MIR17HG Polymorphisms Contribute to High Altitude Pulmonary Edema Susceptibility in Chinese Population

Lining Si
Qinghai University Affiliated Hospital
Haiyuan Wang
Qinghai Red Cross Hospital
Rong Wang
Qinghai University
Qifu Long
Qinghai University
Yanli Zhao (yanlizhao_69@163.com)
Qinghai University

Research Article

Keywords: High altitude pulmonary edema, MIR17HG, polymorphism, case-control study, Chinese population

Posted Date: June 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-654161/v1

Version of Record: A version of this preprint was published at Scientific Reports on March 14th, 2022. See the published version at https://doi.org/10.1038/s41598-022-06944-8.

Abstract

Background: High-altitude pulmonary edema (HAPE) is a common acute altitude sickness. The results from existing studies have shown that the occurrence of HAPE is related to genetic factors. Therefore, six locis of MIR17HG were selected to study its effect on HAPE of Chinese population.

Methods: All subjects were genotyped by the Agena MassARRAY, and the relationship between polymorphisms on *MIR17HG* and HAPE risk was evaluated using a χ^2 test with an odds ratio (OR) and 95% confidence intervals (CIs) in multiple genetic models.

Results: In the allele model, we observed that lower risk (OR = 0.74, p = 0.036) of the A allele for rs7318578 on the *MIR17HG* compared to the people with the C allele. Logistic regression analysis of four models for all selected *MIR17HG* SNPs between cases and controls showed significant differences for rs7318578 (OR = 0.74, p = 0.037) and rs17735387 (OR = 1.51, p = 0.036) in the HAPE population.

Conclusion: Rs7318578 and rs17735387 on *MIR17HG* were associated with the genetic susceptibility of HAPE in Chinese population.

Introduction

Among plateau-related diseases, acute mountain sickness (AMS) is the most common. There are regional differences in the incidence of AMS. Generally speaking, the higher the altitude, the higher the incidence of AMS and the more severe the clinical symptoms. AMS includes two types: light and heavy. High-altitude pulmonary edema (HAPE) is a severe form of AMS [1]. It is a serious life-threatening non-cardiogenic pulmonary edema caused by highaltitude hypoxic environment. And it is a special and common disease after a short period of exposure to altitudes above 2250 m,characterized by acute onset, rapid progress and serious consequences [1,2]. The high-altitude and low-oxygen environment easily leads to the occurrence of HAPE. More and more evidences proved that genetic factors also play a key role in the occurrence of HAPE. For example, IL1R2[3], NR3C2[4], NR3C1[5], NOS3[6] affect the occurrence of high altitude pulmonary edema.

The pathogenesis of HAPE is more complex, and it is unclear. It is generally believed that the key to its occurrence is the pulmonary vasoconstriction and increased pulmonary arterial pressure caused by hypoxia at high altitude. Studies have found that lncRNA polymorphisms were associated with genetic susceptibility to pulmonary hypertension [7]. Therefore, we believe that lncRNA polymorphisms may also be associated with genetic susceptibility to high altitude pulmonary edema. *MIR17HG* (known as miR-17-92) was derived from the mir-17-92a-1 cluster host gene, and was a class of pri-miRNAs located in the 800 base pair region of human chromosome 13, whose sole function is to produce six miRNAs (miR-17, miR-18a, miR − 19a, miR − 20a, miR-19b-1 and miR-92-1) [8,9]. Studies have confirmed that it participates in various growth and development processes such as cell proliferation and differentiation, angiogenesis, and plays an important role [10–12]. More and more studies have shown that the polymorphism of *MIR17HG* gene was associated with breast cancer[13], colorectal cancer[14], Multiple Myeloma[15].

However, the genetic susceptibilityof *MIR17HG* polymorphism and high-altitude pulmonary edema is unknown. Therefore, six locis of MIR17HG were selected to study its effect on HAPE of Chinese population.

