Genome-wide association study of high-altitude pulmonary edema in a Han Chinese population

Xun Li1,*, Tianbo Jin2,3,*, Mingxia Zhang3, Hua Yang3, Xuewen Huang1, Xiaobo Zhou1, Wenchao Huang1, Lipeng Qin1, Longli Kang2, Ming Fan4, Suzhi Li1

1Center of Altitude Disease, General Hospital of Tibet Military Area Command, Lhasa 850003, China
2Key Laboratory of High Altitude Environment and Genes Related to Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi 712082, China
3School of Life Sciences, Northwest University, Xi’an, Shaanxi 710069, China
4Department of Brain Protection and Plasticity, Institute of Basic Medical Sciences, Beijing 100850, China

*These authors contributed equally to this work and are joint first authors

Correspondence to: Ming Fan, email: fanming1973@126.com
Suzhi Li, email: suzhilixizang@163.com

Keywords: high-altitude pulmonary edema (HAPE), single nucleotide polymorphisms (SNPs), genome wide association analysis (GWAS), susceptibility gene

ABSTRACT

A two-stage genome-wide association study (GWAS) was performed to identify and analyze genes and single nucleotide polymorphisms (SNPs) associated with high-altitude pulmonary edema (HAPE) in a Han Chinese patient population. In the first stage, DNA samples from 68 patients with recurrent HAPE were scanned using Affymetrix SNP Array 6.0 Chips, and allele frequencies were compared to those of 84 HapMap CHB samples to identify candidate SNPs. In the second stage, the 77 identified candidate SNPs were examined in an independent cohort of samples from 199 HAPE patients and 304 controls. Associations between SNPs and HAPE risk were tested using various genetic models. Of the 77 original SNPs, 7 were found to be associated with HAPE susceptibility in the second stage of the study. GO and pathway enrichment analysis of the 7 SNPs revealed 5 adjacent genes involved in various processes, including regulation of nucleoside diphosphate metabolism, thyroid hormone catabolism, and low-density lipoprotein receptor activity. These results suggest the identified SNPs and genes may contribute to the physiopathology of HAPE.

INTRODUCTION

High altitude pulmonary edema (HAPE) is a non-cardiogenic form of pulmonary edema that develops in unacclimatized healthy individuals at altitudes above 2500–3000 m [1]. It is a potentially fatal medical condition and the most common cause of death among high-altitude illnesses [2]. However, the pathogenesis of HAPE remains poorly understood. Previous studies suggest that uneven hypoxic pulmonary vasoconstriction, pulmonary capillary damage, and increased pulmonary artery pressure play important roles in the pathogenesis of HAPE [3, 4].

HAPE is caused by the interaction of both genetic and environmental risk factors. Previous studies have shown that family history and race influence individual susceptibilities to HAPE [5]. Some people are susceptible to high-altitude pulmonary edema, whereas others are resistant to this condition [6, 7]. The prevalence of HAPE in the Han Chinese population in Tibet, which is about 0.4%–2% [8] and differs depending on age, gender, and occupation, is higher than that observed in native Tibetans. Rate of ascent, altitude reached, pre-acclimatization, and individual susceptibility are the major factors that contribute to high-altitude maladies [9]. In addition, patients who have previously developed HAPE are more likely to experience recurrence, which suggests the presence of a constitutional, and possibly a genetic, component in its etiology [10].

Several recent studies have examined the genetic basis of HAPE, focusing mainly on genetic polymorphisms
in the beta2-adrenergic receptor [11], vascular endothelial growth factor [12], the renin angiotensin system [13], and pulmonary surfactant proteins A1 and A2 [14] in subjects susceptible to HAPE. Polymorphisms within these genes may explain individual variation in hypoxic responses and perhaps indicate susceptibility to high-altitude disease. However, the precise role of these genes in HAPE pathogenesis remains unclear.

To identify variant genes across the whole genome that are specifically related to HAPE risk, we conducted a two-stage GWAS analysis in 68 patients with recurrent HAPE and in 84 HapMap CHB populations as references. We further evaluated potential associations with HAPE risk in a replication cohort with a total of 199 HAPE patients and 304 healthy controls from a Han Chinese population. While previous GWAS studies were based on case-control samples only, here we examined a large number of cases to identify genes that might be related to HAPE susceptibility.

RESULTS

A total of 571 subjects, including 267 HAPE patients (246 males, 21 females; mean age 32.6 ± 10.7) and 304 controls (290 males, 14 females; mean age 36.2 ± 4.5), were examined in this analysis. Age distribution differed between the patient and control groups (p < 0.05). Participant characteristics are listed in Table 1.

We first scanned DNA samples from 68 patients with recurrent HAPE using Affymetrix Genome-Wide Human SNP Array 6.0 Chips. After filtering with standard quality-control procedures, 502,689 SNPs with an overall call rate of 99.92% were qualified for further GWAS analysis. To identify SNPs that might be associated with the risk of HAPE, we compared SNP allele frequencies in the 68 patients to those of the 84 HapMap CHB controls and found that frequencies differed for 77 SNPs. Information regarding these 77 SNPs and their associated genes is shown in Table 2. A Manhattan plot was generated for the SNPs in patients with recurrent HAPE under the allelic and genotypic model (Figure 1). MDS and QQ-plot revealed that there was no obvious population stratification in this experiment (Figure 2 and Figure 3).

Of the 77 SNPs, 68 were qualified after Sequenom MassARRAY Assay Design. In a second experiment, we confirmed the results of the first experiment by genotyping the 68 SNPs in 199 HAPE patients and 304 controls of Han Chinese descent. Table 3 summarizes the characteristics of the tested SNPs and their predicted associations with HAPE risk in crude analysis. Three SNPs (rs17484974, rs725050, and rs10178082) were excluded at the 5% significance level for Hardy-Weinberg equilibrium (HWE). A χ² test revealed that two SNPs, rs1075355 (OR = 1.825; 95% CI = 1.04–2.40, p = 0.057 for the “A/G” genotype), dominant (OR = 1.62; 95% CI = 1.07–2.44, p = 0.022 for the “A/G-G/G” genotype) and recessive (OR = 1.87; 95% CI = 1.06–2.32, p = 0.017) models. The rs10984811 SNP increased HAPE risk in both the co-dominant (OR = 3.95; 95% CI = 1.33–11.73, p = 0.032 for the “C/C” genotype) and recessive (OR = 3.97; 95% CI = 1.34–11.75, p = 0.0089 for the “C/C” genotype) models. The rs17777329 SNP was also associated with an increased risk of HAPE in the co-dominant (OR = 1.88; 95% CI = 1.07–3.30, p = 0.051), dominant (OR = 1.95; 95% CI = 1.12–3.37, p = 0.018) and log-additive (OR = 1.87; 95% CI = 1.06–2.37, p = 0.03) models. Additionally, the rs12226072 SNP was associated with increased HAPE risk in the co-dominant (OR = 1.65; 95% CI = 1.04–2.62, p = 0.093) and over-dominant (OR = 1.66; 95% CI = 1.04–2.63, p = 0.032) models.

