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ORIGINAL RESEARCH

The Association of Methylation Level in the *CYP39A1* Gene with High Altitude Pulmonary Edema in the Chinese Population

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Background: High altitude pulmonary edema (HAPE) is still the most common fatal disease at high altitudes. DNA methylation proceeds with an important role in HAPE progression. This study was designed to investigate the association between *CYP39A1* methylation and HAPE.

Methods: Peripheral blood samples were enrolled from 106 participants (53 HAPE patients and 53 healthy subjects) to study the association of *CYP39A1* methylation with HAPE. DNA methylation site in the promoter region of *CYP39A1* was detected by Sequenom MassARRAY EpiTYPER platform.

Results: Probability analysis showed that the methylation probabilities of CYP39A1_1_CpG_5 and CYP39A1_3_CpG_21 are significant differences between the cases and controls ($p< 0.05$). The methylation level analysis indicated that CYP39A1_1_CpG_2.3.4, CYP39A1_5_CpG_6.7, and CYP39A1_5_CpG_9.10 were higher methylation in HAPE compared to the controls ($p< 0.05$). CYP39A1_3_CpG_21 and CYP39A1_4_CpG_3 exhibited a lower methylation level in HAPE than that in the controls ($p< 0.05$). The association analysis given that CYP39A1_1_CpG_2.3.4 (OR 2.56, $p= 0.035$), CYP39A1_5_CpG_6.7 (OR 3.99, $p= 0.003$), CYP39A1_5_CpG_9.10 (OR 3.99, $p= 0.003$), CYP39A1_5_CpG_16.17.18 (OR 2.53, $p= 0.033$), and CYP39A1_5_CpG_20 (OR 3.05, $p= 0.031$) are associated with an increased risk of HAPE. Whereas CYP39A1_1_CpG_5 (OR 0.33, $p= 0.016$) and CYP39A1_3_CpG_21 (OR 0.18, $p= 0.005$) have a protective role in HAPE. Besides, age-stratification analysis showed that CYP39A1_1_CpG_5 (OR 0.16, $p= 0.014$) and CYP39A1_3_CpG_21 (OR 0.08, $p= 0.023$) had a protective impact on HAPE in people aged ≤32 years. CYP39A1_5_CpG_6.7 (OR 6.70, $p= 0.008$) and CYP39A1_5_CpG_9.10 (OR 6.70, $p= 0.008$) were related to an increased susceptibility to HAPE aged >32 years. Moreover, the diagnostic value of CYP39A1_3_CpG_21 (AUC = 0.712, $p< 0.001$) was significantly better than other CpG sites.

Conclusion: The methylation level of *CYP39A1* was associated with a risk of HAPE in the Chinese population, which provided new perspective for preventing and diagnosing of HAPE.

Keywords: HAPE, DNA methylation, *CYP39A1*, CpG, the Chinese population

Introduction

High altitude pulmonary edema (HAPE) is a kind of rapid noncardiogenic pulmonary edema, which usually occurs in people who visit to altitudes above 2500–3000 m within 2–4 days.[1] The incidence of HAPE is high and the symptoms are severe.[2] If not addressed in a timely manner, it can develop into a coma in a relatively short time and even lead to death.[3] There is the absence of effective ways to evaluate the prevention and treatment of HAPE. It is assumed that the occurrence of HAPE is a complex process that involved in multi-factor and many genes.[4] The happening and development of HAPE is related to observable individual and racial diversity, and it is affected by the environmental and genetic factors. However, the pathogenic mechanism of HAPE is not yet clear. It is a focus topic in high altitude medical research to study the pathogenesis of acute

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HAPE and explore the genetic markers that can predict high altitude pulmonary edema so as to prevent in advance and reduce the incidence. Previous studies reported that genetic polymorphisms in some genes such as *IL6, ACYP2, RTEL1*, and *NR3C2* are significantly associated with the risk of HAPE.[5–8] DNA methylation is one of the main regulatory mechanisms of epigenetics, which can regulate the expression of target genes without changing the DNA sequence by silencing promoters and altering the transcription of the regulatory genome.[9] The methylation of the CpG site near the gene promoter is linked to the transcriptional activity and expression of the gene.[10,11] An increasing study has shown that DNA methylation significantly contributes to the occurrence of lung diseases.[12–15] These studies indicated that DNA methylation also plays a potential role in the development of HAPE.

Cytochrome P450 family 39 Subfamily A Member 1 (*CYP39A1*), a member of the cytochrome P450 superfamily of enzymes, mainly involves in the drug metabolism and biosynthesis of cholesterol. Besides, previous studies indicated that *CYP39A1* contributes to the occurrence and development of numerous human diseases. Li et al showed that down regulation of *CYP39A1* was related to hepatocellular carcinoma carcinogenesis, tumor differentiation, and poor overall survival in the Chinese population.[16] Huang et al observed that hypermethylation of *CYP39A1* correlated with an increase rate of relapsing in ovarian cancer among European ancestry individuals.[17] In addition, it was found that *CYP39A1* G204E mutation had a significantly increased risk of blindness, higher occurrence of exfoliation glaucoma, and severe glaucoma in the Japanese individuals.[18] *CYP39A1* rs7761731 polymorphism was linked to a higher incidence of leucopenia and infections or death during induction chemotherapy of head and neck cancer in European ancestry participants.[19] Moreover, *CYP39A1* exhibited significant underexpression in adenocarcinoma and squamous cell carcinoma compared to normal lung tissues, and differential expression of *CYP39A1* might be due to hypermethylation of *CYP39A1* gene in lung tumor cells.[20] To sum up, we guess that *CYP39A1* methylation may play an important role in lung-related diseases such as HAPE. The molecular mechanism of *CYP39A1* methylation in HAPE is unclear. Whether the DNA methylation in the *CYP39A1* gene can impact the occurrence of HAPE is needed to be explored.