Methods

Participants

Based on the strategy of the case-control for explored the gene polymorphisms on *MIR17HG* gene, we recruited 487 healthy participants from the Affiliated Hospital of Qinghai University. All participants lived in low altitude areas below 2500 m and reached high altitude areas above 3000 m due to work or travel. By carrying out symptom (dyspnea), signs (cyanosis at rest) and imaging examination (like X-ray radiograph, computed tomography (CT) of the patient chest) for all participants, and combining with the diagnostic criteria of HAPE [16], found that 244 participants had high altitude pulmonary edema as a case group, while 243 participants had no symptoms of high altitude pulmonary edema, as the control group.

Genotyping

Combined previous studies, six polymorphisms (rs75267932, rs72640334, rs7336610, rs7318578, rs17735387, and rs1428) in *MIR17HG* gene with minor allele frequencies > 5% in the Chinese Han Beijing population were selected. Using the Agena MassARRAY Assay Design 3.0 Software to design the primer based on the design principles of primer (Table 1). 5 ml of peripheral venous blood was routinely collected in an EDTA-containing anticoagulation tube and stored in an ultra-low temperature freezer for DNA extraction. Extraction of DNA from whole blood samples using the GoldMag–Mini Purification Kit (GoldMag Co. Ltd. Xi'an, China). Quality monitoring of extracted DNA using a spectrophotometer, including concentration and purity. Finally, genotyping of the six gene polymorphisms on *MIR17HG* gene was performed by the standard protocol from the Agena MassARRAY RS1000 manufacturer, and Agena Typer 4.0 Software was used to analyze the experimental results and obtain genotyping data, which was same as the genotyping method used in previous published articles [3,17].

Table 1
Primer Sequences of MIR17HG Gene SNPs

SNP_ID	2nd-PCRP	1st-PCRP	UEP_SEQ
rs75267932	ACGTTGGATGTTAGAGAGAATGCCGCTCTG	ACGTTGGATGCCCAACCCTAAATTCCATGC	cccCCGCTCTGTTTAAAGCAATGTGTA
rs72640334	ACGTTGGATGGCTTAAGAACTCTGCTAATG	ACGTTGGATGCTATCATTCTGGAGTTGATG	CACTGTTCATTTCACATCCT
rs7336610	ACGTTGGATGACAGCGTTTCACCATGTCGG	ACGTTGGATGAAAAAGTTCCGGCTGGACAC	CTCCTGACCTCAGGTAATCC
rs7318578	ACGTTGGATGGAAATCATCCAGCAGGCTTC	ACGTTGGATGCAGCATGGTCTGGTAGTTTG	TCCTATCACACTGTTCCA
rs17735387	ACGTTGGATGGCTTTCTTTCCAAATATAGGC	ACGTTGGATGAGCCTTAACTATTTGGAGGG	AATAGAAAGTTGTACATGCAAA
rs1428	ACGTTGGATGTCAATATTCTCGTTCTGGAC	ACGTTGGATGTGGCTGTTTGAGTTCTAGCG	tACAATTTCTTAACAGCTTTAAAAT

Statistical Analysis

The analysis of the general characteristics of the subjects, including Chi-square test was used to compare the difference between the case group and the control group in classified variables (gender). The T-test was used to compare the difference in continuous variables (age) between the case group and the control group. The goodness of fit chi-square test was used to calculate the HWE of each polymorphism in the control group to test the representativeness and randomness of the study population. Chi-square test was used to detect the distribution difference of genotype and allele frequency of each polymorphism between the case group and the control group. Logistic regression was used to calculate the OR value and 95% confidence interval to evaluate the strength of association between each genotype and HAPE by PLINK software. All statistical tests were bilateral, with a significance threshold of 0.05.

