Summary & Conclusions
HA places are amongst the most inhospitable on earth. The two main challenges to life at HA come from hypobaric hypoxia and the low ambient temperatures. Sojourners, when exposed to such a stressful environment, a good percentage of them suffer from HA-induced diseases like HAPE etc. Contrary to this, many people live and work at altitude with no apparent adverse effects. This attributes to that of natural selection of over thousands of years resulting in some populations being genetically more suited to the stresses at HA. The relative contribution of the environmental factors and genetic heritage towards acquiring those traits is yet to be resolved as identification of genetic variants which help adapt at HA is crucial in order to avoid the detrimental consequences of hypoxia. In both, pathogenesis of HA disorders or HA adaptation/performance, BP/hypertension is one of the physiological parameters, which plays a critical homeostatic function. The Renin-Angiotensin-Aldosterone System (RAAS) contributes significantly to the regulation of BP, vascular tone, cardiovascular remodeling, electrolyte and volume homeostasis and the earlier studies have explored the association analysis of allelic variants of the RAAS genes in addition to Nitric Oxide Synthases with HA adaptation/disorder. They have been discovered to be influencing physical performance of HA natives and/or the pathogenesis of HA disorders in sojourners. However, each individual genetic variant generally has only a modest effect because of the multifactorial nature of the complex trait. The interaction of genetic variants with each other or with environmental factors can potentially be quite important in determining the observed phenotype.

In this thesis, we attempted to highlight the genetic component (RAAS genes) interacting with environment (HA), thus leading to a probable phenotypic outcome in the form of hypertension among the HA natives and HAPE among sojourners. We, therefore, undertook an association study in two well-characterized cohort by recruiting native subjects comprising healthy controls and hypertensive patients with case-control design and a consecutive ethnic-matched unrelated Indians sojourners visiting HA consisting of HAPE patients and resistants. The major conclusions of the work are as follows:

In Chapter 2, we investigated three genes of RAAS i.e. ACE, AGT and AGTR1, and its association with hypertension among the native population of HA. As the fundamental features of hypertension are still quite ambiguous, it is difficult to differentiate whether the disease in itself is merely an umbrella-like diagnostic phrase covering several different pathologic states with the same measurable phenotype (chronically heightened BP) or whether established hypertension is just common end-
point of several pathways crossing and interacting with each other. Additionally as it is a multifactor disorder, both environmental as well as genetic factors are reported to play an effective and interactive role in elucidating the resulting phenotype. Since the HA environment itself is acting as a strong environmental selection pressure, the alleles are hypothesized to have prominent role in associating with HA environment rather than being associated with any additional disease causing factor. The high prevalence of I allele of ACE among the natives (both cases and controls) depicts the genetic selection under hypobaric-hypoxia as this allele is reported more favorable to this environment. Besides ACE I/D, the additional polymorphisms analyzed in the present study were G–6A, T174M and M235T from AGT and A1166C from AGTR1. All polymorphisms were analyzed individually, in combination and as haplotypes, and their correlations with biochemical parameters. The results from these SNPs do not associate with hypertension among HA natives. Steeping further for haplotype analysis for the combine effect of the 3 studied SNPs of AGT for a possible disease association both 2 and 3 locus haplotype analysis did not associate with hypertension. The 2-locus haplotype consisting -6A and 235T, both reported to be associated with hypertension were equally distributed in cases and controls. Similarly the 3-locus haplotype also depicted insignificant association among the studied HA native population. A possible combined contribution of genetic and environmental factors through gene-gene and gene-environment interactions were also analyzed using a multi-dimensional reduction (MDR) method. Through this approach a strong synergistic interaction was observed between I/D of ACE and G–6A of AGT and redundancy between T174M of AGT and A1166C polymorphisms of AGTR1. Thus it is concluded that the studied Alu and SNPs of RAAS do not associate with hypertension among HA natives.