To identify genes that might be involved in HAPE invasion, we also performed gene annotation and functional classification for the 7 significant loci we identified in the replication study. GO and KEGG pathway enrichment analyses identified 5 potential candidate genes located within ± 500kb of these SNPs (Table 5). These genes were mainly involved in cellular tight junctions, oxidation and reduction, extracellular matrix metabolism, pulmonary development, and pulmonary smooth muscular tension adjustment.

DISCUSSION

In this study, we conducted a two-stage GWAS analysis to investigate the genetic factors associated with HAPE in a Han Chinese population. Seven loci, including four susceptibility loci and three protective loci, were found to be associated with HAPE in this analysis. Gene annotation and functional classification of these loci revealed that five of the candidate genes are potentially involved in the pathogenesis of HAPE. To the best of our knowledge, this is one of the largest studies to explore the genetic factors underlying the development of HAPE in a Han Chinese population.
The rs10789097 locus contained no annotated genes, and the gene nearest to it was INADL, which encodes inactivation no-after potential (INAD) protein, also known as protein associated with Lin seven 1 (Pals1) -associated tight junction protein (PATJ). INAD contains multiple PDZ domains, which are protein-protein interaction modules that typically bind to short peptide sequences at the carboxyl terminus of target proteins. Proteins containing multiple PDZ domains often bind to different transmembrane and intracellular proteins and play central roles as organizers of multimeric complexes [15]. PATJ is a polarity protein and plays a complex role in the maintenance of epithelial polarity [16]. Considering that stress failure in pulmonary capillaries is an important contributor to HAPE pathogenesis, we speculate that the INADL gene may also impact the pathogenesis of HAPE.

The KCNV2 gene, which encodes the Kcnv2 protein, belongs to a group of potassium channel

Table 1: Basic characteristics of cases and controls in this study

| Variables   | Case N (%) | Control N (%) | p-value |
|-------------|------------|---------------|---------|
| Age (years) | 32.6 ± 10.7| 36.2 ± 4.5    | < 0.005a |
| Sex         |            |               | > 0.005b |
| Male        | 246 (92.0%)| 290 (95.4%)   |         |
| Female      | 21 (8.0%)  | 14 (4.6%)     |         |
| Total       | 267        | 304           |         |

aP values were calculated from two-sided chi-square tests.
bP values were calculated by Student t tests.

Figure 1: Manhattan plot for the whole SNPs in recurrent HAPE subjects of Chinese Han decent. Chromosomes are shown in alternate colors. (A) Allelic model; (B) Genotypic model.
| SNP ID        | Chromosome | Gene (s) | Alleles | MAF       | Position | Band   | Role     |
|--------------|------------|----------|---------|-----------|----------|--------|----------|
| rs4908427    | 1          | CAMTA1   | G/A     | 0.059     | 6976226  | 1p36.31| Intron   |
| rs9661274    | 1          |          | G/A     | 0.059     | 30149249 | 1p35.3 |
| rs17484974   | 1          |          | C/T     | 0.132     | 39186794 | 1p34.3 |
| rs12406517   | 1          | PPAP2B   | G/C     | 0.110     | 56974278 | 1p32.2 |
| rs1694212    | 1          |          | T/C     | 0.132     | 61480000 | 1p31.3 |
| rs10789097   | 1          |          | C/G     | 0.066     | 62119978 | 1p31.3 |
| rs17188846   | 1          | KCNH1    | C/G     | 0.184     | 211261821| 1q32.2 |
| rs2577156    | 1          | EPRS     | C/A     | 0.051     | 220190845| 1q41   |
| rs3008613    | 1          | MIA3     | G/A     | 0.110     | 222795769| 1q41   |
| rs4491711    | 2          | RASGRP3  | G/A     | 0.103     | 33776743 | 2p22.3 |
| rs11125567   | 2          | CCDC88A  | A/G     | 0.081     | 55627913 | 2p16.1 |
| rs11898268   | 2          |          | C/A     | 0.000     | 154622125| 2q23.3 |
| rs10167840   | 2          | RASGRP3  | G/A     | 0.110     | 145325196| 3q24   |
| rs17598758   | 2          |          | C/T     | 0.213     | 112916203| 3q13.2 |
| rs11924340   | 3          | A/G     | 0.060    | 145325196| 3q24   |
| rs12504325   | 3          | C4orf6   | A/G     | 0.103     | 5537184  | 4p16.2 |
| rs17598758   | 3          |          | C/T     | 0.110     | 21090068 | 4p15.31|
| rs6535838    | 4          | C/T     | 0.147    | 117082198 | 4q26   |
| rs7688505    | 4          |          | A/C     | 0.199     | 153023402| 4q31.3 |
| rs41417552   | 4          | CMBL     | G/A     | 0.110     | 10305452 | 5p15.2 |
| rs2161592    | 5          | A/G     | 0.162    | 50772554  | 5q11.2 |
| rs3777207    | 5          | ELL2     | A/G     | 0.118     | 95231115 | 5q15   |
| rs6595114    | 5          | C/T     | 0.118    | 117676709 | 5q23.1 |
| rs2193963    | 5          | C/T     | 0.096    | 121596196 | 5q23.2 |
| rs17652561   | 5          | SLC6A7   | A/G     | 0.162     | 149584197| 6q32   |
| rs2937582    | 5          | A/G     | 0.434    | 166465008 | 6q34   |
| rs2984100    | 6          | C/G     | 0.125    | 8592499  | 6p24.3 |
| rs7762263    | 6          | T/C     | 0.110    | 118582318| 6p31.3 |
| rs4715938    | 6          | G/C     | 0.103    | 14944857 | 6p23   |
| rs725050     | 6          | C/T     | 0.162    | 89267376 | 6q15   |
| rs1419722    | 7          | EIF3B    | C/T     | 0.142     | 2413258  | 7p22.3 |
| rs10178082   | 7          | T/A     | 0.199    | 10706912 | 7p21.3 |
| rs4947936    | 7          | C/A     | 0.103    | 50906752 | 7p12.1 |
| rs12226072   | 7          | A/T     | 0.294    | 96443614 | 7q21.3 |
| rs2956956    | 8          | DLGAP2   | C/T     | 0.066     | 1553118  | 8q23.3 |
| rs2980508    | 8          | SGK223   | C/T     | 0.096     | 8171732  | 8q23.1 |
| rs310282     | 8          | C/A     | 0.132    | 23614369 | 8p21.2 |
| rs4573320    | 8          | C/T     | 0.343    | 65128758 | 8q12.3 |
| rs1568828    | 8          | PREX2    | A/G     | 0.081     | 69122128 | 8q13.2 |
| rs1006698    | 9          | KCNV2    | T/G     | 0.206     | 2725283  | 9p24.2 |
| rs1011531    | 9          | A/G     | 0.110    | 13755192 | 9p23   |
| rs13289064   | 9          | C/G     | 0.228    | 16897685 | 9p22.2 |
| rs10984811   | 9          | ANP32B   | C/A     | 0.149     | 100784050| 9q22.33|

