system; BD Biosciences Clontech, Palo Alto, California) to provide a control for cDNA synthesis, labeling efficiency and cDNA microarray hybridization. Labeled cDNAs were purified through FluorTrap matrix (Atlas Powerscript labeling system; BD Biosciences Clontech, Palo Alto, California) and eluted through 0.22mm spin filters. Microarray hybridization was performed on BD Atlas Glass Microarrays (Human 3.8 I K, Clontech catalogue no. 634638). Hybridization was conducted for 18 hours at 50°C. Following hybridization, cDNA microarrays were washed as per the manufacturer's protocol and air dried by centrifugation in a cushioned 50-ml conical centrifuge tube at 3000 x g for 1 minute.

Image processing and Data Analysis

Hybridization signals were collected by Axon microarray scanner (GenePix Pro 3.0) and raw spot

intensity report was created by Gene Pix analyzer software. Average pixel intensity within each circle was determined and local background was computed for each spot. Net signal was determined by subtracting local background from the average intensity. Genespring GX V 7.3 software (Agilent Technologies) was used for data analysis. A Lowess curve was fit to the log-intensity versus log-ratio plot. 10% of the data was used to adjust the control value for each measurement. Gene Annotation sources included Unigene, Entrez Gene, Genbank, and KEGG Database. Hierarchical clustering was done using Cluster 3.0 program and visualized using Java Tree View. Genes that showed a minimum of 0.7 fold change (to capture even the weak signals on the array) was considered as differentially regulated. Functional Annotation clustering was done by Database for Annotation, Visualization and Integrated Discovery (DAVID v 6.7 available at http://david.abcc.ncifcrf.gov) [4, 5].

Results

Of the 3800 sequences in the gene array, about 297 transcripts showed expression on the 3.8 K array (expressed in at least one condition), 64 transcripts expressed in all three conditions, 49 transcripts expressed in at least 2 conditions and 184 transcripts expressed in only condition. About 89 transcripts showed a change in gene expression after acute induction to altitude and were protein coding type. The differentially regulated genes belonged to both biological functions and cellular component. Seventy three gene transcripts had a decreased expression and about fifteen transcripts were upregulated under the high altitude hypoxic stress (Table 1). Genes of G-protein coupled receptor protein signaling pathway were downregulated on altitude induction: these included guanine nucleotide binding protein (*GNA11*) and regulator of G-protein signaling 11 (*RS11*). Calcitonin/calcitonin-related polypeptide (*CGRP*), angiotensin II receptor type 2 (*ATGR2*), olfactory receptor family 6 (*OR6A2P*) and gonadotropin releasing hormone receptor (*GRHR*) were also downregulated. Among the other downregulated genes were present genes for cyclic nucleotide gated channel (*CNG1*) and mitochondrial solute carrier family 25 (*ARALAR1*). Genes involved in RNA processing, regulation of transcription, RNA processing/catabolism (*PLAGL2*), (*APP1*), zinc finger proteins (*ZNF124, MZF1*), retinoic acid receptor (*RAR*), genes involved in mRNA cleavage (*RNS4*), RNA splicing gene [DEAH (Asp-Glu-Ala_His) box polypeptide 16] (*DBP2*), mRNA capping RNA (guanine-7-methyltransferase) were also downregulated. Also downregulated was dual-specificity tyrosine (Y) phosphorylated kinases (*DYRK5, DYRK2*). Genes involved in defence response like interferon alpha 14 (*MGC125756*), apolipoproein H (*APOH*) andforkhead box N1 (*FKHL20*) were downregulated on exposure to high altitude. Transcripts involved in cell adhesion like calcium/calmodulin dependent serine protein kinase (*LIN2*) and scavenger receptor class F (*SREC*) were downregulated. Antigen presenting major histocompatibility complex class 1 (*HLA-JY3* or *D6S204*), blood coagulation factor glycoprotein V (*CD42d*) and neurotransmitter synapsin II (*SYNII*) were also downregulatedon high altitude induction.

