Table of Contents

List of Tables i
List of Figures iii
Abbreviations vi
Abstract ix

Chapter 1 Review of literature 1
1.1 Environment 2
  1.1.1 High-Altitude Environment 2
  1.1.2 Medically how high is high-altitude? 3
1.2 Gene 7
  1.2.1 Renin-Angiotensin-Aldosterone System 7
    1.2.1.1 Angiotensin-I Converting Enzyme 9
    1.2.1.2 Angiotensinogen 11
    1.2.1.3 Angiotensin-II type 1 receptor (AGTR1) 12
    1.2.1.4 Angiotensin-II type 2 receptor (AGTR2) 13
1.3 Gene-Environment Interaction 14
  1.3.1 Adaptation and Mal-adaptation to high-altitude 15
    1.3.1.1 Adaptation: Native population 15
    1.3.1.1.1 BP at high-altitude 16
    1.3.1.2 Mal-adaptation: Chronic Mountain Sickness 18
  1.3.2 Acclimatization and Deterioration 19
    1.3.2.1 Acclimatization 19
    1.3.2.1.1 HbO₂ Association/Dissociation 19
    1.3.2.1.2 Polycythemia 20
    1.3.2.1.3 Blood Flow 20
    1.3.2.1.4 O₂ diffusion 21
    1.3.2.2 Deterioration: Disease at HA 21
    1.3.2.2.1 Acute Mountain Sickness 21
    1.3.2.2.2 High-Altitude Pulmonary Edema 22
    1.3.2.2.3 High-Altitude Cerebral Edema 25
  1.3.3 Intermittent hypoxia: “Living low - working high” 25
1.4 Study approaches 26
  1.4.1 Genetic association studies 27
    1.4.1.1 Candidate gene approach 28
    1.4.1.2 Analysis methods 28
  1.4.2 Biostatistical analysis 31
    1.4.2.1 Testing for Hardy-Weinberg equilibrium 31
    1.4.2.2 ORs for disease from case-control studies 31
    1.4.2.3 Linkage Disequilibrium Analysis 32
Chapter 2  RAAS genes in high-altitude hypertension  

2.1  Introduction  

2.2  Materials and methods  

2.2.1  Patients and healthy volunteers  

2.2.2  Demographic evaluation  

2.2.3  Blood pressure measurements  

2.2.4  Biochemical Analysis  

2.2.5  Genomic DNA extraction  

2.2.6  Genotyping  

2.2.6.1  Angiotensin-I converting enzyme  

2.2.6.2  Angiotensinogen  

2.2.6.3  Angiotensin-II type 1 receptor  

2.2.7  Gene–gene and gene–environment interaction  

2.3  Results  

2.3.1  Baseline characteristics of study groups  

2.3.2  Single-locus analysis of ACE, AGT and AGTR1 gene  

2.3.3  Correlation between ACE genotype and activity  

2.3.4  Haplotype association analysis of AGT  

2.3.5  Gene–gene and gene–environment interaction  

2.4  Discussion  

Chapter 3  RAAS genes in HAPE  

3.1  Introduction  

3.2  Materials and methods  

3.2.1  Patients and healthy volunteers  

3.2.2  Demographic evaluation  

3.2.3  Blood pressure measurements  

3.2.4  Biochemical Analysis: Estimation of plasma ACE activity  

3.2.5  Genomic DNA extraction  

3.2.6  Genotyping
3.2.6.1  ACE I/D polymorphism 94
3.2.6.2  AGT G–6A polymorphism 94
3.2.6.3  AGT T174M polymorphism 94
3.2.6.4  AGT M235T polymorphism 94
3.2.6.5  AGTR1 A1166C polymorphism 94
3.2.6.6  AGTR2 SNP rs5193, rs5194 & rs12845035 94
  3.2.6.6.1  SNaPshot assay 94
      3.2.6.6.1.1  Primers for SNapShot 95
      3.2.6.6.1.2  PEG purification 95
      3.2.6.6.1.3  Principle of SNapshot 95
      3.2.6.6.1.4  Protocol 97
3.2.7  Gene–gene and gene–environment interaction 98
3.2.8  Haplotype analysis 98
3.2.9  Statistical analysis 99

3.3  Results 99
3.3.1  Baseline characteristics of study groups 99
3.3.2  Clinical characteristics & correlation analysis 99
3.3.3  Single–locus analysis 99
3.3.3.1  Association of ACE I/D and HAPE 99
3.3.3.2  Association of AGT polymorphism with HAPE 103
3.3.3.3  Ang-II receptors polymorphisms and HAPE 103
3.3.4  Haplotype association analysis of AGT and AGTR2 110
3.3.5  Gene-gene and gene-environment interaction 110

3.4  Discussion 120

Chapter 4  RAAS genes and high-altitude environment 122
4.1  Introduction 123
4.2  Role of ACE I/D polymorphism at HA 127
  4.2.1  Materials and methods 127
      4.2.1.1  Study populations 127
      4.2.1.2  Population sampling 129
      4.2.1.3  Alu typing 129
      4.2.1.4  Statistical Analysis 130
  4.2.2  Results 131
      4.2.2.1  Alu diversity and distance analyses 131
      4.2.2.2  Genomic affinities among world populations 135
      4.2.3  Discussion 138
4.3  Comparison with the mainland low altitude populations 141
  4.3.1  Materials and Methods 141
      4.3.1.1  Selection of population and sample collection 141
      4.3.1.2  High throughput SNP genotyping platforms 144
      4.3.1.3  Statistical Analysis 144
  4.3.2  Result 145
      4.3.2.1  LD pattern of ACE gene among HA population 145
4.3.2.2 SNPs of AGT, AGTR1 & AGTR2 in Indian populations 149
4.3.3 Discussion 154

Summary and Conclusions 159
Bibliography 164
Credentials 191