Table 2: Basic information of the significantly different SNPs between 68 recurrent HAPE cases and 84 Hapmap CHB subjects in the first stage.
| SNP          | Chromosome | Gene | Allele | MAF (Minor) | MAF (Major) | Position | Location                  |
|--------------|------------|------|--------|-------------|-------------|----------|---------------------------|
| rs12554842   | 9          | COL5A1 | T/C    | 0.081       | 0.071       | 137573407| Intron                    |
| rs11593009   | 10         | T/A   |        | 0.051       | 0.065       | 31974946 | 10p11.22                  |
| rs12243354   | 10         | T/C   |        | 0.125       | 0.131       | 70411536 | 10q21.3                   |
| rs7923700    | 10         | T/C   |        | 0.162       | 0.190       | 87843290 | 10q23.1                   |
| rs2239153    | 12         | T/C   |        | 0.338       | 0.399       | 6186667 | 12p13.31                  |
| rs7303062    | 12         | T/C   |        | 0.074       | 0.084       | 22990450 | 12p12.1                   |
| rs10879780   | 12         | T/C   |        | 0.235       | 0.226       | 74837984 | 12q21.1                   |
| rs1316571    | 13         | T/C   |        | 0.081       | 0.095       | 68320718 | 13q21.32                  |
| rs9550256    | 13         | T/C   |        | 0.265       | 0.220       | 114494675| 13q34                     |
| rs17435983   | 14         | G/A   |        | 0.169       | 0.101       | 27860597 | 14q12                     |
| rs8007744    | 14         | G/A   |        | 0.265       | 0.262       | 28329396 | 14q12                     |
| rs17777329   | 14         | G/A   |        | 0.081       | 0.060       | 101934762| 14q32.31                  |
| rs4787426    | 16         | G/T   |        | 0.059       | 0.065       | 27384731 | 16p12.1                   |
| rs1075355    | 16         | C/G   |        | 0.147       | 0.107       | 77874149 | 16q23.1                   |
| rs12931468   | 16         | G/C   |        | 0.074       | 0.054       | 84495301 | 16q24.1                   |
| rs8067836    | 17         | G/T   |        | 0.081       | 0.071       | 37081707 | 17q12                     |
| rs16955841   | 17         | G/T   |        | 0.105       | 0.107       | 53364146 | 17q22                     |
| rs12450240   | 17         | T/G   |        | 0.265       | 0.235       | 80423712 | 17q25.3                   |
| rs9961715    | 18         | C/T   |        | 0.029       | 0.054       | 3824312 | 18q11.31                  |
| rs12606093   | 18         | C/A   |        | 0.044       | 0.065       | 46077295 | 18q21.1                   |
| rs6074799    | 20         | G/C   |        | 0.110       | 0.101       | 14771472 | 20p12.1                   |
| rs9617661    | 22         | G/T   |        | 0.029       | 0.060       | 18595352 | 22q11.21                  |
| rs5758913    | 22         | C/T   |        | 0.154       | 0.161       | 43148259 | 22q13.2                   |

Notes: A/B stands for minor/major alleles on the entire sample frequencies.

Figure 2: Multidimensional scaling approach (MDS) analysis for the first stage.
Table 3: Allele frequencies in cases and controls and odds ratio estimates for HAPE for the replication stage

