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# Association between genetic polymorphism of telomere-associated gene *ACYP2* and the risk of HAPE among the Chinese Han population

## A Case–control study

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**Abstract**

High-altitude pulmonary edema (HAPE) is a hypoxia-induced, life-threatening, pulmonary edema, which is characterized by exaggerated pulmonary hypertension caused by stress failure. *ACYP2* was found to associated with telomere length, the aim of this study was to identify whether *ACYP2* polymorphisms increase or decrease HAPE risk in the Chinese Han individuals.

In present study, we have genotyped 7 single-nucleotide polymorphisms (SNPs) in *ACYP2* to determine the haplotypes in a case–control study with 265 HAPE patients and 303 healthy individuals. Genotypes were determined using the Sequenom MassARRAY method. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression with adjustment for gender and age. We found 3 SNPs yielded significant evidence for association with HAPE risk which had not been investigated before. Rs6713088 was found to have a 1.85- and 1.30-fold increased risk of HAPE in the recessive and additive model. The GT of rs843752 also conferred an increased risk of HAPE (GT/TT: OR = 1.51, 95% CI: 1.05–2.16, $P = 0.026$) and the genotype frequency distributions of rs843752 had significant difference between cases and controls. The CC genotype of rs17045754 had a protect effect on HAPE patients, and it was found to have a 0.29-fold reduced risk of HAPE in the recessive model.

Although additional, larger population-based studies are needed to confirm these findings, our study shed light on the association between ACYP2 variant and HAPE risk in Han Chinese population for the first time.

**Abbreviations:** CI = confidence interval, HAPE = high-altitude pulmonary edema, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, SNP = single-nucleotide polymorphism.

**Keywords:** *ACYP2*, association, genetic polymorphism, HAPE, telomere length

## 1. Introduction

High-altitude pulmonary edema (HAPE) is one of idiopathic mountain sickness that may develop in otherwise healthy individuals when first quickly ascended and exposed to altitude above 2500 m.[1] It is a hypoxia-induced pulmonary edema which is characterized by exaggerated pulmonary hypertension caused by stress failure.[2] It is considered as a life-threatening respiratory disease because it occurs and processes quickly, and it can develop to coma even death in a short time if it cannot be treated in time. However, the exact mechanisms underlying the pathogenesis of HAPE are still unclear. Hotta et al[3] revealed that HAPE is the result of a combination of genetic and environmental factors, besides the environment susceptive factors are influenced by genetic factors. Meanwhile, several genetic studies have demonstrated that a genetic susceptibility may play a role in the development of HAPE.[4,5] So, the genetic factors are crucial in the study of HAPE.

Hypoxia-induced was one of the important environmental factors for HAPE, which would influence the quantity of liquid escaping from the pulmonary vasculature. Some recent plateau researches make hypoxia act as a kind of optional factors to explore the telomere length changes, which gradually attracted the scholars' interest. Wang et al[6] reported that the telomeres were significantly elongated in a mildly rather than highly hypoxic environment, and that the expression of TERT and HIF-1α changed in a similar manner as the leukocyte telomere length

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**Table 1**

**Characteristics of cases and controls in this study.**

| Variable | Case (n = 265) | Control (n = 303) | *P* |
|---|---|---|---|
| Sex | | | 0.102* |
| Male | 244 (92.1) | 289 (95.4) | |
| Female | 21 (7.9) | 14 (4.6) | |
| Age, year (mean ± SD) | 32.59 ± 10.756 | 36.18 ± 4.474 | <0.001† |

$P \leq 0.05$ indicates statistical significance.
* *P* was calculated by Pearson $\chi^2$ test.
† *P* was calculated by Welch *t* test.