Taken together, we speculated DNA methylation in the *CYP39A1* gene has a possible role in the progression of HAPE. Thus, this study was conducted to investigate the association of DNA methylation in *CYP39A1* with HAPE in the Chinese population. Our study will provide additional perspective of understanding the epigenetic modifications of *CYP39A1* in HAPE pathophysiology.

Materials and Methods

Study Subjects

Before recruiting study subjects, we calculated the sample size using G.Power software (version 3.0), and followed by the conditions: Effect size = 0.50, α= 0.05, power (1-β)= 0.95, the calculated total sample size was 54. In this study, we recruited 106 (53 HAPE patients and 53 healthy volunteers) unrelated Chinese population from the Affiliated Hospital of Xizang Minzu University. All subjects with prior cardiovascular disease, acute altitude disease, and other lung diseases were excluded in this study before entering high-altitude area. The patients were sojourners, chronically living in low-altitude area, who had recently arrived at the high-altitude area (Tibet altitude: 4000–5000 m). After exposure to the high-altitude area within 7 days, they returned to the plain area and were first diagnosed with HAPE based on chest X-rays and standard diagnostic criteria.[21] The diagnostic criteria of HAPE were as follows: (1) the clinical symptoms were cough, dyspnea at rest, hypoxemia, lung rale and cyanosis, and subnormal SaO2; (2) The clinical diagnosis was based on chest radiography, and the specific manifestations were dotted or flocculent infiltrating shadows in unilateral or bilateral lung fields with hilum as the center, which could form large pulmonary edema with diffuse distribution. Controls, chronically residing in low-altitude, had no HAPE or related diseases after exposure to the high-altitude within 7 days at the same time as the case. Their identity (sojourner), native place (low altitude area or high altitude area), age, gender, physical activities (carried out routine strenuous physical activities or not), and way of entering the plateau (hiking or not) were all matched with the cases. All participants agreed to the informed consent and interviewed using a self-administered questionnaire that included a complete medical history, demographic data, and physical condition. Besides, the clinical characteristics such as identity, native place, age, gender, and way of entering the plateau were obtained by a self-administered questionnaire. Our research was approved by the Ethics Committee of

the Affiliated Hospital of Xizang Minzu University. All experiments were carried out in accordance with the protocol of Helsinki's declaration.

DNA Isolation and Methylation Analyses

Genomic DNA was extracted from peripheral blood samples of all participants by the GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xi'an, China). PCR primers for PCR amplification were designed by Agena Bioscience EpiDesigner (http://www.epidesigner.com/), and are listed in Table S1. The genomic region of CpGs in *CYP39A1* was chr6:-46652805–46653452. The sequencing region for methylation was in the promoter region of *CYP39A1*. The prediction evaluation of each CpG site in different *CYP39A1* gene fragments was shown in Figure S1. MassARRAY Epityper DNA platform and MALDI-TOF detection were performed to detect and analyze the methylation of *CYP39A1*.[22]

Statistical Analyses

The comparisons of age and gender between cases and controls were, respectively, detected by a Student's *t*-test and Pearson's X^2 test. Besides, the Pearson's X^2 test was performed to compare the methylation probability between cases and controls. In probability analysis, when the methylation value is greater than or equal to 0.05, it is considered that methylation occurs at this site. When the methylation value is lesser than 0.05, no methylation occurs at this site. The differences of methylation levels were compared by non-parametric Wilcoxon rank-sum test since they were not normally distributed between the case and control group. To evaluate the effect of methylation level on case-control status, multivariable unconditional logistic regression was used to determine odds ratio (OR) and its 95% confidence interval (CI) for each individual CpG site, adjusted for age and gender. In logistic analysis, methylation levels were divided into two groups (high methylation and low methylation) using the median of the methylation value, based on the distribution for that site in the controls, with the low methylation group (methylation level < median value) as the reference. Model formula for logistic regression analysis was $\text{logit}(p)=a+b_1x_1+.b_nx_n$. Receiver operating characteristic (ROC) curve analysis, including calculation of the area under the ROC curve (AUC), was used to evaluate the ability of methylation at individual CpG sites to separate individuals with disease from controls. A *p*- value< 0.05 (two-sided) means statistical significance. All *p* values were corrected for multiple testing by the false discovery rate (FDR) method. All analyses were performed with SPSS statistics software (version 20.0).

Results

Study Population

Fifty-three patients with HAPE and 53 healthy subjects were enrolled in our study. The general characteristics of the participants are shown in Table 1. The mean age was 32.47 ± 10.18 years old in cases and 32.60 ± 10.50 years old in controls. There was no significant difference in age and gender between the cases and controls (p= 0.948, p= 1.000, respectively).

Methylation Levels of *CYP39A1* in HAPE

A total of 43 sites in the promoter region of *CYP39A1* were detected, 12 of which were missing, and 31 sites were finally analyzed in our study. The methylation degree of each CpG site in case and control groups was presented in Figure S2. Pearson 's X^2 test was performed to compare methylation probability of *CYP39A1* between the cases and controls. The number after the

Table 1 General Characteristics of Study Participants

Variables	Cases (n= 53)	Controls (n= 53)	*p*
Age, years (mean ± SD)[a]	32.47 ± 10.18	32.60 ± 10.50	0.948
Gender[b]			1.000
Male	49	49	
Female	4	4	

Notes: [a]Student's *t*-test is used. [b]Pearson's X^2 test is used. $p > 0.05$ indicates no statistical significance.
Abbreviation: SD, standard deviation.

gene name represents the gene segment (we designed five pairs of methylation primers), the number after CpG represents the site, and the multiple numbers after CpG are due to the relatively close distance of these sites. Due to technical limitations, methylation levels of these sites were mixed for detection. As is presented in Table 2, the methylation probabilities of CYP39A1_1_CpG_5 and CYP39A1_3_CpG_21 are significant difference between the cases and controls (p= 0.015, p= 0.003; respectively). We calculated the average methylation value of each CpG site and methylation levels between cases and controls were further compared by non-parametric Wilcoxon rank-sum test. As shown in Table 3, the methylation levels of CYP39A1_1_CpG_2.3.4 (p= 0.013), CYP39A1_5_CpG_6.7 (p= 0.006), and CYP39A1_5_CpG_9.10 (p= 0.006) were higher in HAPE compared to the controls. CYP39A1_3_CpG_21 (p= 0.000) and CYP39A1_4_CpG_3 (p= 0.002) exhibited a lower methylation level in HAPE than that in the controls.