Upregulated transcripts on altitude induction were for various binding molecules viz., heme binding (hemoglobin alpha 1), hemoglobin alpha 2 (*HBA1*), GTP binding (ADP-ribosylation factor like 4A, *ARL4*), GTP binding septin 5 (*H5*), RNA binding ribosomal protein L3 type (*RPL3L*), protein binding (syntaxin1A, *STX1A*), parathymosin (*PTMS*) which is known to be involved in cellular defense response, transporter activity related to excretion (aquaporin 5, *AQP5*), gene involved in carbohydrate metabolism (ST8 alpha-n-acetyl-neuraminide alpha

2, 8 sialytransferase, *GD3S*), cytochrome c oxidase subunit VIa polypeptide involved in electron transport (*COX6AH*), cell adhesion molecule protein tyrosine phosphatase receptor (*PTPSIGMA*), actin related protein 2/3 complex involved in actin related polymerization (*ARC20*) as well as chromosome 10 open reading frame 116 of unknown biological function. The prominent functional clusters were regulation of apoptosis, T cell activation, oxygen transport, neurotransmitter secretion, regulation of blood pressure, regulation of body fluid levels, cell-cell signaling, transcripts of calcium ion binding etc (Table 2). The pathways which were found to be affected were antigen processing and presentation (hsa04612), h_ctlPathway: CTL mediated immune response against target cells, GnRH signaling pathway (hsa04912), vascular smooth muscle contraction (hsa04270), ubiquitin mediated proteolysis (hsa04120), regulation of actin cytoskeleton (hsa04810), calcium signaling pathway (hsa04020), neuroactive ligand-receptor interaction (hsa04080) and cytokine-cytokine receptor interaction (hsa04060) (Table 3).

In individuals with HAPE, thirty one transcripts were downregulated and fourteen transcripts were upregulated when compared to URR. In resistant control samples, twenty six genes were downregulated and eighteen genes were upregulated compared to URR. Although the pattern of gene expression was distinct in the three groups, there was overlapping also (Fig 1). Genes like alpha 2-HS glycoproein (*AHSG*), neurotansmiter transporter (*SLC6A2*), ADAM metallopeptidase domain 12 (*ADAM12*), UDP-glucose ceramideglycosyltransferase (*UGCG*) gonadotropin releasing hormone receptor (*GNRHR*), solute carrier family 6 (*SLC6A2*), protein coupled receptor CD3 antigen (*T3E*), aquaporin 2 (*AQP2*), mitochodrial ribosomal protein L49 (*MRPL49*), ATP binding cassette sub family C (CFTR/MRP), member 6 (*ABCC6*), lymphocyte cytosolic protein 1 (*LCP1*), distal less homeobox 3 (*DLX3*), keratin 13 (*KRT13*), a transmembrane glycoprotein A33 (*GPA33*) major histocompatibility complex class I C (*HLA-C*) adenine phosphorybosyltransferase (*APRT*) were more pronounced in HAPE than in resistant controls. Downregulated transcripts in HAPE were lysyl oxidase-like 1 (*LOXL1*), Wiskott-Aldrich syndrome protein interacting protein (*WSPIP*), pancreatic popeptide (*PPY*), hepatic transcription factor 1 (*TCF1*), actin gama 2 (*ACTG2*), solute carrier family 30 (zinc transporter) (*SLC30A3*), protein tyrosine phosphatase receptor type S (*PTPRS*) and protein tyrosine phosphatase receptor type N (*PPRN*).