under the hypoxic conditions in their Wistar rats study. Recent genome-wide association study had found that *ACYP2* gene had association with telomere length.[7] To our knowledge, the study about *ACYP2* which can influence the length of telomere was relative less. Du et al[8] revealed the association between rs11125529 polymorphism of ACYP2 and lung cancer, and Ding et al[9] investigated the rs11125529 polymorphism of ACYP2 associated with coronary heart disease in a Chinese population. But neither of them had significant findings. Therefore, 7 tag single-nucleotide polymorphisms (SNPs) (rs6713088, rs12621038, rs1682111, rs843752, rs10439478, rs17045754, and rs843720) located in intron regions of *ACYP2* on 2p16.2 were stochastically selected for our study in an attempt to investigate the association between HAPE and 7 SNPs polymorphism in the *ACYP2*. The study sheds light on the association between HAPE risk and *ACYP2* genetic polymorphism in the Chinese population.

## 2. Materials and methods

### 2.1. Study participants

The characteristics of the HAPE patients and controls in our study are shown in Table 1. A number of 265 patients were recruited between January 2011 and January 2016 at the Affiliated Hospital of Xizang Minzu University. Our study protocol was reviewed and approved by the Ethics Committee of Xizang Minzu University. HAPE diagnosis was based on standard criteria (Hultgren and Marticoremna, 1978), including cough, dyspnea, cyanosis at rest, absence of infection, and the presence of pulmonary rales. We recruited a total of 303 healthy unrelated samples from the individuals seeking health care in the outpatient departments at the hospital. All of the chosen subjects were Han Chinese and residents living in Northwest China.

The cases (244 female, 21 male) and controls (289 female, 14 male) were well matched by sex ($P = 0.102$). All HAPE and control subjects were unrelated to each other and permitted to rest after rapidly inducted to high altitude (Tibet altitude: 4000–5000 m). Each subject we recruited in present study was healthy people without any previous history of cancers, cardiopulmonary, and infectious diseases or any other genetic diseases. They did not use prophylactic medications, and the rate and altitude of ascent are almost similar among the cases and controls groups. Controls were unrelated to each other and had no HAPE or related diseases after exposure to high altitude (≥4000 m) within 7 days. Those with reported mtDNA-related diseases (such as diabetes, Parkinson disease, and Alzheimer disease) in their medical or family histories were excluded. All of the participants were agreed to the informed consent and interviewed using a self-administered questionnaire includes a complete medical history, demographic data, and physical condition. We collected blood samples from all participants after a period of 72 hours of exposure to high-altitude conditions (≥4000 m), and the use of samples were approved by the Human Research Committee of the Affiliated Hospital of Xizang Minzu University for Approval of Research Involving Human Subjects.

### 2.2. SNPs selection and genotyping

Blood samples were collected in EDTA tubes and stored at −80° C after centrifugation by 2000 rpm in 10 minutes. GoldMag extraction method (GoldMag Co Ltd, Xi'an, China) was used to extract genomic DNA from whole blood. Seven tag SNPs (rs6713088, rs12621038, rs1682111, rs843752, rs10439478, rs17045754, and rs843720) were selected for our study, and these SNPs were with minor allele frequencies >5% in the HapMap Chinese Han Beijing population (http://www.hapmap.org). All selected polymorphisms are located in intron regions. Sequenom MassARRAY Assay Design 3.0 Software (Sequenom, Inc, San Diego, CA) was used to design a Multiplexed SNP MassEXTEND assay. Sequenom MassARRAY RS1000 was utilized to perform the SNP genotyping according to the manufacturer's protocol.[10] Primers of PCR which were used for each SNP in our study are listed in Table 2. Sequenom Typer 4.0 Software (Sequenom, Inc) was used for data analyses.[11,12]

### 2.3. Statistical analyses

Allele and genotype frequencies of ACYP2 polymorphisms were obtained by direct counts. The genotype frequencies of each SNP in the control subjects were checked using the Hardy–Weinberg equilibrium (HWE) before analysis. Chi-squared test/Fisher exact test was used to calculate the allele and genotype frequencies of cases and controls. The statistical power of the case–control study was calculated using Power and Sample Size Calculation software (available on line: http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Associations between the genotypes of the ACYP2 polymorphisms and the risk of HAPE were evaluated by