Results

As Table 2 shown that 487 healthy participants were recruited. And 244 participants had high altitude pulmonary edema (female 19, male 225), while 243 participants had no symptoms of high altitude pulmonary edema (female 19, male 224). The mean of age in the HAPE group and healthy group were 32.41 ± 10.81 and 32.70 ± 9.52 years. No significant differences were found in sex and age between the case and control groups (both $p > 0.05$). Table 3 shows that the basic information of six SNPs on the *MIR17HG* gene in this study. All the SNPs were found to be at Hardy–Weinberg equilibrium based on exact test. In the allele model, we observed that lower risk (OR = 0.74, 95%CI: 0.56–0.98, $p = 0.036$) of the A allele for rs7318578 on the *MIR17HG* gene for HAPE compared with the people with the C allele.

Table 2
Characteristics of participants

Variables	case (244)	control (243)	*p*
Age ± SD	32.41 ± 10.81	32.70 ± 9.52	0.761
Sex			0.869
Female	19	19	
male	225	224	
$p < 0.05$ indicates statistical significance			

Table 3
Basic information of six SNPs on the MIR17HG gene in this study

SNP	Chromosome	BP	Alleles	Gene	MAF-Case	MAF-Control	HWE-p	OR (95%CI)	*p*
rs75267932	13	91351812	A/G	MIR17HG	0.125	0.115	0.750	1.10 (0.75–1.62)	0.639
rs72640334	13	91352674	A/C	MIR17HG	0.102	0.103	0.726	1.00 (0.66–1.51)	0.983
rs7336610	13	91352883	C/T	MIR17HG	0.471	0.484	0.608	0.95 (0.74–1.22)	0.703
rs7318578	13	91353215	A/C	MIR17HG	0.256	0.317	0.882	0.74 (0.56–0.98)	0.036
rs17735387	13	91353800	A/G	MIR17HG	0.199	0.160	0.229	1.30 (0.93–1.80)	0.120
rs1428	13	91354516	G/T	MIR17HG	0.475	0.484	0.608	0.97 (0.75–1.25)	0.800
HWE, Hardy-Weinberg Equilibrium; ORs, odds ratios; CI: confidence interval. $p < 0.05$ indicates statistical significance									

Finally, logistic regression analysis of four models for all selected *MIR17HG* SNPs between cases and controls showed significant differences for rs7318578 and rs17735387 in the HAPE population. For rs7318578, under the log-additive model, we found that the people carrier the “A” allele

decreased the risk of HAPE, when compared with the C allele carriers, which plays a protective role (OR = 0.74, 95%CI: 0.56–0.98, $p = 0.037$; Table 4). Individuals carrying the rs17735387 "A" allele have a 51% increased risk of HAPE than those with G allele (OR = 1.51, 95%CI: 1.03–2.21, $p = 0.036$; Table 4).

Table 4
Correlation of MIR17HG SNPs with HAPE risk

SNP	Model	Genotype	Number		Crude analysis		adjusted by age and sex	
			Case	Control	OR (95%CI)	*p*	OR (95%CI)	*p*
rs7318578	Genotype	CC	134	114	1		1	
		AC	95	104	0.78 (0.53–1.13)	0.186	0.78 (0.54–1.14)	0.198
		AA	15	25	0.51 (0.26–1.02)	0.055	0.51 (0.26–1.01)	0.053
	dominant	CC/AC + AA	134/110	114/129	0.73 (0.51–1.04)	0.078	0.73 (0.51–1.04)	0.081
	recessive	CC + AC/AA	229/15	228/25	0.57 (0.29–1.11)	0.100	0.57 (0.29–1.10)	0.094
	log-additive	/	/	/	0.74 (0.56–0.98)	0.037	0.74 (0.56–0.98)	0.037
rs17735387	Genotype	GG	153	174	1		1	
		AG	85	60	1.61 (1.09–2.39)	0.018	1.62 (1.09–2.41)	0.018
		AA	6	9	0.76 (0.26–2.18)	0.607	0.76 (0.27–2.20)	0.617
	dominant	GG/AG + AA	153/91	174/69	1.50 (1.03–2.20)	0.037	1.51 (1.03–2.21)	0.036
	recessive	GG + AG/AA	238/6	234/9	0.66 (0.23–1.87)	0.430	0.66 (0.23–1.88)	0.434
	log-additive	/	/	/	1.30 (0.93–1.81)	0.119	1.31 (0.94–1.82)	0.117
ORs, odds ratios; CI: confidence interval. $p < 0.05$ indicates statistical significance								