The Association Between *CYP39A1* Methylation Sites and HAPE

We further investigated the correlations between *CYP39A1* methylation sites and HAPE and the results are shown in Table 4. Our study indicated that CYP39A1_1_CpG_2.3.4 (OR 2.56, 95% CI = 1.07–6.13, p= 0.035), CYP39A1_5_CpG_6.7 (OR 3.99, 95%

Table 2 Probability Analysis of Methylation of *CYP39A1* in Case and Control

CpG Site	Case		Controls		*p*	*p* (FDR)
	Without	With	Without	With		
CYP39A1_1_CpG_1	26.4%	73.6%	29.4%	70.6%	0.828	1.167
CYP39A1_1_CpG_2.3.4	86.4%	13.6%	93.9%	6.1%	0.299	1.324
CYP39A1_1_CpG_5	79.1%	20.9%	54.2%	45.8%	**0.015**	0.233
CYP39A1_1_CpG_12.13.14.15	9.5%	90.5%	12.8%	87.2%	0.743	1.280
CYP39A1_1_CpG_17.18	100.0%	0.0%	95.9%	4.1%	0.497	1.185
CYP39A1_2_CpG_1.2.3	87.8%	12.2%	86.3%	13.7%	1.000	1.192
CYP39A1_2_CpG_4	56.0%	44.0%	52.8%	47.2%	0.844	1.047
CYP39A1_2_CpG_11.12.13.14	4.1%	95.9%	2.0%	98.0%	0.614	1.269
CYP39A1_2_CpG_16.17	98.0%	2.0%	100.0%	0.0%	0.490	1.266
CYP39A1_3_CpG_1.2.3	57.7%	42.3%	60.8%	39.2%	0.842	1.088
CYP39A1_3_CpG_4	73.1%	26.9%	80.4%	19.6%	0.486	1.507
CYP39A1_3_CpG_11.12.13.14	3.8%	96.2%	2.0%	98.0%	1.000	1.192
CYP39A1_3_CpG_16.17	98.1%	1.9%	88.2%	11.8%	0.060	0.465
CYP39A1_3_CpG_20	94.2%	5.8%	92.0%	8.0%	0.713	1.300
CYP39A1_3_CpG_21	92.3%	7.7%	68.6%	31.4%	**0.003**	0.093
CYP39A1_3_CpG_22	80.8%	19.2%	84.0%	16.0%	0.797	1.235
CYP39A1_4_CpG_2	93.6%	6.4%	87.8%	12.2%	0.487	1.372
CYP39A1_4_CpG_3	80.9%	19.1%	69.4%	30.6%	0.242	1.250
CYP39A1_4_CpG_4	100.0%	0.0%	98.0%	2.0%	1.000	1.192
CYP39A1_5_CpG_3	62.0%	38.0%	72.1%	27.9%	0.379	1.469
CYP39A1_5_CpG_4	28.0%	72.0%	30.2%	69.8%	0.823	1.215
CYP39A1_5_CpG_5	93.3%	6.7%	97.7%	2.3%	0.617	1.195
CYP39A1_5_CpG_6.7	82.0%	18.0%	95.3%	4.7%	0.058	0.599
CYP39A1_5_CpG_8	87.8%	12.2%	85.7%	14.3%	1.000	1.192
CYP39A1_5_CpG_9.10	82.0%	18.0%	95.3%	4.7%	0.058	0.599
CYP39A1_5_CpG_11.12	16.7%	83.3%	32.6%	67.4%	0.091	0.564
CYP39A1_5_CpG_13.14	17.9%	82.1%	13.2%	86.8%	0.755	1.232
CYP39A1_5_CpG_15	28.6%	71.4%	30.6%	69.4%	1.000	1.192
CYP39A1_5_CpG_16.17.18	10.0%	90.0%	16.3%	83.7%	0.537	1.189
CYP39A1_5_CpG_20	28.6%	71.4%	40.0%	60.0%	0.450	1.550
CYP39A1_5_CpG_21.22	56.0%	44.0%	53.5%	46.5%	0.837	1.128

Notes: With: when the methylation value is greater than or equal to 0.05, it is considered that methylation occurs at this site. Without: when the methylation value is lesser than 0.05, no methylation occurs at this site. The p value was calculated by Pearson's X^2 test. Bold values represent statistically significant ($p < 0.05$).
Abbreviation: FDR, false discovery rate.

Table 3 Difference of Methylation Levels of *CYP39A1* Between the Case and Control