| SNP ID  | Gene (s)  | Alleles | MAF | HWE | ORs   | 95% CI  | p-value |
|---------|-----------|---------|-----|-----|-------|---------|---------|
|         |           | A*/B    |     |     |       |         |         |
| rs4908427 | CAMTA1    | G/A     | 0.035 | 0.048 | 1     | 0.728   | 0.380   | 1.395   | 0.337   |
| rs9661274 |           | G/A     | 0.065 | 0.067 | 0.3787 | 0.967   | 0.581   | 1.607   | 0.896   |
| rs17484974 | PPAP2B    | C/T     | 0.111 | 0.112 | 7.851E-47 | 0.987   | 0.660   | 1.476   | 0.949   |
| rs12406517 |           | T/C     | 0.139 | 0.133 | 0.8025 | 1.049   | 0.726   | 1.517   | 0.798   |
| rs10789097 |           | C/G     | 0.075 | 0.043 | 1     | 1.825   | 1.062   | 3.135   | 0.027*  |
| rs17188846 | KCNH1     | C/G     | 0.139 | 0.130 | 0.198  | 1.080   | 0.746   | 1.564   | 0.683   |
| rs2577156 | EPRS      | C/A     | 0.076 | 0.077 | 0.08963 | 0.978   | 0.607   | 1.576   | 0.928   |
| rs3008613 | MIA3      | G/A     | 0.093 | 0.105 | 0.552  | 0.871   | 0.569   | 1.334   | 0.526   |
| rs4491711 | RASGRP3   | G/A     | 0.063 | 0.097 | 1     | 0.624   | 0.384   | 1.014   | 0.055   |
| rs1125567 | CCDC88A   | A/G     | 0.111 | 0.127 | 1     | 0.857   | 0.578   | 1.271   | 0.443   |
| rs11898268 |           | C/A     | 0.003 | 0.000 | 1     | -       | -       | -       | -       |
| rs10167840 |           | T/G     | 0.088 | 0.095 | 1     | 0.914   | 0.589   | 1.420   | 0.690   |
| rs7612512 |           | G/C     | 0.151 | 0.172 | 1     | 0.857   | 0.606   | 1.212   | 0.382   |
| rs1846594 |           | C/T     | 0.193 | 0.232 | 0.6287 | 0.794   | 0.582   | 1.085   | 0.148   |
| rs11924340 |           | A/G     | 0.111 | 0.086 | 0.4683 | 1.329   | 0.871   | 2.029   | 0.186   |
| rs12504325 | C4orf6    | G/T     | 0.101 | 0.105 | 1     | 0.950   | 0.626   | 1.441   | 0.808   |
| rs17598758 |           | C/T     | 0.143 | 0.191 | 1     | 0.709   | 0.502   | 1.002   | 0.051   |
| rs7677143 |           | A/C     | 0.133 | 0.137 | 0.4691 | 0.972   | 0.671   | 1.407   | 0.879   |
| rs6535838 |           | T/A     | 0.156 | 0.140 | 0.8133 | 1.135   | 0.796   | 1.619   | 0.483   |
| rs7688505 |           | A/G     | 0.108 | 0.102 | 0.7525 | 1.067   | 0.707   | 1.609   | 0.758   |
| rs41417552 | CMBL      | G/A     | 0.169 | 0.127 | 0.4419 | 1.404   | 0.985   | 2.003   | 0.060   |
| rs2161592 |           | A/G     | 0.108 | 0.107 | 0.5511 | 1.012   | 0.673   | 1.521   | 0.955   |
| rs3777207 | ELL2      | A/G     | 0.108 | 0.107 | 0.5511 | 1.012   | 0.673   | 1.521   | 0.955   |
| rs6595114 |           | C/T     | 0.118 | 0.097 | 0.5042 | 1.246   | 0.830   | 1.870   | 0.288   |
| rs2193963 |           | C/T     | 0.106 | 0.095 | 1     | 1.130   | 0.742   | 1.721   | 0.568   |
| rs17652561 | SLC6A7    | A/G     | 0.145 | 0.151 | 0.6541 | 0.949   | 0.663   | 1.357   | 0.773   |
| rs2937582 |           | A/G     | 0.439 | 0.434 | 0.6413 | 1.021   | 0.791   | 1.318   | 0.871   |
| rs2984100 |           | C/G     | 0.184 | 0.156 | 0.2747 | 1.220   | 0.873   | 1.707   | 0.244   |
| rs7762263 |           | T/C     | 0.111 | 0.123 | 0.594  | 0.883   | 0.595   | 1.312   | 0.539   |
| rs4715938 |           | G/C     | 0.161 | 0.155 | 0.3873 | 1.048   | 0.741   | 1.481   | 0.792   |
| rs7225050 |           | C/T     | 0.249 | 0.243 | 0.04164 | 1.029   | 0.767   | 1.381   | 0.849   |
| rs1419722 | EIF3B     | C/T     | 0.143 | 0.149 | 0.648  | 0.958   | 0.669   | 1.372   | 0.816   |
| rs10178082 |           | T/A     | 0.141 | 0.161 | 0.0001595 | 0.852   | 0.597   | 1.216   | 0.378   |
| rs4947936 |           | C/A     | 0.163 | 0.150 | 0.6502 | 1.109   | 0.784   | 1.569   | 0.559   |
| rs12226072 |           | A/T     | 0.317 | 0.340 | 0.7994 | 0.897   | 0.685   | 1.175   | 0.431   |
| rs2956956 | DLGAP2    | C/T     | 0.078 | 0.092 | 0.7288 | 0.833   | 0.527   | 1.317   | 0.433   |
| rs2989508 |           | C/T     | 0.146 | 0.135 | 1     | 1.097   | 0.763   | 1.576   | 0.619   |
| rs310282  |           | C/A     | 0.096 | 0.135 | 0.3231 | 0.678   | 0.451   | 1.019   | 0.061   |
| rs4573320 |           | C/T     | 0.279 | 0.299 | 0.8912 | 0.907   | 0.685   | 1.200   | 0.493   |
| SNP          | Gene   | Allele | Minor Allele Frequency | Minor Allele Frequency (Control) | Minor Allele Frequency (Case) | Odds Ratio (Case/Control) | 95% CI (Case) | 95% CI (Control) |
|--------------|--------|--------|------------------------|----------------------------------|-------------------------------|--------------------------|--------------|------------------|
| rs1568828    | PREX2  | A/G    | 0.108                  | 0.109                            | 0.3729                        | 0.995                    | 0.662        | 1.494            |
| rs1006698    | KCNV2  | T/G    | 0.216                  | 0.263                            | 0.2387                        | 0.772                    | 0.572        | 1.041            |
| rs1011531    | A/G    | 0.118  | 0.120                  | 0.981                            | 0.664                         | 1.450                    | 0.925        |                  |
| rs13289064   | C/G    | 0.231  | 0.183                  | 1.346                            | 0.986                         | 1.837                    | 0.060        |                  |
| rs10984811   | ANP32B | C/A    | 0.178                  | 0.148                            | 0.6479                        | 1.250                    | 0.889        | 1.757            |
| rs12554842   | COL5A1 | T/C    | 0.095                  | 0.109                            | 0.2273                        | 0.867                    | 0.569        | 1.320            |
| rs11593009   | T/A    | 0.078  | 0.076                  | 0.6833                           | 1.032                         | 0.642                    | 1.658        | 0.896            |
| rs12243354   | TET1   | A/G    | 0.138                  | 0.137                            | 0.809                         | 1.014                    | 0.703        | 1.464            |
| rs7923700    | GRID1  | G/A    | 0.116                  | 0.117                            | 0.399                         | 0.988                    | 0.666        | 1.467            |
| rs2239153    | VWF    | C/T    | 0.415                  | 0.428                            | 0.4145                        | 0.948                    | 0.734        | 1.224            |
| rs7303062    | FAM70B | A/T    | 0.050                  | 0.061                            | 0.6129                        | 0.817                    | 0.467        | 1.428            |
| rs17435983   | A/G    | 0.143  | 0.128                  | 0.7988                           | 1.136                         | 0.786                    | 1.640        | 0.497            |
| rs8007744    | G/A    | 0.261  | 0.267                  | 0.7692                           | 0.973                         | 0.729                    | 1.299        | 0.852            |
| rs17777329   | G/A    | 0.085  | 0.049                  | 0.5287                           | 1.800                         | 1.083                    | 2.991        | 0.022*           |
| rs4787426    | IL4R   | G/T    | 0.083                  | 0.066                            | 1.284                         | 0.795                    | 2.073        | 0.306            |
| rs1075355    | VAT1L  | C/G    | 0.131                  | 0.092                            | 0.1553                        | 1.481                    | 0.992        | 2.212            |
| rs12931468   | ATP2C2 | G/C    | 0.055                  | 0.044                            | 1.259                         | 0.707                    | 2.244        | 0.434            |
| rs8067836    | LASP1  | G/T    | 0.111                  | 0.082                            | 0.7057                        | 1.387                    | 0.906        | 2.125            |
| rs16955841   | HLF    | G/A    | 0.133                  | 0.109                            | 0.1143                        | 1.255                    | 0.830        | 1.898            |
| rs12450240   | NARF   | T/G    | 0.242                  | 0.281                            | 0.8872                        | 0.818                    | 0.612        | 1.093            |
| rs9961715    | DLGAP1 | C/T    | 0.055                  | 0.041                            | 0.4021                        | 1.364                    | 0.758        | 2.455            |
| rs12606093   | KIAA0427 | C/A | 0.063 | 0.066 | 0.3739 | 0.952 | 0.568 | 1.595 | 0.851 |
| rs6074799    | MACRON2 | G/C    | 0.113                  | 0.140                            | 0.2313                        | 0.784                    | 0.533        | 1.154            |
| rs9617661    | TUBA8  | G/T    | 0.050                  | 0.033                            | 1.556                         | 0.826                    | 2.930        | 0.168            |
| rs5758913    | TUBA8  | C/T    | 0.156                  | 0.151                            | 0.6541                        | 1.035                    | 0.729        | 1.469            |

Notes: a Minor allele; *p value ≤ 0.05 indicates statistical significance; site with HWE p ≤ 0.05 is excluded; Abbreviations: HWE, Hardy-Weinberg Equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism; ORs, odds ratios; CI, confidence interval.