In Chapter 3, given the reported functional impact of the RAAS in cardiovascular phenotypes as well as pulmonary diseases, we hypothesized that these variants individually and in combination may be associated with HAPE. However, decades of serious work have not been fruitless, and the puzzle of the pathogenesis is slowly getting completed. Our focus on HAPE in this chapter revealed an inverse correlation between SaO2 and PR for both controls and patients. The correlation was more severe among HAPE patients ($R^2=−0.292$ in HAPE-p and $R^2=−0.01$ in HAPE-r). Additionally lower DBP and higher BMI were found to be associated with HAPE. The genetic variants of RAAS genes were significantly associated with the disease. The ACE I allele in consistent to its role in $O_2$ saturation at HA, endurance
performances among athletes and adaptation to HA among natives was also observed to be significantly associated with HAPE resistant. The D allele was significantly overrepresented in HAPE-p. Some interesting results were observed for SNP of AGT gene. The M allele of M235T and G allele of G-6A were significantly associated with HAPE. The literature is scant for these alleles associating with the disease, while ACE gene from the same pathway is a prominent example where the I allele is reportedly associated with HA adaptation among the Himalayans while the same allele associating with HA pulmonary hypertension among the Kyrgyz populations. Subsequent analysis of these polymorphisms for its possible role in combination with other genetic variants consistently depicted a visible role of I/D of ACE and M235T and G-6A of AGT in disease manifestation. For example the distribution of genotype-combinations between the SNPs resulting from best MDR model depicted M235T as the best disease predicting model with CVC of 10/10.

The Chapter 4 highlights stratification of population hence is an indispensable part of the earlier chapters. The first section of chapter 4 investigated the role of ACE I/D polymorphism at HA. This chapter is indeed a comparative study for functional and non-functional genetic variants. Here we studied six Alus, viz, ACE, A25, B65, D1, Fx111B and PV92. We observed that the role of ACE alu polymorphism stands prominent as a functional variant having important role in HA adaptation. This chapter also introduces for the first time a unique HA population called Brokpas. It highlights the genetic fitness of native population to its native environment with consistency of genetic data to that of linguistic pattern. A systematic comparison of this population along with two other HA populations, with approximately 32 populations from worldwide depict the spatial distribution of entire populations on a PC plot. The distribution on the PC plot was consistent to their similar ethnicity, geographic location and historical evidences as well as similar environment they share.

In the later second part of Chapter 4 we report the results of analyses of data from IGV consortium on 24 populations with an approximately 23 samples from each population totaling 548 unrelated individuals. As an effort made to cover the diverse populations of India both large and isolated populations were studied. Population stratification, a consequence of differences in allele frequencies across populations arising mainly due to natural selection and genetic drift, is a major problem in association studies. Analyses of frequency profiles of markers of genes from RAAS associated with disease or drug-response related genes in diverse populations were the
major thrust as they are important for the dissection of common diseases. The LD pattern of *ACE* constructed from 18 SNPs selected at equidistance, covering the entire gene region depicted that when a trend line is drawn for LD between two SNPs based on $r^2$ value, the HA population had the highest value compared to any of the other populations from LA, irrespective of their ethnic groups (both from India as well as HapMap). We also report the allelic frequency distribution in these 24 populations for other important SNPs studied in earlier chapters viz, T174M and M235T of *AGT*, and A1166C of *AGTR1*. The results on frequency distribution of SNPs from RAAS genes depicted that the M235 allele association with HAPE was least among the African population, followed by Chinese and Japanese. The HA population had frequency lesser than European and Indo-European. Overall the Indian populations not only overlap with the diversity of HapMap populations, but also contain population groups that are genetically distinct. These data and results are useful for addressing stratification and study design issues in complex traits like hypertension especially for heterogeneous populations.

It has thus depicted that the candidate gene of RAAS are subjected to strong environmental selection pressure of HA hypobaric-hypoxia. The work included in this thesis aimed at understanding the inherent genetic variability due to gene-environmental interaction and a step towards identifying susceptible biomarkers for disease or predisposition to HA environment. As the tools are becoming more precise and powerful, parallelly, technical improvements provide tremendous enhancement in deciphering the knowledge on gene-environment interaction. Together these advances offer promises for the future of genetic medicine, especially for the identification of genetic variations influencing multifactorial diseases, particularly those of public health importance, and response to treatment. Such identification will provide avenues for the search of specific treatment and prevention that could be tailored to the genetic constitution of each individual.