CpG site	Case	Control	*p*	*p* (FDR)
	Mean ± Std	Mean ± Std		
CYP39A1_1_CpG_1	0.430 ± 0.417	0.418 ± 0.409	0.786	0.902
CYP39A1_1_CpG_2.3.4	0.056 ± 0.146	0.036 ± 0.064	**0.013**	0.101
CYP39A1_1_CpG_5	0.035 ± 0.043	0.064 ± 0.067	0.086	0.381
CYP39A1_1_CpG_12.13.14.15	0.092 ± 0.037	0.100 ± 0.138	0.320	0.763
CYP39A1_1_CpG_17.18	0.025 ± 0.013	0.022 ± 0.016	0.096	0.372
CYP39A1_2_CpG_1.2.3	0.042 ± 0.031	0.038 ± 0.015	0.366	0.756
CYP39A1_2_CpG_4	0.039 ± 0.034	0.044 ± 0.052	0.907	1.004
CYP39A1_2_CpG_11.12.13.14	0.082 ± 0.02	0.086 ± 0.023	0.654	0.845
CYP39A1_2_CpG_16.17	0.028 ± 0.013	0.023 ± 0.008	0.059	0.305
CYP39A1_3_CpG_1.2.3	0.042 ± 0.011	0.044 ± 0.01	0.765	0.912
CYP39A1_3_CpG_4	0.028 ± 0.028	0.033 ± 0.044	0.421	0.768
CYP39A1_3_CpG_11.12.13.14	0.08 ± 0.026	0.079 ± 0.026	0.222	0.626
CYP39A1_3_CpG_16.17	0.03 ± 0.009	0.030 ± 0.012	0.686	0.851
CYP39A1_3_CpG_20	0.019 ± 0.018	0.020 ± 0.016	0.436	0.751
CYP39A1_3_CpG_21	0.031 ± 0.013	0.042 ± 0.021	**0.000**	**0.000**
CYP39A1_3_CpG_22	0.05 ± 0.162	0.018 ± 0.031	0.350	0.775
CYP39A1_4_CpG_2	0.027 ± 0.014	0.031 ± 0.024	0.650	0.876
CYP39A1_4_CpG_3	0.037 ± 0.015	0.049 ± 0.037	**0.002**	**0.031**
CYP39A1_4_CpG_4	0.002 ± 0.006	0.006 ± 0.012	0.164	0.508
CYP39A1_5_CpG_3	0.069 ± 0.104	0.042 ± 0.035	0.562	0.830
CYP39A1_5_CpG_4	0.144 ± 0.227	0.127 ± 0.206	1.000	1.033
CYP39A1_5_CpG_5	0.013 ± 0.018	0.029 ± 0.093	0.132	0.455
CYP39A1_5_CpG_6.7	0.043 ± 0.075	0.018 ± 0.014	**0.006**	0.062
CYP39A1_5_CpG_8	0.021 ± 0.028	0.022 ± 0.023	0.475	0.775
CYP39A1_5_CpG_9.10	0.043 ± 0.075	0.019 ± 0.016	**0.006**	0.062
CYP39A1_5_CpG_11.12	0.120 ± 0.169	0.080 ± 0.069	0.419	0.812
CYP39A1_5_CpG_13.14	0.176 ± 0.167	0.186 ± 0.138	0.554	0.859
CYP39A1_5_CpG_15	0.272 ± 0.339	0.204 ± 0.293	0.577	0.813
CYP39A1_5_CpG_16.17.18	0.150 ± 0.179	0.09 ± 0.086	0.055	0.341
CYP39A1_5_CpG_20	0.173 ± 0.231	0.129 ± 0.19	0.293	0.757
CYP39A1_5_CpG_21.22	0.047 ± 0.025	0.054 ± 0.054	0.987	1.055

Notes: Methylation levels between cases and controls were compared by non-parametric Wilcoxon rank-sum test. Bold values represent statistically significant ($p < 0.05$).
Abbreviation: FDR, false discovery rate.

Table 4 Association of *CYP39A1* Methylation Levels with HAPE by Multivariate Analysis

CpG Site	β	S.E.	Wald	OR (95% CI)	*p*	*p* (FDR)
CYP39A1_1_CpG_1	0.006	0.403	0.000	1.01 (0.46–2.21)	0.989	1.022
CYP39A1_1_CpG_2.3.4	0.941	0.445	4.464	**2.56 (1.07–6.13)**	**0.035**	0.181
CYP39A1_1_CpG_5	−1.114	0.461	5.839	**0.33 (0.13–0.81)**	**0.016**	0.165
CYP39A1_1_CpG_12.13.14.15	0.162	0.443	0.134	1.18 (0.49–2.80)	0.714	1.165
CYP39A1_1_CpG_17.18	0.258	0.438	0.346	1.29 (0.55–3.05)	0.557	1.151
CYP39A1_2_CpG_1.2.3	−0.126	0.600	0.044	0.88 (0.27–2.86)	0.834	1.034
CYP39A1_2_CpG_4	−0.119	0.412	0.083	0.89 (0.40–1.99)	0.773	1.089
CYP39A1_2_CpG_11.12.13.14	−0.057	0.457	0.016	0.94 (0.39–2.31)	0.901	1.034
CYP39A1_2_CpG_16.17	0.597	0.413	2.091	1.82 (0.81–4.08)	0.148	0.574
CYP39A1_3_CpG_1.2.3	0.130	0.403	0.104	1.14 (0.52–2.51)	0.747	1.158

(*Continued*)

Table 4 (Continued).

CpG Site	β	S.E.	Wald	OR (95% CI)	*p*	*p* (FDR)
CYP39A1_3_CpG_4	−0.112	0.408	0.075	0.89 (0.40–1.99)	0.784	1.057
CYP39A1_3_CpG_11.12.13.14	0.587	0.400	2.153	1.80 (0.82–3.94)	0.142	0.629
CYP39A1_3_CpG_16.17	0.060	0.431	0.020	1.06 (0.46–2.47)	0.888	1.059
CYP39A1_3_CpG_20	−0.050	0.464	0.012	0.95 (0.38–2.36)	0.914	1.012
CYP39A1_3_CpG_21	−1.716	0.607	7.995	**0.18 (0.05–0.59)**	**0.005**	0.078
CYP39A1_3_CpG_22	0.324	0.405	0.642	1.38 (0.63–3.06)	0.423	0.937
CYP39A1_4_CpG_2	−0.160	0.548	0.086	0.85 (0.29–2.49)	0.770	1.137
CYP39A1_4_CpG_3	−0.622	0.513	1.472	0.54 (0.20–1.47)	0.225	0.581
CYP39A1_4_CpG_4	−0.768	0.543	2.000	0.46 (0.16–1.34)	0.157	0.541
CYP39A1_5_CpG_3	0.577	0.433	1.774	1.78 (0.76–4.16)	0.183	0.567
CYP39A1_5_CpG_4	0.189	0.423	0.199	1.21 (0.53–2.77)	0.655	1.194
CYP39A1_5_CpG_5	−0.245	0.452	0.294	0.78 (0.32–1.90)	0.588	1.139
CYP39A1_5_CpG_6.7	1.384	0.464	8.876	**3.99 (1.61–9.91)**	**0.003**	0.093
CYP39A1_5_CpG_8	−0.426	0.457	0.867	0.65 (0.27–1.60)	0.352	0.839
CYP39A1_5_CpG_9.10	1.384	0.464	8.876	**3.99 (1.61–9.91)**	**0.003**	0.093
CYP39A1_5_CpG_11.12	0.168	0.428	0.154	1.18 (0.51–2.74)	0.695	1.197
CYP39A1_5_CpG_13.14	−0.037	0.458	0.007	0.96 (0.39–2.37)	0.936	1.001
CYP39A1_5_CpG_15	0.605	0.466	1.681	1.83 (0.73–4.57)	0.195	0.550
CYP39A1_5_CpG_16.17.18	0.927	0.434	4.563	**2.53 (1.08–5.92)**	**0.033**	0.205
CYP39A1_5_CpG_20	1.117	0.518	4.651	**3.05 (1.11–8.43)**	**0.031**	0.240
CYP39A1_5_CpG_21.22	−0.092	0.425	0.047	0.91 (0.40–2.10)	0.829	1.071