**Figure 3: QQ plot for the whole SNPs for the first stage.**
Table 4: Logistic regression analysis of the associations between SNPs and HAPE risk

| SNP         | Model           | Genotype | Controls | Cases | OR (95 % CI) | P-value | AIC   | BIC   |
|-------------|-----------------|----------|----------|-------|--------------|---------|-------|-------|
| rs7677143   | Co-dominant     | T/T      | 199 (65.5%) | 145 (72.9%) | 1            | 0.11    | 661.6 | 682.7 |
|             | T/C             | 94 (30.9%) | 51 (25.6%) | 0.72 (0.48–1.09) | 0.062 | 660.6 | 677.5 |
|             | C/C             | 11 (3.6%)  | 3 (1.5%)  | 0.38 (0.10–1.40) | 0.16 | 662.1 | 678.9 |
|             | Dominant        | T/T      | 199 (65.5%) | 145 (72.9%) | 1            | 0.062 | 660.6 | 677.5 |
|             | T/C-C/C         | 105 (34.5%) | 54 (27.1%) | 0.69 (0.46–1.02) | 0.16 | 662.1 | 678.9 |
|             | Recessive       | T/T-T/C  | 293 (96.4%) | 196 (98.5%) | 1            | 0.062 | 660.6 | 677.5 |
|             | C/C             | 11 (3.6%)  | 3 (1.5%)  | 0.41 (0.11–1.53) | 0.16 | 662.1 | 678.9 |
|             | Over-dominant   | T/T-C/C  | 210 (69.1%) | 148 (74.4%) | 1            | 0.062 | 660.6 | 677.5 |
|             | T/C             | 94 (30.9%) | 51 (25.6%) | 0.75 (0.50–1.12) | 0.039 | 659.8 | 676.7 |
|             | Log-additive    | —        | —        | —        | 0.69 (0.48–0.99) | 0.039 | 659.8 | 676.7 |
| rs12226072  | Co-dominant     | T/T      | 131 (43.1%) | 103 (51.8%) | 1            | 0.017  | 657.9 | 679   |
|             | A/T             | 139 (45.7%) | 66 (33.2%) | 0.61 (0.41–0.90) | 0.017 | 657.9 | 679   |
|             | A/A             | 34 (11.2%)  | 30 (15.1%) | 1.19 (0.67–2.09) | 0.017 | 657.9 | 679   |
|             | Dominant        | T/T      | 131 (43.1%) | 103 (51.8%) | 1            | 0.077  | 660.9 | 677.8 |
|             | A/T-A/A         | 173 (56.9%) | 96 (48.2%) | 0.72 (0.50–1.04) | 0.077 | 660.9 | 677.8 |
|             | Recessive       | T/T-T/A  | 270 (88.8%) | 169 (84.9%) | 1            | 0.077  | 660.9 | 677.8 |
|             | A/A             | 34 (11.2%)  | 30 (15.1%) | 1.48 (0.86–2.54) | 0.077 | 660.9 | 677.8 |
|             | Over-dominant   | T/T-A/T  | 165 (54.3%) | 133 (66.8%) | 1            | 0.053  | 656.3 | 673.2 |
|             | A/T             | 139 (45.7%) | 66 (33.2%) | 0.92 (0.71–1.20) | 0.053 | 656.3 | 673.2 |
|             | Log-additive    | —        | —        | —        | 0.58 (0.40–0.86) | 0.053 | 656.3 | 673.2 |
| rs6074799   | Co-dominant     | C/C      | 222 (73%)  | 159 (79.9%) | 1            | 0.03   | 659   | 680.1 |
|             | C/G             | 79 (26%)  | 35 (17.6%) | 0.60 (0.38–0.95) | 0.03 | 659   | 680.1 |
|             | G/G             | 3 (1%)    | 5 (2.5%)  | 2.57 (0.59–11.13) | 0.03 | 659   | 680.1 |
|             | Dominant        | C/C      | 222 (73%)  | 159 (79.9%) | 1            | 0.068  | 660.7 | 677.6 |
|             | C/G-G/G         | 82 (27%)  | 40 (20.1%) | 0.67 (0.43–1.04) | 0.068 | 660.7 | 677.6 |
|             | Recessive       | C/C-C/G  | 301 (99%)  | 194 (97.5%) | 1            | 0.15   | 662   | 678.9 |
|             | G/G             | 3 (1%)    | 5 (2.5%)  | 2.88 (0.67–12.40) | 0.15 | 662   | 678.9 |
|             | Over-dominant   | C/C-G/G  | 225 (74%)  | 164 (82.4%) | 1            | 0.02   | 658.7 | 675.6 |
|             | C/G             | 79 (26%)  | 35 (17.6%) | 0.59 (0.37–0.93) | 0.02 | 658.7 | 675.6 |
|             | Log-additive    | —        | —        | —        | 0.78 (0.52–1.15) | 0.21 | 662.5 | 679.4 |
| rs41417552  | Co-dominant     | A/A      | 230 (75.7%) | 135 (68.2%) | 1            | 0.057  | 658.3 | 679.4 |
|             | A/G             | 71 (23.4%) | 59 (29.8%) | 1.58 (1.04–2.40) | 0.057 | 658.3 | 679.4 |
|             | G/G             | 3 (1%)    | 4 (2%)    | 2.68 (0.58–12.38) | 0.057 | 658.3 | 679.4 |
|             | Dominant        | A/A      | 230 (75.7%) | 135 (68.2%) | 1            | 0.022  | 656.7 | 673.6 |
|             | A/G-G/G         | 74 (24.3%) | 63 (31.8%) | 1.62 (1.07–2.44) | 0.022 | 656.7 | 673.6 |
|             | Recessive       | A/A-A/G  | 301 (99%)  | 194 (98%)  | 1            | 0.27   | 660.8 | 677.7 |
|             | G/G             | 3 (1%)    | 4 (2%)    | 2.35 (0.51–10.80) | 0.27 | 660.8 | 677.7 |
|             | Over-dominant   | A/A-G/G  | 233 (76.6%) | 139 (70.2%) | 1            | 0.042  | 657.9 | 674.8 |
|             | A/G             | 71 (23.4%) | 59 (29.8%) | 1.54 (1.02–2.34) | 0.042 | 657.9 | 674.8 |
|             | Log-additive    | —        | —        | —        | 1.59 (1.09–2.32) | 0.017 | 656.3 | 673.2 |
| rs10984811  | Co-dominant     | A/A      | 219 (72%)  | 139 (69.8%) | 1            | 0.032  | 659.2 | 680.3 |
|             | C/A             | 80 (26.3%) | 49 (24.6%) | 0.97 (0.64–1.49) | 0.032 | 659.2 | 680.3 |
|             | C/C             | 5 (1.6%)  | 11 (5.5%)  | 3.95 (1.33–11.73) | 0.032 | 659.2 | 680.3 |
modulatory subunits that are electrically silent and cannot form functional homotetramers. These silent subunits form heterotetramers that modulate the properties of other subunits, increasing the functional diversity of channel subfamilies [17]. Voltage-gated K\(^+\) (K\(_V\)) channel activity in pulmonary artery smooth muscle cells (PASMC) is important for the control of apoptosis and proliferation as well as the regulation of membrane potential and pulmonary vascular tone [18]. A previous study demonstrated that KNCV2 contributes to susceptibility to and was considered a genetic modifier of epilepsy [17]. However, the role of KNCV2 in HAPE remains unknown, and additional studies are needed.