Discussion

Low cellular oxygen tension (hypoxia) is a feature of high altitude. An integral part of the human cellular response to hypoxia is changes in gene expression [6, 7]. Till date, more than 100 genes have been identified that show a change in expression during hypoxic exposure, including a number of genes that are thought to be part of a

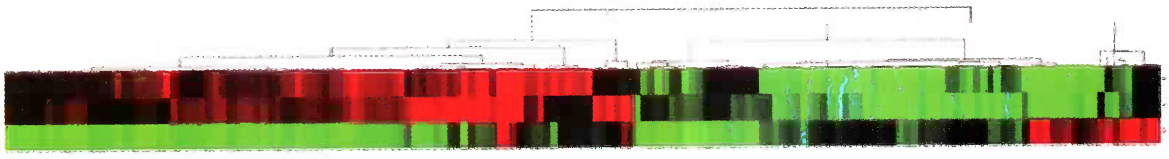

Fig 1. Hierarchical clustering of gene expression from individuals who developed HAPE labeled with Cy3 compared to Universal Reference RNA labeled with Cy5 (Group I), matched controls who did not developed HAPE labeled with Cy3 compared to Universal Reference RNA labeled with Cy5 (Group II) and individuals at sea level labeled with Cy3 and at high altitude after acclimatization labeled with Cy5 (Group III).

Table 1: List of differentially expressed gene transcripts during high altitude acclimatization

Gene symbol	Ref Seq Accession no	Gene	Fold change	Gene symbol	Ref Seq Accession no	Gene	Fold
HBA1	NM_000558	hemoglobin, alpha 1	2.411	LALBA	NM_002289	lactalbumin, alpha-	0.695
AQP5	NM_001651	aquaporin 5	2.24	HLA-C	NM_002117	major histocompatibility complex, class I, C	0.695
ARL4A	NM_001037164	ADP-ribosylation factor-like 4A	1.744	AURKC	NM_001015878	aurora kinase C	0.695
RPL3L	NM_005061	ribosomal protein L3-like	1.667	ARCN1	NM_001655	archain 1	0.694
STX1A	NM_004603	syntaxin 1A (brain)	1.609	PSMC5	NM_002805	proteasome (prosome, macropain) 26S subunit, ATPase, 5	0.694
PITX1	NM_002653	paired-like homeodomain transcription factor 1	1.604	SSNA1	NM_003731	Sjogren's syndrome nuclear autoantigen 1	0.693
ST8SIA1	NM_003034	ST8 alpha-N-acetyl-neuraminide alpha-2, 8-sialyltransferase 1	1.59	PLAGL2	NM_002657	pleiomorphic adenoma gene-like 2	0.692
				CNGA1	NM_000087	cyclic nucleotide gated channel alpha 1	0.692
HBA1	NM_000517	hemoglobin, alpha 2	1.519	RGS11	NM_003834	regulator of G-protein signalling 11	0.69
PTMS	NM_002824	parathymosin	1.504	PABPC4	NM_003819	poly(A) binding protein, cytoplasmic 4 (inducible form)	0.688
C10orf116	NM_006829	chromosome 10 open reading frame 116	1.501				
SEPT5	NM_001009939	septin 5	1.499	APRT	NM_000485	adenine phosphoribosyltransferase	0.688
COX6A2	NM_005205	cytochrome c oxidase subunit VIa polypeptide 2	1.431	DYRK2	NM_003583	dual-specificity tyrosine-(Y) -phosphorylation regulated kinase 2	0.687
ARSE	NM_000047	arylsulfatase E (chondrodysplasia punctata 1)	1.423	CASK	NM_003688	calcium/calmodulin-dependent serine protein kinase (MAGUK family)	0.687
PTPRS	NM_002850	protein tyrosine phosphatase, receptor type, S	1.409	CALM2	NM_001743	calmodulin 2 (phosphorylase kinase, delta)	0.682
ARPC4	NM_001024959	actin related protein 2/3 complex, subunit 4, 20kDa	1.407	DYRK3	NM_001004023	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	0.681
PLA2G4C	NM_003706	phospholipase A2, group IVC (cytosolic, calcium-independent)	0.7	SSR1	NM_003144	signal sequence receptor, alpha (translocon-associated protein alpha)	0.68
TNFSF12	NM_003809	tumor necrosis factor (ligand) superfamily, member 12	0.699	UBE2D3	NM_003340	ubiquitin-conjugating enzyme E2D 3 (UBC4/5 homolog, yeast)	0.677
GNA11	NM_002067	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	0.699	TMEFF1	NM_003692	transmembrane protein with EGF-like and two follistatin-like domains 1	0.677
KRT31	NM_002277	keratin, hair, acidic, 1	0.699	IFNA14	NM_002172	interferon, alpha 14	0.676
RFXANK	NM_003721	regulatory factor X-associated ankyrin-containing protein	0.698	DOC2A	NM_003586	double C2-like domains, alpha	0.673
				ATXN2L	NM_007245	ataxin 2-like	0.672
GPA33	NM_005814	glycoprotein A33 (transmembrane)	0.697	ZNF124	NM_003431	zinc finger protein 124 (HZF-16)	0.672