Discussion

Six polymorphisms on *MIR17HG* were selected to investigate the effect of mutations on *MIR17HG* on HAPE in Chinese population. In our investigative study, we found that *MIR17HG* rs7318578 minimized the risk and rs17735387 increased the risk.

One research about *MIR17HG* polymorphism and the risk of breast cancer, revealed that rs4824505 "A" allele, rs7336610 "C" allele, and rs4824505/rs7336610 "AC" haplotype were related to the risk of breast cancer, via 244 breast cancer patients and 187 healthy females [13]. In Multiple Myeloma (MM), *MIR17HG* rs7336610 T allele and *MIR17HG* rs4284505 G added the risk of MM, while rs7336610-rs4284505 "CA" haplotype decreased the risk of MM. While rs17735387 and rs1428 did not share any relationship with MM risk [15]. In colorectal cancer, rs7336610 and rs1428 were significantly related to increased risk of colorectal cancer. While, rs7318578 was associated with a decreased risk of colorectal cancer [14]. In our research, we observed that the people carrier the "A" allele decreased the risk of HAPE, when compared with the C allele carriers; individuals carrying the rs17735387 "A" allele have a 51% increased risk of HAPE than those with G allele. This is the first time we have found that the *MIR17HG* mutation was associated with the genetic susceptibility to high altitude pulmonary edema.

The promoter of the miR-17-92 gene cluster host gene *MIR17HG* is highly conserved, and the promoter region has multiple conserved transcription factor binding sites [18]. Studies have confirmed that p53 and HIF-1α can inhibit the expression of miR-17-92 gene cluster[19]. And study finds increased expression of HIF-1a is associated with hypoxia adaptation [20]. The *MIR17HG* gene locus encodes the miR-17-92 gene cluster (*MIR17HG*), the family members of this gene cluster are widely involved in the functional regulation of vascular ECs [21]. Single-nucleotide polymorphisms (SNPs) could potentially impact biological processes involved in the production or functional effects of the microRNA 17–92 cluster host gene. We found that *MIR17HG* rs7318578 minimized the risk and rs17735387 increased the risk. Therefore, we speculate that the mutation may affect the expression of *MIR17HG* in high altitude pulmonary edema, and then affect the occurrence of high altitude pulmonary edema.

Although we found that the *MIR17HG* mutation was associated with the genetic susceptibility to altitude pulmonary edema. But we need to further verify with large samples. The effects of mutations on the expression of *MIR17HG* and the molecular mechanisms of *MIR17HG* in the occurrence of high altitude pulmonary edema were explored through molecular and cell biological methods.

Conclusion

Through our research, we found that *MIR17HG* rs7318578 minimized the risk and rs17735387 increased the risk in Chinese population, which may be novel biomarkers for susceptibility to HAPE.

List Of Abbreviations

Abbreviations	English full name
HAPE	High altitude pulmonary edema
AMS	acute mountain sickness
SNP	Single nucleotide polymorphism
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
95%CI	95% confidence intervals
CT	computed tomography
MAF	Minor allele frequency
MM	Multiple Myeloma
miR-17-92 gene cluster	MIR17HG
LD	linkage disequilibrium

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Affiliated Hospital of Qinghai University, and in compliance with the Declaration of Helsinki. The purpose of this study was well informed to the all participants and written informed consents were obtained from all participants prior to biological material collection in this study. All subsequent study analyses were conducted in accordance with the approved guidelines and regulations.

Consent for publication

Written informed consent was obtained from the patient for publication of this report.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no conflict of interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81460284, No. 81460051, No. 31560039) and Natural Science Foundation of Qinghai Provincial (No. 2014-ZJ-944Q), which provided financial support for the smooth progress of this research.