The rs1075355 SNP had the strongest association in this study. It is located in the intron of the VAT1L gene and encodes a vesicle amine transport 1 homologue; its cellular localization and functions have not yet been researched. An association study suggested that a locus on chromosome 16q23-24 (including VAT1L) affected HDLC levels in two independent French-Canadian populations [19]. Additionally, a genome-wide association study of the rate of cognitive decline in Alzheimer’s disease indicated that rs9934540 genetic variants in the VAT1L gene intron were positively associated with the development of Alzheimer’s disease [20]. Two different genes, ADAMTS18 and WWOX, are adjacent to the rs1075355 SNP. ADAMTS18 is a member of the ADAMTS protease family, which is comprised of complex secreted enzymes containing a prometalloprotease domain attached to an ancillary domain with a highly-conserved structure including at least one thrombospondin type 1 repeat. Known functions of ADAMTS proteases include processing procollagens and von Willebrand factor and catabolism of aggrecan, versican, and brevican. ADAMTS also play important roles in connective tissue organization,
coagulation, inflammation, arthritis, angiogenesis, and cell migration [21, 22] and are regulated by the Tissue Inhibitor of Metalloproteinase 3 Gene (TIMP3). Furthermore, Kobayashi et al.’s study in a Japanese population demonstrated that TIMP3 was associated with susceptibility to HAPE [23]. TIMPs play a crucial role in the physiological turnover of the extracellular matrix (ECM) by tightly regulating matrix metalloproteinase (MMP) activity [24]. TIMP3 is the only TIMP that binds tightly to the ECM, and the balance between MMPs and TIMPs plays an important role in maintaining the integrity of healthy tissues. Disturbances of the TIMP/MMP system are implicated in various pathologic conditions in lungs, including pulmonary inflammation, edema, emphysema, and fibrosis, where loss of ECM integrity is a principal feature [25]. Our findings together with those of previous studies demonstrate that the balance between MMPs and TIMPs plays an important role in HAPE pathogenesis.

The human WWOX gene encodes a putative tumor suppressor WW domain-containing oxidoreductase WOX1.

Table 5: Go and pathway analysis of the top genes of GWAS

| Function                                      | p-value     | Adjusted p-value | Genes                              |
|-----------------------------------------------|-------------|------------------|------------------------------------|
| zinc ion binding                              | 7.99E-07    | 1.60E-06         | ADAMTS18; VAT1L                    |
| protein binding                               | 1.59E-05    | 1.06E-05         | INADL; KCNV2                       |
| thyroxine 5-deiodinase activity               | 7.56E-05    | 3.36E-05         | DIO3                               |
| very-low-density lipoprotein receptor activity| 2.27E-04    | 6.52E-05         | VLDLR                              |
| thyroxine 5'-deiodinase activity              | 2.27E-04    | 6.52E-05         | DIO3                               |
| metal ion binding                             | 2.45E-04    | 6.52E-05         | ADAMTS18                           |
| low density lipoprotein receptor activity     | 8.31E-04    | 1.45E-04         | VLDLR                              |
| peptidase activity                            | 8.69E-04    | 1.45E-04         | ADAMTS18                           |
| oxidoreductase activity                       | 0.001153    | 1.58E-04         | WWOX                               |
| selenium binding                              | 0.002265    | 2.01E-04         | DIO3                               |
| ATP binding                                   | 0.004538    | 3.70E-04         | CCT5                               |
| voltage-gated potassium channel activity      | 0.007231    | 5.26E-04         | KCNV2                              |
| metalloendopeptidase activity                 | 0.007756    | 5.35E-04         | ADAMTS18                           |
| unfolded protein binding                      | 0.008356    | 5.52E-04         | CCT5                               |
| potassium ion binding                         | 0.00948     | 6.12E-04         | KCNV2                              |
| nucleotide binding                            | 0.010312    | 6.34E-04         | CCT5                               |
| manganese ion binding                         | 0.011276    | 6.63E-04         | NUDT7                              |
| coenzyme binding                              | 0.0115      | 6.67E-04         | WWOX                               |
| hydrolase activity                            | 0.012359    | 6.83E-04         | NUDT7                              |
| voltage-gated ion channel activity            | 0.013964    | 7.35E-04         | KCNV2                              |
| protein dimerization activity                 | 0.031265    | 0.001374         | WWOX                               |
| magnesium ion binding                         | 0.032144    | 0.001398         | NUDT7                              |
| hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides | 0.053236 | 0.002117 | NUDT7 |
| calcium ion binding                           | 0.06698     | 0.002528         | VLDLR                              |
| receptor activity                             | 0.121001    | 0.00436          | VLDLR                              |

| Pathways                                      | p-value     | Adjusted p-value | Genes      |
|-----------------------------------------------|-------------|------------------|------------|
| 1,4-Dichlorobenzene degradation               | 0.000371    | 0.000742         | CMBL       |
| gamma-Hexachlorocyclohexane degradation       | 0.006661    | 0.001665         | CMBL       |
| Maturity onset diabetes of the young          | 0.008871    | 0.001971         | IAPP       |
| Tight junction                                | 0.049301    | 0.002641         | INADL      |
| Wnt signaling pathway                         | 0.054948    | 0.002641         | PPP2R5C    |
(also known as WWOX or FOR). High frequencies of loss of heterozygosity (LOH) in this gene have been observed in prostate, lung, breast, and other cancers [27]. A recent genome-wide association analysis identified WWOX as one of the loci associated with forced vital capacity (FVC), a spirometric measure of pulmonary function used to diagnose and monitor lung diseases [27]. These findings indicate that the WWOX gene may be involved in lung development and the pathogenesis of restrictive lung disease; future studies are needed to determine whether WWOX is similarly associated with HAPE pathogenesis.