Gene symbol	Ref Seq Accession no	Gene	Fold change
TNFRSF6B	NM_003823	tumor necrosis factor receptor superfamily, member 6b, decoy	0.67
SCARF1	NM_003693	scavenger receptor class F, member 1	0.666
SLC25A12	NM_003705	solute carrier family 25 (mitochondrial carrier, Aralar), member 12	0.665
RNMT	NM_003799	RNA (guanine-7-) methyltransferase	0.663
STC1	NM_003155	stanniocalcin 1	0.662
HIST2H2BE	NM_003528	histone 2, H2be	0.662
CUL3	NM_003590	cullin 3	0.66
BTG1	NM_001731	B-cell translocation gene 1, anti-proliferative	0.66
MZF1	NM_003422	zinc finger protein 42 (myeloid-specific retinoic acid-responsive)	0.658
MADD	NM_003682	MAP-kinase activating death domain	0.657
SRPK2		synonym: SFRSK2; isoform b is encoded by transcript variant 2; H_RG152G17.1a; WUGSC:H_RG152G17.1a; serine kinase SRPK2; H_RG152G17.1b; go_component: nucleus [goid 0005634] [evidence IDA] [pmid 9472028]; go_component: cytoplasm [goid 0005737] [evidence IDA	0.651
RARA	NM_000964	retinoic acid receptor, alpha	0.65
CUL2	NM_003591	cullin 2	0.646
HSD17B10	NM_003725	hydroxysteroid (17-beta) dehydrogenase 6	0.646
RUVBL1	NM_003707	RuvB-like 1 (E. coli)	0.645
OFD1	NM_003611	oral-facial-digital syndrome 1	0.642
TRP1	NM_002769	protease, serine, 1 (trypsin 1)	0.641
SCGB2A2	NM_002411	secretoglobin, family 2A, member 2	0.641
CALCA	NM_001033952	calcitonin/calcitonin-related polypeptide, alpha	0.641
SYN2	NM_003178	synapsin II	0.631
RDH16	NM_003708	retinol dehydrogenase 16 (all-trans and 13-cis)	0.625
DYRK1A	NM_001396	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	0.622
GP5	NM_004488	glycoprotein V (platelet)	0.621
BCL7B	NM_001707	B-cell CLL/lymphoma 7B	0.62
APCL	NM_005883	adenomatosis polyposis coli 2	0.62
FBLN2	NM_001004019	fibulin 2	0.613
MRPL49	NM_004927	mitochondrial ribosomal protein L49	0.609
TPST1	NM_003596	tyrosylprotein sulfotransferase 1	0.607
FABP5	NM_001444	fatty acid binding protein 5 (psoriasis-associated)	0.607
PRKRA	NM_003690	protein kinase, interferon-inducible double stranded RNA dependent activator	0.605
APOH	NM_000042	apolipoprotein H (beta-2-glycoprotein I)	0.595
FOXN1	NM_003593	forkhead box N1	0.594
AQP2	NM_000486	aquaporin 2 (collecting duct)	0.583
BAG6	NM_004639	HLA-B associated transcript 3	0.577
AGTR2	NM_000686	angiotensin II receptor, type 2	0.573
PPAP2B	NM_003713	phosphatidic acid phosphatase type 2B	0.569
PRKX	NM_005044	protein kinase, X-linked	0.566
FKBP1A	NM_000801	FK506 binding protein 1A, 12kDa	0.56
DHX16	NM_003587	DEAH (Asp-Glu-Ala-His) box polypeptide 16	0.547
UBE2L3	NM_003347	ubiquitin-conjugating enzyme E2L 3	0.542
RNASE4	NM_002937	ribonuclease, RNase A family, 4	0.54
PPAP2A	NM_003711	phosphatidic acid phosphatase type 2A	0.539
OR6A2	NM_003696	olfactory receptor, family 6, subfamily A, member 2	0.538
GNRHR	NM_000406	gonadotropin-releasing hormone receptor	0.537
STK16	NM_001008910	serine/threonine kinase 16	0.514