**Table 2**

**Primers used for this study.**

| SNP_ID | 1st-PCRP | 2nd-PCRP | UEP_SEQ |
|---|---|---|---|
| rs6713088 | ACGTTGGATGACACACACAGACTCCTTCAC | ACGTTGGATGGTCACCAAAACACGTAATG | gaggcCAGAATGGTCCACTAGAGA |
| rs12621038 | ACGTTGGATGATTGTGCTAGGCACTTTAGG | ACGTTGGATGGGCATAAGTTTTATTGCCTC | ccATTGCCTCAGCTAGACT |
| rs1682111 | ACGTTGGATGGAATTGCTGGGTTATTTGGC | ACGTTGGATGGCCAGTGGGAATGCAAAATG | tgtcATGCAAAATGAAACAGACACTT |
| rs843752 | ACGTTGGATGTCCTCTTTTCAGAAACCTGC | ACGTTGGATGGAGACAACATAATGGAGGTC | cGAGTTTGGGTTTGAGGT |
| rs10439478 | ACGTTGGATGTAGCACAAGACCTACACTGG | ACGTTGGATGCTACACTCTCCAGAGGAATG | TTGCTGTTTTCCCAGAA |
| rs17045754 | ACGTTGGATGCTGTAAAAGTTCTGGCATGG | ACGTTGGATGGAAATCAGGGATATTAGTGC | caggTATTCAGCTTCCTAGAGTTA |
| rs843720 | ACGTTGGATGCTTCACAACACTCCTGTAAG | ACGTTGGATGAGTCAGAGCTAGACCTCTGG | ccccAATCTGTCTCAGGGTCTT |

**Table 3**

**Comparisons of genotypes of some special SNPs and their associations with HAPE risk.**

| SNP | Genes | Band | Position | Role | Alleles A/B | $P^*$-HWE | OR (95% CI) | $P^\dagger$ |
|---|---|---|---|---|---|---|---|---|
| rs6713088 | *ACYP2* | 2p16.2 | 54345469 | Intron | G/C | 0.075 | 1.22 (0.97–1.55) | 0.095 |
| rs12621038 | *ACYP2* | 2p16.2 | 54391113 | Intron | T/C | 0.646 | 0.84 (0.67–1.07) | 0.152 |
| rs1682111 | *ACYP2* | 2p16.2 | 54427979 | Intron | A/T | 0.778 | 1.10 (0.85–1.42) | 0.457 |
| rs843752 | *ACYP2* | 2p16.2 | 54446587 | Intron | G/T | 0.028 | 1.10 (0.85–1.43) | 0.474 |
| rs10439478 | *ACYP2* | 2p16.2 | 54459450 | Intron | C/A | 0.641 | 0.88 (0.70–1.11) | 0.289 |
| rs17045754 | *ACYP2* | 2p16.2 | 54496757 | Intron | C/G | 0.591 | 0.82 (0.61–1.11) | 0.202 |
| rs843720 | *ACYP2* | 2p16.2 | 54510660 | Intron | G/T | 0.897 | 0.92 (0.72–1.19) | 0.539 |

CI = confidence interval, HAPE = high-altitude pulmonary edema, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, SNP = single-nucleotide polymorphism.

* *P* was calculated by exact test.

† *P* was calculated by Pearson Chi-squared test.

3 genetic models (dominant, recessive, and additive model). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis with an adjustment for age. We determined *P* values for trend by entering the variable as a single term in the model (ie, 1 degree of freedom) and testing using the Wald test. We evaluated the risk in the dominant model (AA + AB vs BB; A represents the minor allele, B represents the major allele), the recessive model (AA vs AB + BB) and the allele model (A vs B) and the additive model (AA/AB vs AB/BB). A *P* value $<0.05$ was considered as achieving the threshold of statistical significance, and all statistical tests were 2 sided. Bonferroni correction was used on our data. All of the statistical analyses were performed with the SPSS 18.0 software for Windows (PASW Statistics, SPSS Inc, Chicago, IL).