Although the statistical power of the present study was sufficient, some limitations should be considered when interpreting these results. First, the patient sample sizes were relatively small, and the association between the identified polymorphisms and HAPE susceptibility should be confirmed in future studies with larger sample sizes. Secondly, the mechanisms by which the potential candidate genes contribute to the pathogenesis of HAPE remain unclear, and functional studies of these candidate genes are needed. In conclusion, our study provides new evidence regarding the pathogenesis of HAPE in the Han Chinese population. Although the genetic factors that contribute to the development of HAPE remain largely unknown, we identified candidate genes that contribute to HAPE susceptibility. However, polymorphisms in these genes should be examined further before definitive conclusions regarding their role in HAPE pathogenesis can be made.

MATERIALS AND METHODS

Study populations

In this two-stage case-control study, we evaluated associations between genetic variants across the human genome and the risk of HAPE. All participants included in the study were from the Han Chinese population. Study subjects for both GWAS scan of HAPE and the replication phase of the experiment were selected according to detailed inclusion and exclusion criteria. Briefly, patients who lived on the Tibet Plateau and were diagnosed with HAPE were recruited from the General Hospital of Tibet Military Region. Control subjects were Han Chinese immigrants living in Lhasa, Tibet, and their medical histories and physical examinations confirmed that they were in good health. Demographic information was collected through interviews using a standard questionnaire. Ultimately, 267 HAPE cases (89 recurrent HAPE cases; mean age 32.6 ± 10.7 years) and 304 controls (mean age 36.2 ± 4.5 years) were selected for the study. Two mL of venous blood were collected from each individual into tubes containing 2% EDTA-K2, centrifuged, and stored at -80°C until analysis. DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini kit (Qiagen), and DNA concentrations were measured using a NanoDrop 2000. Informed consent was obtained from all subjects, and the Human Ethics Committee of our institute approved the investigation.

Study design

For the GWAS scan experiment, we scanned DNA samples from 68 patients with recurrent HAPE using Affymetrix SNP Array 6.0 Chips. The allele frequencies of the 68 patients were then compared to those of 84 HapMap CHB subjects to identify significant differences in SNP frequencies. In the replication experiment, associations between the SNPs identified in the GWAS scan and risk of HAPE where examined in 199 HAPE patients and 304 unrelated healthy controls. Furthermore, to identify candidate genes that might underlie HAPE susceptibility, we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for the genes involved in the associated genetic loci.

Quality control (QC) in GWAS

A total of 906,660 SNPs were genotyped in 68 patients with recurrent HAPE during the GWAS experiment using Affymetrix Genome-Wide Human SNP Array 6.0 Chips as described previously [28]. A systematic quality control (QC) procedure was applied to both SNPs and samples prior to the association analysis. SNPs were excluded if they (i) did not map onto autosomal chromosomes; (ii) had a call rate of less than 95%; (iii) had a minor allele frequency (MAF) less than 0.05; or (iv) deviated from Hardy-Weinberg equilibrium ($p < 0.001$). Sixty-eight HAPE cases and 84 controls with 502,689 SNPs remained after QC.

SNP selection and genotyping in the replication study

After genome-wide association analysis, we compared the allele frequencies of the 502,689 SNPs in the 68 recurrent HAPE cases to those in the 84 HapMap CHB controls using a chi-squared ($\chi^2$) test. Allele frequencies differed significantly between HAPE cases and controls for 77 SNPs. In the replication study, these 77 SNPs were genotyped in 199 HAPE patients and 304 normal controls. SNPs that were significantly associated with HAPE risk ($p < 0.05$) in the replication study were selected for GO and KEGG pathway enrichment analyses. Genotyping was performed using Sequenom MassARRAY Assay Design 3.0 Software [29] with a genotype success rate greater than 97.3%.

Statistical analysis

SPSS 17.0 statistical software was used for statistical analysis. An exact test was used to test the departure of each SNP frequency from Hardy–Weinberg equilibrium.
(HWE) in control subjects. Differences in SNP genotype distribution between HAPE patients and controls were compared using a χ² test [30]. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using unconditional logistic regression analysis with adjustments for age and gender [31]. All p values presented in this study are two-sided; p < 0.05 indicated a statistically significant difference.

Associations between SNPs and HAPE risk were tested using various genetic models (co-dominant, dominant, over-dominant, recessive, and log-additive) and analyzed using SNP Stats software (obtained from http://bioinfo.iconcologia.net, Catalan Institute of Oncology, Barcelona, Spain). To reduce population stratification, a multidimensional scaling approach (MDS) was used and a QQ-plot was generated using PLINK software (version 1.07) (http://www.cog-genomics.org/plink2/) [32]. R software (version 2.11.1) was used for statistical analysis and to generate plots, including Manhattan plots. GO analysis were performed using Bingo software [33], and pathway enrichment analyses were performed using Mas 3.0 software (http://bioinfo.capitalbio.com/mas3/).

Authors’ contributions

Not applicable.

ACKNOWLEDGMENTS

We are grateful to all of the patients and other individuals who made this work possible. We would also like to thank the clinicians and hospital staff who contributed to data collection for this study.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

FUNDING

This work was supported by the Science and Technology Project of Tibet Autonomous Region (2009Z-3) and by a general financial grant from the China Postdoctoral Science Foundation (2012M512186).