Notes: The *p* value was calculated by multivariable unconditional logistic regression with adjustment by age and gender. Bold values represent statistically significant association ($p < 0.05$).
Abbreviations: HAPE, high altitude pulmonary edema; OR, odds ratio; 95% CI, 95% confidence interval; FDR, false discovery rate.

CI = 1.61–9.91, $p = 0.003$), CYP39A1_5_CpG_9.10 (OR 3.99, 95% CI = 1.61–9.91, $p = 0.003$), CYP39A1_5_CpG_16.17.18 (OR 2.53, 95% CI = 1.08–5.92, $p = 0.033$), and CYP39A1_5_CpG_20 (OR 3.05, 95% CI = 1.11–8.43, $p = 0.031$) are associated with an increased risk of HAPE. While CYP39A1_1_CpG_5 (OR 0.33, 95% CI = 0.13–0.81, $p = 0.016$) and CYP39A1_3_CpG_21 (OR 0.18, 95% CI = 0.05–0.59, $p = 0.005$) have a protective role in the risk of HAPE.

The Association of *CYP39A1* Methylation with HAPE Under Age-Stratification Analysis

We further studied the correlations between *CYP39A1* methylation and HAPE stratified by age. As shown in Table 5, our study indicated that CYP39A1_1_CpG_5 (OR 0.16, 95% CI = 0.40–0.69, $p = 0.014$) and CYP39A1_3_CpG_21 (OR 0.08, 95% CI = 0.01–0.70, $p = 0.023$) had a protective impact on HAPE in people aged ≤32 years. Besides, CYP39A1_5_CpG_6.7 (OR 6.70, 95% CI = 1.63–27.58, $p = 0.008$) and CYP39A1_5_CpG_9.10 (OR 6.70, 95% CI = 1.63–27.58, $p = 0.008$) were related to an increased susceptibility to HAPE aged >32 years.

The Diagnostic Value of *CYP39A1* DNA Methylation Sites for HAPE

ROC analysis was used to evaluate the diagnostic value of *CYP39A1* methylation for HAPE. The value of AUC was computed to assess the ability of *CYP39A1* methylation to diagnose HAPE. As presented in Table 6 and Figure 1, we found that the diagnostic value of CYP39A1_3_CpG_21 (AUC = 0.712, $p < 0.001$) was significantly better than other CpG sites (AUC < 0.700, $p < 0.05$), indicating that CYP39A1_3_CpG_21 may be used as a diagnostic indicator for HAPE compared to other CpG sites. Besides, we observed that the AUC of the combination of the significant CpG sites (CYP39A1_1_CpG_2.3.4+ CYP39A1_3_CpG_21+ CYP39A1_4_CpG_3+ CYP39A1_5_CpG_6.7+ CYP39A1_5_CpG_9.10) was 0.713 ($p < 0.05$) (Figure 2).

Table 5 The Correlation Between *CYP39A1* Methylation and HAPE Risk Stratified by Age