## 3. Results

A total of 7 SNPs were analyzed in present study. Chromosomal position, gene, role, the alleles, and HWE test results for all the SNPs are presented in Table 3. All 7 SNPs were in HWE among control subjects ($P > 0.01$). The differences of frequency distributions of alleles between cases and controls were compared by Pearson Chi-squared test, but no significant difference was found.

Next, comparisons of the SNP genotypes were shown in Table 4. First, we compared the genotype frequency distributions of the 2 groups again and found that the genotype frequency distributions of rs843752 among cases had significantly different from controls. Then, using a Wald test by unconditional logistic regression adjusted for age and gender, we found that compared with the CC genotype, the GG and GG + GC genotype of rs6713088 polymorphism of cases were significantly different from the controls (GG/CC: OR = 1.84, 95% CI: 1.10–3.07, $P = 0.020$; GG + GC/CC: $P = 0.029$); compared with the TT genotype, the GT genotype of rs843752 polymorphism of cases were significantly different from the controls (GT/TT: OR = 1.51, 95% CI: 1.05–2.16, $P = 0.026$); compared with the GG genotype, the CC genotype of rs17045754 polymorphism of cases were significantly different from the controls (CC/GG: OR = 0.28, 95% CI: 0.09–0.89, $P = 0.030$) (Table 5).

Furthermore, 3 genetic models (dominant, recessive, and additive) were used to further identify the associations between the SNPs and the HAPE risk. No significant difference was discovered between the 2 groups in the dominant model. However, in the recessive and additive model, rs6713088 was found to be associated with an increased risk of HAPE (OR = 1.85, 95% CI: 1.18–2.91, $P = 0.008$; OR = 1.30, 95% CI: 1.01–1.66, $P = 0.045$, respectively) and the CC genotype of rs17045754 had a significantly reduced risk of HAPE in the recessive model (OR = 0.29, 95% CI: 0.09–0.90, $P = 0.032$).

## 4. Discussion

HAPE is a disorder experienced by unacclimatized sojourners when first quickly ascended and exposed to altitude above 2500 m because of the hypobaric hypoxic condition of high altitude.[13] Epidemiologic studies had suggested that HAPE presents not only a racial family specificity and susceptibility but also an individual susceptibility. The HAPE incidence of local Tibetans were lower than those Han migrants, and only 21 HAPE cases of 3184 cases

**Table 4**

**Comparisons of genotypes of some special SNPs and their associations with HAPE risk.**

| | Genotype (Fre) | | Crude | | | Adjusted | | |
|---|---|---|---|---|---|---|---|---|
| SNP_ID | $P^\dagger$ | Test | OR | 95%CI | $P^\ddagger$ | OR | 95%CI | $P^\ddagger$ |
| rs6713088 | 0.073 | GG/CC | 1.64 | 1.00–2.70 | 0.049* | 1.84 | 1.10–3.07 | 0.020* |
| | | GC/CC | 0.99 | 0.68–1.44 | 0.949 | 0.99 | 0.67–1.46 | 0.953 |
| | | (GG + GC)/CC | – | – | 0.076 | – | – | 0.029* |
| rs843752 | 0.039* | GG/TT | 0.79 | 0.42–1.47 | 0.452 | 0.95 | 0.50–1.80 | 0.868 |
| | | GT/TT | 1.47 | 1.04–2.09 | 0.030* | 1.51 | 1.05–2.16 | 0.026* |
| | | (GG + GT)/TT | – | – | 0.040* | – | – | 0.063 |
| rs17045754 | 0.106 | CC/GG | 0.31 | 0.10–0.97 | 0.044* | 0.28 | 0.09–0.89 | 0.030* |
| | | CG/GG | 0.97 | 0.68–1.38 | 0.856 | 0.95 | 0.65–1.37 | 0.774 |
| | | (CC + CG)/GG | – | – | 0.131 | – | – | 0.096 |

* $P \leq 0.05$. CI = confidence interval, HAPE = high-altitude pulmonary edema, OR = odds ratio, SNP = single-nucleotide polymorphism.