Table 2: Functional clusters obtained from the differentially expressed gene transcripts during high altitude acclimatization

Term	Count	%	P Value	Genes	Fold Enrichment
GO:0043067~regulation of programmed cell death	24	15.4	1.06E-05	TRAF1, LALBA, TNFRSF6B, CEBPB, CD3G, MADD, CD3E, ACTN1, SOX4, TNFSF14, TNFSF12, CUL3, CUL2, PEA15, AGTR2, SSTR3, PSMC5, DYNLL1, BTG1, PRKRA, APOH, TPT1, DYRK2, PLAGL2	2.81
GO:0042110~T cell activation	7	4.48	0.002	CD3G, CD3E, FYN, TNFSF14, SOX4, FKBP1A, LCP1	5.29
GO:0008092~cytoskeletal protein binding	14	8.97	0.002	STX1A, APC2, BAIAP2, ACTN1, ARPC4, AQP2, YWHAH, SYN1, FYN, SORBS2, ARPC2, CALM2, LCP1, BCL7B	2.61
oxygen transport	3	1.92	0.004	HBA2, HBA1, HBE1, HBB	30.82
GO:0046649~lymphocyte activation	8	5.12	0.004	CD3G, CD3E, FYN, TNFSF14, SOX4, FKBP1A, CD79A, LCP1	3.82
GO:0007269~neurotransmitter secretion	4	2.56	0.005	STX1A, DOC2A, SYN1, SYN2	11.2
GO:0005856~cytoskeleton	25	16	0.008	APC2, GNA11, AURKC, CASK, ARPC4, CCT3, OFD1, ACTG2, PEA15, DYNLL1, SPRR2D, SORBS2, ARPC2, TPT1, TUBG1, STX1A, KIF5A, ACTN1, KRT13, KRT17, SGCG, RUVBL1, SSNA1, LCP1, CALM2	1.72
calcium binding	5	3.2	0.009	DOC2A, ACTN1, CALM2, CALB2, LCP1	5.98
GO:0007267~cell-cell signaling	14	8.97	0.009	LALBA, INSL3, EGR3, STX1A, GLRA1, KIF5A, SLC6A2, CTF1, CALCA, DOC2A, SSTR3, SYN1, SYN2, STC1	2.22
calcium	14	8.97	0.013	LALBA, ARSE, PRSS1, ACTN1, CALB2, SSR1, ATP2B1, SLC25A12, DOC2A, FBLN2, TPT1, ARSA, LCP1, CALM2	2.14
GO:0060191~regulation of lipase activity	5	3.2	0.012	CALCA, AGTR2, GNA11, APOH, FKBP1A	5.47
GO:0051004~regulation of lipoprotein lipase activity	3	1.92	0.013	AGTR2, APOH, FKBP1A	16.81
GO:0006706~steroid catabolic process	3	1.92	0.019	YWHAH, HSD17B6, SCARF1	13.6
GO:0008217~regulation of blood pressure	5	3.2	0.02	CALCA, ACTG2, AGTR2, HBB, AQP2	4.76
IPR002290:Serine/threonine protein kinase	7	4.48	0.03	DYRK1A, MAP4K2, AURKC, CASK, DYRK3, DYRK2, PRKX	2.96
immune response	6	3.84	0.035	HLA-C, CD79A, TNFSF12, PTMS, HLA-G, B2M	3.3
GO:0002684~positive regulation of immune system process	7	4.48	0.038	CD3E, FYN, TNFSF14, RARA, CD79A, TNFSF12, B2M	2.8
GO:0003073~regulation of systemic arterial blood pressure	3	1.92	0.051	CALCA, AGTR2, AQP2	8.16
GO:0050878~regulation of body fluid levels	5	3.2	0.06	GP5, GP1BB, PABPC4, APOH, AQP2	3.37
ribonucleoprotein	6	3.84	0.075	SRP14, RPL3L, MRPL49, RPL37, RPL38, SNRPF	2.65
GO:0032844~regulation of homeostatic process	4	2.56	0.116	CALCA, FKBP1A, CALM2, AHSG	3.34
GO:0005509~calcium ion binding	14	8.97	0.171	LALBA, ARSE, PRSS1, ACTN1, CALB2, SSR1, ATP2B1, SLC25A12, DOC2A, FBLN2, TPT1, ARSA, LCP1, CALM2	1.43