CpG Site	Age≤ 32		Age> 32	
	OR (95% CI)	*p*	OR (95% CI)	*p*
CYP39A1_1_CpG_1	1.07 (0.38–3.03)	0.903	0.93 (0.28–3.12)	0.905
CYP39A1_1_CpG_2.3.4	1.97 (0.64–6.04)	0.236	3.77 (0.92–15.50)	0.066
CYP39A1_1_CpG_5	**0.16 (0.40–0.69)**	**0.014**	0.56 (0.16–1.96)	0.359
CYP39A1_1_CpG_12.13.14.15	0.53 (0.17–1.69)	0.285	1.67 (0.42–6.56)	0.465
CYP39A1_1_CpG_17.18	0.68 (0.22–2.06)	0.492	3.43 (0.83–14.21)	0.089
CYP39A1_2_CpG_1.2.3	0.81 (0.17–4.01)	0.799	0.93 (0.16–5.42)	0.939
CYP39A1_2_CpG_4	1.05 (0.37–2.98)	0.933	0.65 (0.18–2.37)	0.512
CYP39A1_2_CpG_11.12.13.14	1.68 (0.50–5.69)	0.401	0.44 (0.11–1.82)	0.256
CYP39A1_2_CpG_16.17	1.75 (0.60–5.13)	0.307	2.01 (0.57–7.06)	0.275
CYP39A1_3_CpG_1.2.3	0.68 (0.24–1.98)	0.484	2.18 (0.64–7.42)	0.213
CYP39A1_3_CpG_4	1.22 (0.42–3.56)	0.713	0.57 (0.16–1.95)	0.367
CYP39A1_3_CpG_11.12.13.14	1.90 (0.66–5.46)	0.232	1.78 (0.53–5.93)	0.348
CYP39A1_3_CpG_16.17	0.95 (0.30–3.03)	0.934	0.92 (0.25–3.40)	0.902
CYP39A1_3_CpG_20	0.72 (0.21–2.42)	0.590	1.29 (0.32–5.19)	0.718
CYP39A1_3_CpG_21	**0.08 (0.01–0.70)**	**0.023**	0.30 (0.07–1.36)	0.117
CYP39A1_3_CpG_22	1.41 (0.51–3.92)	0.514	1.50 (0.42–5.34)	0.533
CYP39A1_4_CpG_2	3.26 (0.54–19.56)	0.197	0.27 (0.06–1.22)	0.089
CYP39A1_4_CpG_3	0.64 (0.16–2.58)	0.527	0.35 (0.08–1.63)	0.181
CYP39A1_4_CpG_4	0.26 (0.06–1.10)	0.067	1.16 (0.23–5.93)	0.858
CYP39A1_5_CpG_3	1.47 (0.46–4.64)	0.514	2.37 (0.63–8.84)	0.200
CYP39A1_5_CpG_4	1.04 (0.33–3.27)	0.944	1.42 (0.40–4.99)	0.585
CYP39A1_5_CpG_5	0.69 (0.21–2.30)	0.541	0.94 (0.25–3.52)	0.929
CYP39A1_5_CpG_6.7	2.55 (0.77–8.43)	0.125	**6.70 (1.63–27.58)**	**0.008**
CYP39A1_5_CpG_8	0.70 (0.21–2.36)	0.567	0.59 (0.16–2.24)	0.438
CYP39A1_5_CpG_9.10	2.55 (0.77–8.43)	0.125	**6.70 (1.63–27.58)**	**0.008**
CYP39A1_5_CpG_11.12	1.78 (0.55–5.77)	0.336	0.75 (0.21–2.69)	0.663
CYP39A1_5_CpG_13.14	0.54 (0.16–1.87)	0.329	1.70 (0.42–6.91)	0.462
CYP39A1_5_CpG_15	1.59 (0.48–5.31)	0.452	2.05 (0.47–9.02)	0.343
CYP39A1_5_CpG_16.17.18	2.64 (0.84–8.31)	0.096	2.42 (0.67–8.67)	0.176
CYP39A1_5_CpG_20	2.29 (0.64–8.15)	0.202	6.01 (0.97–37.30)	0.054
CYP39A1_5_CpG_21.22	0.88 (0.28–2.80)	0.832	0.87 (0.25–3.00)	0.821

Notes: The *p* value was calculated by logistic regression with adjustment by gender. Bold values represent statistically significant association ($p < 0.05$).

Abbreviations: HAPE, high altitude pulmonary edema; OR, odds ratio; 95% CI, 95% confidence interval; FDR, false discovery rate.

Table 6 Analysis of the Diagnostic Value of Each CpG Site in *CYP39A1* for HAPE

CpG Site	AUC	*p*	*p* (FDR)
CYP39A1_1_CpG_1	0.485	0.787	0.904
CYP39A1_1_CpG_2.3.4	**0.355**	**0.016**	0.124
CYP39A1_1_CpG_5	0.603	0.091	0.403
CYP39A1_1_CpG_12.13.14.15	0.439	0.322	0.832
CYP39A1_1_CpG_17.18	0.401	0.105	0.407
CYP39A1_2_CpG_1.2.3	0.452	0.408	0.843
CYP39A1_2_CpG_4	0.493	0.908	1.005
CYP39A1_2_CpG_11.12.13.14	0.526	0.659	0.888
CYP39A1_2_CpG_16.17	0.398	0.079	0.408

(*Continued*)

Table 6 (Continued).

CpG Site	AUC	*p*	*p* (FDR)
CYP39A1_3_CpG_1.2.3	0.516	0.777	0.926
CYP39A1_3_CpG_4	0.545	0.429	0.782
CYP39A1_3_CpG_11.12.13.14	0.432	0.231	0.716
CYP39A1_3_CpG_16.17	0.478	0.700	0.868
CYP39A1_3_CpG_20	0.543	0.449	0.773
CYP39A1_3_CpG_21	**0.712**	**< 0.001**	**< 0.001**
CYP39A1_3_CpG_22	0.451	0.392	0.868
CYP39A1_4_CpG_2	0.526	0.663	0.856
CYP39A1_4_CpG_3	**0.672**	**0.004**	0.062
CYP39A1_4_CpG_4	0.558	0.324	0.773
CYP39A1_5_CpG_3	0.466	0.569	0.840
CYP39A1_5_CpG_4	0.500	1.000	1.033
CYP39A1_5_CpG_5	0.590	0.148	0.510
CYP39A1_5_CpG_6.7	**0.336**	**0.007**	0.072
CYP39A1_5_CpG_8	0.543	0.484	0.790
CYP39A1_5_CpG_9.10	**0.337**	**0.007**	0.072
CYP39A1_5_CpG_11.12	0.451	0.422	0.818
CYP39A1_5_CpG_13.14	0.539	0.555	0.860
CYP39A1_5_CpG_15	0.463	0.578	0.814
CYP39A1_5_CpG_16.17.18	0.385	0.057	0.353
CYP39A1_5_CpG_20	0.427	0.296	0.834
CYP39A1_5_CpG_21.22	0.499	0.988	1.056

Notes: The value of AUC was calculated by ROC analysis. Bold values represent statistically significant ($p < 0.05$).
Abbreviations: HAPE, high altitude pulmonary edema; AUC, Area under a curve; FDR, false discovery rate.

Discussion

In this study, we explored the associations of *CYP39A1* methylation with HAPE in the Chinese population. Our results indicated that the DNA methylation in *CYP39A1* has a probable role in the occurrence of HAPE. *CYP39A1* methylation was related to the risk of HAPE. To the best of our knowledge, our study is the first time to detect the association between DNA methylation in the *CYP39A1* gene and HAPE, which may provide a novel biomarker for the diagnosis of HAPE in the Chinese population.