† *P* was calculated by Pearson Chi-squared test.

‡ *P* values were calculated by Wald test by unconditional logistic regression adjusted for age and gender.

**Table 5**

**Logistic regression analysis of the association between SNPs and HAPE risk n (%).**

| | Dominant model | | | Recessive model | | | Additive model | | |
|---|---|---|---|---|---|---|---|---|---|
| SNP_ID | OR | 95% CI | *P* | OR | 95% CI | *P* | OR | 95% CI | *P* |
| rs6713088 | 1.16 | 0.80–1.67 | 0.442 | 1.85 | 1.18–2.91 | 0.008* | 1.30 | 1.01–1.66 | 0.045* |
| rs843752 | 1.39 | 0.99–1.96 | 0.059 | 0.79 | 0.43–1.48 | 0.468 | 1.17 | 0.90–1.52 | 0.251 |
| rs17045754 | 0.85 | 0.60–1.22 | 0.392 | 0.29 | 0.09–0.90 | 0.032* | 0.79 | 0.58–1.01 | 0.140 |

*P* values were calculated by Wald test by unconditional logistic regression adjusted for age and gender.
CI = confidence interval, HAPE = high-altitude pulmonary edema, OR = odds ratio, SNP = single-nucleotide polymorphism.
* $P \leq 0.05$.

in the Tibet military region general hospital records of the past 50 years were local Tibetans.[14] These results indicated that the Han migrants were relative easier to have HAPE and this might related to their different genetic background.[4] Observational studies have shown that some genetic SNPs have significantly association with HAPE risk.[13,15–20] In addition, telomere length was significantly influenced by hypoxic exposure.[21] Recent studies have revealed that the telomere length varies considerably with hypoxia levels,[22–24] and the possible molecular mechanisms underlying hypoxia-induced TERT expression, which indicates that TERT expression is upregulated following the induction of HIF-1α during exposure to hypoxia.[25,26] Furthermore, HIF-1α resulted in elongation of telomeres as a key transactivator for the induction of TERT transcription.[27] Simultaneously, some SNPs in *ACYP2* have been identified to be associated with telomere length.[7,9,28,29] Subsequently, we performed a multivariate analysis adjusted for age and gender among HAPE patients and controls to investigate the association between *ACYP2* genetic polymorphism and HAPE risk.

In the present case–control study, we investigated the associations between 7 SNPs in telomere-associated gene *ACYP2* and risk of HAPE. We demonstrated that 3 *ACYP2* genetic polymorphisms are associated with HAPE risk in Chinese Han population. Rs17045754 was associated with protection from HAPE, but rs6713088 and rs843752 increased HAPE susceptibility. To the best of our knowledge, this is the first time to report about a positive association between *ACYP2* genetic polymorphism and HAPE risk, and our result provided new evidence of the relationship between telomere-associated genes and HAPE risk, and may shed light on the etiology of HAPE.

Our study had some limitations. First, the participants in our study were limited to the Han Chinese people. Second, we did not analyze whether predisposing factors, including cold, drug, alcohol consumption, and postmenopausal obesity, were associated with the risk of HAPE. Because of a lack of such data from both patients with HAPE and controls, the effect of these factors on HAPE risk should be assessed in a future study. Finally, the sample size was relatively small. Further studies with large samples are needed to circumvent these problems and to confirm our results.

To sum up, we provide new evidence for the association between *ACYP2* variant and HAPE risk in Han Chinese population for the first time, which may contribute to screening of HAPE and shed light on the new candidate genes for HAPE susceptibility. Further functional studies and larger population-based prospective studies are required in order to understand the genetic factors underlying HAPE.

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