Term	Count	%	P Value	Genes	Fold Enrichment
GO:0051924~regulation of calcium ion transport	3	1.92	0.161	CALCA, FKBP1A, CALM2	4.14
ubl conjugation	8	5.12	0.197	CUL3, CUL2, CEBPB, HIST2H2BE, SORBS2, COX6A2, HLA-C, CALM2	1.67
GO:0007155~cell adhesion	11	7.05	0.194	CALCA, GP5, GP1BB, CASK, PTPRS, ACTN1, ADAM12, ECM2, CD151, SCARF1, NPHP1	1.49
GO:0019953~sexual reproduction	8	5.12	0.201	INSL3, GLRA1, DYNLL1, ARSA, RUVBL1, PPAP2A, PPAP2B, CNGA1	1.66
palmitate	4	2.56	0.243	STK16, FYN, GPA33, CD151	2.31
GO:0006936~muscle contraction	4	2.56	0.213	ACTG2, GLRA1, GNA11, FKBP1A	2.49
GO:0016887~ATPase activity	6	3.84	0.277	TNFRSF6B, ATP2B1, PSMC5, DHX16, RUVBL1, ABCC6	1.69
GO:0003700~transcription factor activity	12	7.69	0.454	SHOX2, DLX3, TCF21, EGR3, CEBPB, FOXN1, SOX4, RARA, PBX2, RFXANK, PITX1, PLAGL2	1.15
GO:0044057~regulation of system process	5	3.2	0.402	CALCA, STX1A, AGTR2, YWHAH, GLRA1	1.54
GO:0009055~electron carrier activity	3	1.92	0.68	ACOX2, HSD17B6, RDH16	1.27
GO:0055085~transmembrane transport	6	3.84	0.712	SLC25A12, AQP5, SLC30A3, CNGA1, AQP2, ABCC6	1
GO:0055085~transmembrane transport	6	3.84	0.712	SLC25A12, AQP5, SLC30A3, CNGA1, AQP2, ABCC6	1
GO:0050890~cognition	6	3.84	0.964	CALCA, GLRA1, FYN, OR6A2, CNGA1, ABCC6	0.62