Studies on high-altitude diseases have shown that some essential genes related to pharmacogenomics are involved in the occurrence of HAPE (https://www.pharmgkb.org/view/vips.jsp). *CYP450* enzymes are comprised of a group of enzyme proteins encoded by gene superfamily, which are isozymes encoded by a group of structure and function related superfamily genes, and are important pharmacogenomics gene families. *CYP39A1* is the important member of CYP450, which involves the drug metabolism and biosynthesis of cholesterol. Previous study suggested that hypoxia adaptation is a complex trait. In order to deal with hypoxia, the human body will also initiate some apparent regulatory mechanisms such as gene methylation except for activating and stabilizing transcriptional regulatory factors, which can lead to long-term changes in gene expression. The methylation level of *CYP39A1* gene will be changed under unusual conditions (such as tumor, drug therapy, smoking, etc.), which will lead to abnormal expression of CYP39A1.[23–25] The methylation of *CYP39A1* may have a possible role in the development of HAPE. DNA methylation is the methylation modification of carbon atoms under the action of DNA methyltransferase. Cytosine phosphate guanine (CpG) site-specific DNA methylation changes occur in human and mammalian cells. Clustered CpG regions are called CpG islands, which are common in the promoter region of genes and are switches for regulating gene expression.[26,27] Thus, we determined the association between methylation of the promoter region in the *CYP39A1* gene and HAPE. We found that CYP39A1_1_CpG_2.3.4, CYP39A1_5_CpG_6.7, and CYP39A1_5_CpG_9.10 were higher methylation in HAPE

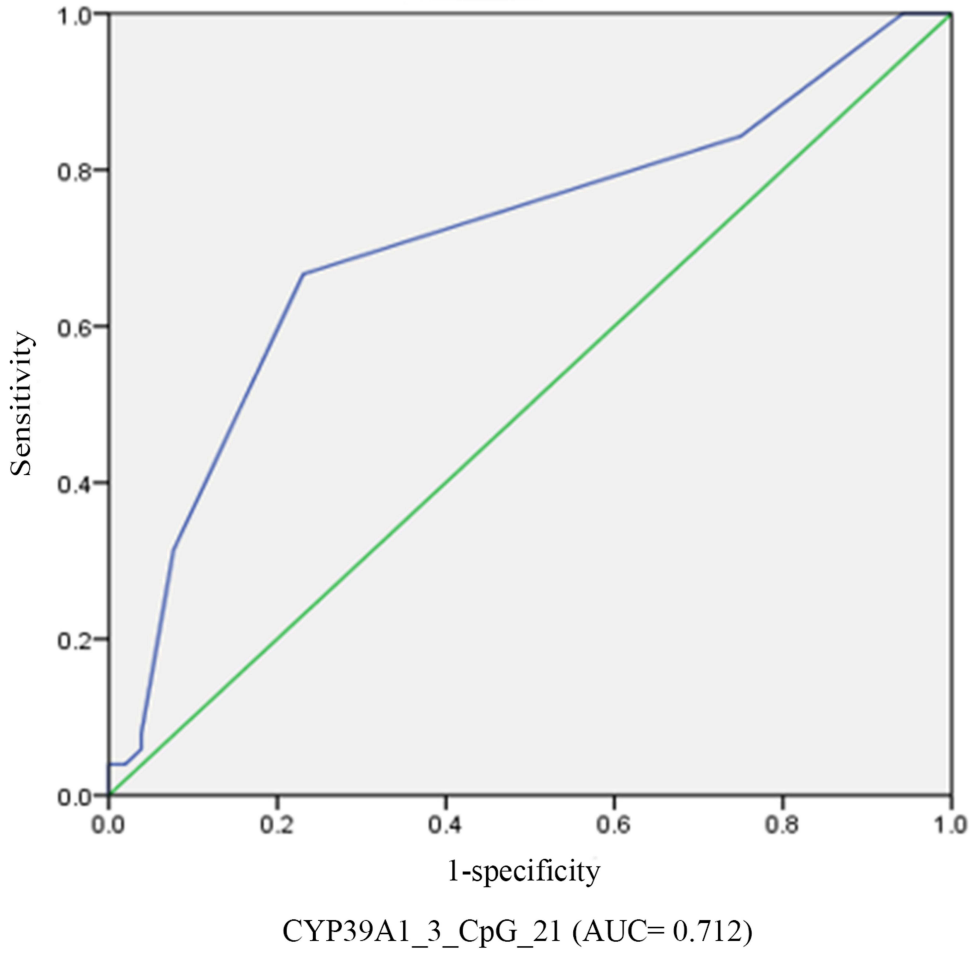

Figure 1 The ROC curves of the diagnostic value of CYP39A1_3_CpG_21 methylation for HAPE. The AUC was 0.712.
Abbreviations: ROC, receiver operating characteristics curve; AUC, the area under the ROC curve.

compared to the controls. CYP39A1_3_CpG_21 and CYP39A1_4_CpG_3 exhibited a lower methylation level in HAPE than that in the controls. Levels of DNA methylation as a promoter-associated CpG island are generally negatively associated with gene expression. For example, Cui et al reported that CpG sites with higher methylation levels in esophageal squamous cell carcinoma tissues exhibited lower expression level of miR-203.[28] Additionally, Zhou et al showed that *CYP2S1* gene expression is negatively associated with DNA methylation in psoriasis.[29] Therefore, we speculate that these high levels of CpG sites may influence the occurrence of HAPE by down regulating the *CY939A1* gene expression, and further study is necessary to confirm this hypothesis.

The association analyses indicated that CYP39A1_1_CpG_2.3.4, CYP39A1_5_CpG_6.7, CYP39A1_5_CpG_9.10, CYP39A1_5_CpG_16.17.18, and CYP39A1_5_CpG_20 were linked to an increased risk of HAPE. While CYP39A1_1_CpG_5 and CYP39A1_3_CpG_21 have a protective role in the risk of HAPE. Our study suggests that the DNA methylation in *CYP39A1* is related to HAPE.