REFERENCES

1. Shrestha P, Pun M, Basnyat B. High altitude pulmonary edema (HAPE) in a Himalayan trekker: a case report. Extreme physiology & medicine. 2014; 3:6.
2. Maggiorini M. Prevention and treatment of high-altitude pulmonary edema.Prog Cardiovasc Dis. 2010; 52:500–506.
3. Sartori C, Allemann Y, Trueb L, Lepori M, Maggiorini M, Nicod P, Scherrer U. Exaggerated pulmonary hypertension is not sufficient to trigger high-altitude pulmonary oedema in humans. Schweiz Med Wschr. 2000; 130:385–389.
4. West JB, Colice GL, Lee YJ, Namba Y, Kurtdak SS, Fu Z, Ou LC, Mathieu-Costelo O. Pathogenesis of high-altitude pulmonary oedema: direct evidence of stress failure of pulmonary capillaries. Eur Respir J. 1995; 8:523–529.
5. Bartsch P. High altitude pulmonary edema. Respiration; international review of thoracic diseases. 1997; 64:435–443.
6. Sartori C, Trueb L, Scherrer U. High-altitude pulmonary edema. Mechanisms and management. Cardiologia (Rome, Italy). 1997; 42:559–567.
7. Bartsch P, Maggiorini M, Ritter M, Noti C, Vock P, Oelz O. Prevention of high-altitude pulmonary edema by nifedipine. New Engl J Med. 1991; 325:1284–1289.
8. Zhang D, Zhou Q, Yang J. Epidemic Characteristics of Acute High Altitude Pulmonary Edema in High Altitude Areas. West China Medical Journal. 2001; 16:316–317.
9. Bhagi S, Srivastava S, Singh SB. High-altitude pulmonary edema: review. Int J Occup Med Env. 2014; 56:235–243.
10. Mortimer H, Patel S, Peacock AJ. The genetic basis of high-altitude pulmonary oedema. Pharmacology & therapeutics. 2004; 101:183–192.
11. Stobdan T, Kumar R, Mohammad G, Thinlas T, Norboo T, Iqbal M, Pasha MA. Probable role of beta2-adrenergic receptor gene haplotype in high-altitude pulmonary oedema. Respirology (Carlton, Vic). 2010; 15:651–658.
12. Hanaoka M, Droma Y, Ota M, Ito M, Katsuyama Y, Kubo K. Polymorphisms of human vascular endothelial growth factor gene in high-altitude pulmonary oedema susceptible subjects. Respirology (Carlton, Vic). 2009; 14:46–52.
13. Hotta J, Hanaoka M, Droma Y, Katsuyama Y, Ota M, Kobayashi T. Polymorphisms of renin-angiotensin system genes with high-altitude pulmonary edema in Japanese subjects. Chest. 2004; 126:825–830.
14. Saxena S, Kumar R, Madan T, Gupta V, Muralidhar K, Sarma PU. Association of polymorphisms in pulmonary surfactant protein A1 and A2 genes with high-altitude pulmonary edema. Chest. 2005; 128:1611–1619.
15. Chen Z, Leibiger I, Katz AI, Bertorello AM. Pals-associated tight junction protein functionally links dopamine and angiotensin II to the regulation of sodium transport in renal epithelial cells. Brit J Pharmacol. 2009; 158:486–493.
16. Shin K, Straight S, Margolis B. PATJ regulates tight junction function and polarity in mammalian epithelial cells. J Cell Biol. 2005; 168:705–711.
17. Jorge BS, Campbell CM, Miller AR, Rutter ED, Gurnett CA, Vanyoe CG, George AL Jr, Kearney JA. Voltage-gated potassium channel KCNV2 (Kv8.2) contributes to epilepsy susceptibility. Proc Natl Acad Sci U S A. 2011; 108:5443–5448.
18. Fantozzi I, Platoshyn O, Wong AH, Zhang S, Remillard CV, Furtado MR, Petrauskene OV, Yuan JX. Bone morphogenetic protein-2 upregulates expression and function of voltage-gated K+ channels in human pulmonary artery smooth muscle cells. Am J Physiol-Lung C. 2006; 291:L993–1004.
19. Dastani Z, Pajukanta P, Marcil M, Rudzicz N, Ruel I, Bailey SD, Lee JC, Lemire M, Faith J, Platko J, Rioux J, Hudson TJ, Gaudet D, et al. Fine mapping and association studies of a high-density lipoprotein cholesterol linkage region on chromosome 16 in French-Canadian subjects. Eur J Hum Genet. 2010; 18:342–347.

20. Sherva R, Tripodis Y, Bennett DA, Chibnik LB, Crane PK, de Jager PL, Farrer LA, Saykin AJ, Shulman JM, Naj A, Green RC. Genome-wide association study of the rate of cognitive decline in Alzheimer’s disease. Alzheimer’s & dementia. 2014; 10:45–52.

21. Cal S, Obaya AJ, Llamazares M, Garabaya C, Quesada V, Lopez-Otin C. Cloning, expression analysis, and structural characterization of seven novel human ADAMTSs, a family of metalloproteinases with disintegrin and thrombospondin-1 domains. Gene. 2002; 283:49–62.

22. Apte SS. A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motifs: the ADAMTS family. Int J Biochem Cell B. 2004; 36:981–985.

23. Kobayashi N, Hanaoka M, Droma Y, Ito M, Katsuyama Y, Kubo K, Ota M. Polymorphisms of the tissue inhibitor of metalloproteinase 3 gene are associated with resistance to high-altitude pulmonary edema (HAPE) in a Japanese population: a case control study using polymorphic microsatellite markers. PLoS One. 2013; 8:e71993.

24. Loffek S, Schilling O, Franzke CW. Series “matrix metalloproteinases in lung health and disease”: Biological role of matrix metalloproteinases: a critical balance. Eur Respir J. 2011; 38:191–208.

25. Clark IM, Swingler TE, Sampieri CL, Edwards DR. The regulation of matrix metalloproteinases and their inhibitors. Int J Biochem Cell B. 2008; 40:1362–1378.

26. Chang NS, Doherty J, Ensign A, Lewis J, Heath J, Schultz L, Chen ST, Oppermann U. Molecular mechanisms underlying WOX1 activation during apoptotic and stress responses. Biochem Pharmacol. 2003; 66:1347–1354.

27. Loth DW, Artigas MS, Gharib SA, Wain LV, Franceschini N, Koch B, Pottinger TD, Smith AV, Duan Q, Oldmadow C, Lee MK, Strachan DP, James AL, et al. Genome-wide association analysis identifies six new loci associated with forced vital capacity. Nat Genet. 2014; 46:669–677.

28. Hu Z, Wu C, Shi Y, Guo H, Zhao X, Yin Z, Yang L, Dai J, Hu L, Tan W, Li Z, Deng Q, Wang J, et al. A genome-wide association study identifies two new lung cancer susceptibility loci at 13q12.12 and 22q12.2 in Han Chinese. Nat Genet. 2011; 43:792–796.

29. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Current protocols in human genetics. 2009; Chapter 2:Unit 2.12.

30. Adamec C. [Example of the use of the nonparametric test X2 for comparison of 2 independent examples]. [Article in Czech]. Ceskoslovenske zdravotnictvi. 1964; 12: 613–619.

31. Bland JM, Altman DG. The odds ratio. Bmj. 2000; 320:1468.

32. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575.

33. Liu M, Wu B, Wang WZ, Lee LM, Zhang SH, Kong LZ. Stroke in China: epidemiology, prevention, and management strategies. Lancet Neurol. 2007; 6:456–464.