Table 3: Pathway specific transcripts of high altitude acclimatization

Term	Count	%	P Value	Genes	Fold Enrichment
hsa04612:Antigen processing and presentation	5	3.2	0.01	HLA-C, IFNA14, RFXANK, HLA-G, B2M	4.71
h_ctlPathway: CTL mediated immune response against target cells	3	1.92	0.03	CD3G, CD3E, B2M	9.91
hsa04912:GnRH signaling pathway	4	2.56	0.12	GNA11, GNRHR, CALM2, PRKX	3.19
hsa04270:Vascular smooth muscle contraction	4	2.56	0.16	ACTG2, GNA11, CALM2, PRKX	2.79
hsa04120:Ubiquitin mediated proteolysis	4	2.56	0.24	CUL3, CUL2, UBE2D3, UBE2L3	2.28
hsa04810:Regulation of actin cytoskeleton	5	3.2	0.28	APC2, ARPC2, BAIAP2, ACTN1, ARPC4	1.81
hsa04020:Calcium signaling pathway	4	2.56	0.38	ATP2B1, GNA11, CALM2, PRKX	1.77
hsa04080:Neuroactive ligand-receptor interaction	5	3.2	0.4	AGTR2, SSTR3, GLRA1, PRSS1, GNRHR	1.52
hsa04060:Cytokine-cytokine receptor interaction	5	3.2	0.42	TNFRSF6B, CTF1, TNFSF14, IFNA14, TNFSF12	1.49'
hsa04080:Neuroactive ligand-receptor interaction	5	3.2	0.4	AGTR2, SSTR3, GLRA1, PRSS1, GNRHR	1.52
hsa04060:Cytokine-cytokine receptor interaction	5	3.2	0.42	TNFRSF6B, CTF1, TNFSF14, IFNA14, TNFSF12	1.49

nonspecific cellular response to stress. In the present study, about 89 transcripts showed a change in gene expression on the 3.8 K gene array after acute induction to altitude and were protein coding type. High altitude hypoxia appears to have a substantial down regulatory effect on transcript expression in peripheral blood cells. The functional clusters of apoptosis, oxygen transport, neurotransmitter secretion, regulation of blood pressure, regulation of body fluid levels, cell-cell signaling, transcripts of calcium ion binding were evident of an hypoxic signature of altitude acclimatization. The pathways which were found to be affected were antigen processing and presentation (hsa04612), h_ctlPathway: CTL mediated immune response against target cells, GnRH signaling pathway (hsa04912), vascular smooth muscle contraction (hsa04270), ubiquitin mediated proteolysis (hsa04120), regulation of actin cytoskeleton (hsa04810), calcium signaling pathway (hsa04020), neuroactive ligand-receptor interaction (hsa04080) and cytokine-cytokine receptor interaction (hsa04060).

It has been reported that continuous residence at moderate heights (2,000-2,500 m) tends to improve oxygen transport capacity by an erythropoietin-induced increase in the hematocrit [8]. An increase in hemoglobin concentration augments maximal O_2 consumption ($VO_{2\,max}$) and enhances exercise performance [9]. In the present study, increase in expression of hemoglobin alpha 1 and hemoglobin alpha 2 was noted on acute altitude induction. The result of the present study suggests that cellular response to hypoxia at the level of transcript expression is quite broad, although it may also be more specific to hypoxia than generally appreciated. Fink and colleagues [10] by applying DNA array technology and real-time PCR in a variety of human hepatocyte cell lines identified several previously unrecognized hypoxia-responsive genes; it was also seen that hypoxic exposure without reoxygenation led to an overall decrease in the number of transcripts expressed by cells, although increase in expression of heat shock proteins was not observed. In a recent study on effect of hypoxia on gene expression in HepG2 cells, it was shown that gene expression was broad, had a significant component of downregulation, and included a relatively small number of genes whose response was independent of cell and stress type [11].

Profiles of gene expression patterns are helping to define the complex biological processes associated with both health and disease *in vivo*. Microarrays can identify changes in gene expression that can be used as biomarkers of environmental and any other stress related exposure and their early effect and can provide information on mechanisms of various biological processes. DNA arrays have increased substantially in power and complexity and application of late-generation arrays would enable identification of more hypoxia-responsive genes. Gene expression is often stochastic [12] because most genes exist at single or low copy number in a cell. Some genes are expressed at high levels and others at low levels. It is now possible to track mRNA expression in a single cell with single molecule sensitivity in real time dynamics providing mechanistic insight into macromolecules [13]. Such kind of real time assays together with other emerging single molecule techniques [14] will yield further insight into not only gene expression and but many other fundamental biological processes. Understanding of this biological phenomenon will strategize therapeutic approaches for combating the harsh environment as well as perform better under the circumstances.

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