It has been shown that certain CpG sites are highly associated with age.[30,31] Some findings demonstrate that promoter-associated CpG islands have a tendency to increase methylation with age.[32,33] Because the average age of the participants was 32 years in the current study, we stratified by 32 years. We observed that CYP39A1_1_CpG_5 and CYP39A1_3_CpG_21 had a protective impact on HAPE in people aged ≤32 years. In addition, high methylation of CYP39A1_5_CpG_6.7 and CYP39A1_5_CpG_9.10 were related to an increased susceptibility to HAPE at aged >32 years. Our data suggest that the association of CYP39A1_5_CpG_6.7 and CYP39A1_5_CpG_9.10 with HAPE may also be affected by age.

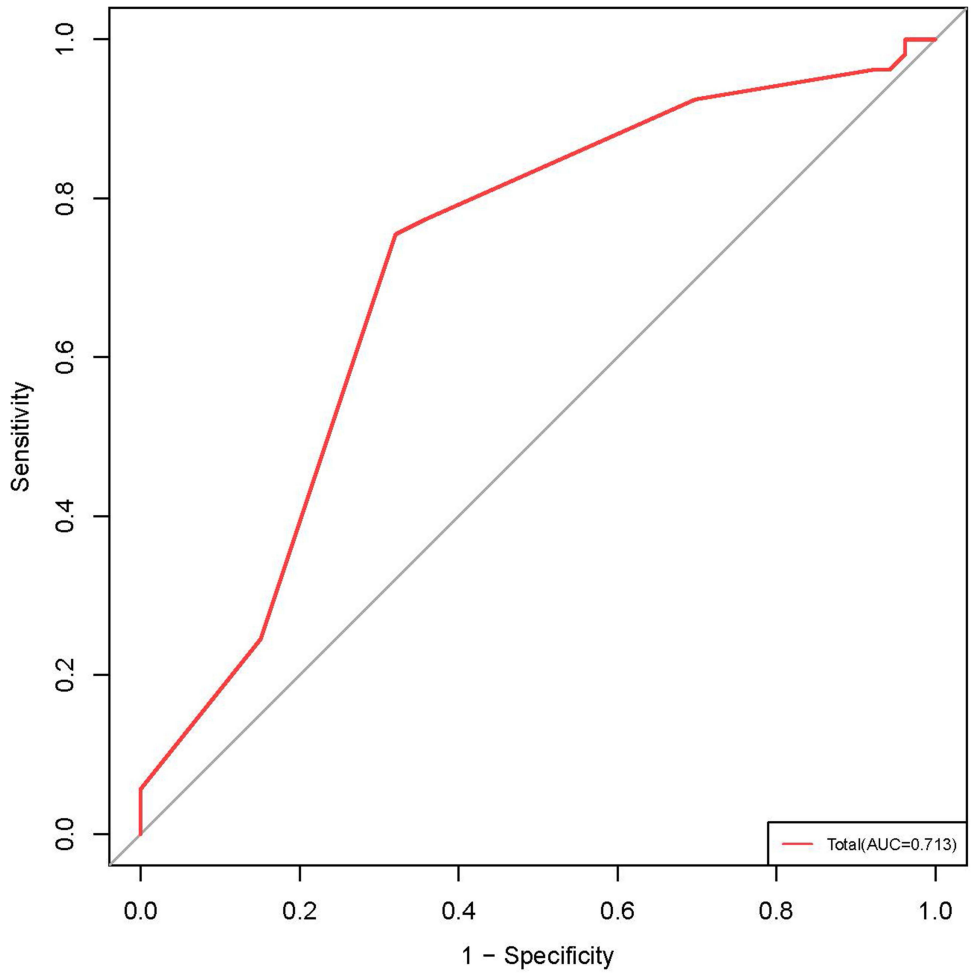

Figure 2 The ROC curves of the diagnostic value of all significant CpGs methylation for HAPE. All significant CpGs including CYP39A1_1_CpG_2.3.4, CYP39A1_3_CpG_21, CYP39A1_4_CpG_3, CYP39A1_5_CpG_6.7 and CYP39A1_5_CpG_9.10, and the AUC was 0.713.
Abbreviations: ROC, receiver operating characteristics curve; AUC, the area under the ROC curve.

HAPE is a fatal disease brought about by acute exposure to high-altitude and hypoxia leading to fluid accumulation in the lungs. Early diagnosis for HAPE is of great importance for its prevention and treatment. DNA methylation can be utilized to detect and diagnose HAPE. We performed ROC analysis to evaluate the diagnostic value of *CYP39A1* methylation for HAPE, and we found that CYP39A1_3_CpG_21 has a higher diagnostic value for HAPE compared to other CpG sites (AUC = 0.712). There is one paper assessing diagnostic value of *CYP2S1* methylation in HAPE, and they showed that the AUC value of CYP2S1_3_CpG_11.12 was 0.672.[22] The comparison suggests that *CYP39A1* could be a candidate diagnostic biomarker for molecular targeting prevention and therapy for HAPE.

Our study had some limitations. First, there is no functional verification in the current research. We will test mRNA expression and functional experiment to further explore the potential role of the *CYP39A1* methylation in HAPE. Second, the correlation between CpG methylation and gene expression has not been conducted, and the association will be determined in the next. Third, the role of *CYP39A1* methylation in HAPE severity has not been explored, we will determine the association between *CYP39A1* methylation and HAPE severity by collecting different severity of HAPE in future. Despite the above disadvantages, our findings firstly provided evidence that *CYP39A1* DNA methylation was correlated with HAPE susceptibility, which may provide a novel biomarker for the prevention and diagnosis of HAPE.

Conclusions

Our present study indicated that *CYP39A1* methylation was related to HAPE susceptibility. DNA methylation in the *CYP39A1* gene has a potential role in the occurrence of HAPE.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Affiliated Hospital of Xizang Minzu University and the 1964 Helsinki declaration. Informed written consent was obtained from each participant before the research.

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Disclosure

There were not any competing interests.

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