Author's Contributions

Yanli Zhao: conceived and designed the experiments;

Lining Si: performed the experiments;

Haiyuan Wang: analyzed the data;

Rong Wang: contributed reagents/materials/analysis tools;

Qifu Long: prepared the figures and/or tables;

Yanli Zhao: drafted the work or revised it critically for important content.

All authors have read and approved the manuscript.

Acknowledgements

We thank all participants for taking part in this study.

References

1. Basnyat, B. & Murdoch, D. R. High-altitude illness., **361**, 1967–1974 https://doi.org/10.1016/S0140-6736(03)13591-X (2003).
2. Maggiorini, M. High altitude-induced pulmonary oedema. *Cardiovasc Res*, **72**, 41–50 https://doi.org/10.1016/j.cardiores.2006.07.004 (2006).
3. Jin, T. *et al.* Association between the IL1R2 rs2072472 polymorphism and high-altitude pulmonary edema risk. *Mol Genet Genomic Med*, **7**, e542 https://doi.org/10.1002/mgg3.542 (2019).
4. Yang, Y. *et al.* NR3C2 Gene is Associated with Susceptibility to High-Altitude Pulmonary Edema in Han Chinese. *Wilderness Environ Med*, **29**, 488–492 https://doi.org/10.1016/j.wem.2018.07.006 (2018).
5. Yang, Y. *et al.* NR3C1 gene polymorphisms are associated with high-altitude pulmonary edema in Han Chinese. *J Physiol Anthropol*, **38**, 4 https://doi.org/10.1186/s40101-019-0194-1 (2019).
6. Wang, P., Ha, A. Y., Kidd, K. K., Koehle, M. S. & Rupert, J. L. A variant of the endothelial nitric oxide synthase gene (NOS3) associated with AMS susceptibility is less common in the Quechua, a high altitude Native population. *High Alt Med Biol*, **11**, 27–30 https://doi.org/10.1089/ham.2009.1054 (2010).
7. Zhuo, Y. *et al.* Functional polymorphism of lncRNA MALAT1 contributes to pulmonary arterial hypertension susceptibility in Chinese people. *Clin Chem Lab Med*, **55**, 38–46 https://doi.org/10.1515/cclm-2016-0056 (2017).
8. Zhu, H., Han, C. & Wu, T. MiR-17-92 cluster promotes hepatocarcinogenesis., **36**, 1213–1222 https://doi.org/10.1093/carcin/bgv112 (2015).
9. He, L. *et al.* A microRNA polycistron as a potential human oncogene. *Nature*, **435**, 828–833 https://doi.org/10.1038/nature03552 (2005).
10. Lu, Y., Thomson, J. M., Wong, H. Y., Hammond, S. M. & Hogan, B. L. Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Developmental biology*, **310**, 442–453 https://doi.org/10.1016/j.ydbio.2007.08.007 (2007).
11. Zhou, M., Ma, J., Chen, S., Chen, X. & Yu, X. MicroRNA-17-92 cluster regulates osteoblast proliferation and differentiation., **45**, 302–310 https://doi.org/10.1007/s12020-013-9986-y (2014).
12. Dews, M. *et al.* The myc-miR-17 ~ 92 axis blunts TGF{beta} signaling and production of multiple TGF{beta}-dependent antiangiogenic factors. *Cancer Res*, **70**, 8233–8246 https://doi.org/10.1158/0008-5472.CAN-10-2412 (2010).
13. Chacon-Cortes, D., Smith, R. A., Lea, R. A., Youl, P. H. & Griffiths, L. R. Association of microRNA 17–92 cluster host gene (MIR17HG) polymorphisms with breast cancer. *Tumour Biol*, **36**, 5369–5376 https://doi.org/10.1007/s13277-015-3200-1 (2015).
14. Chen, P. *et al.* Association between polymorphisms of MIR17HG and risk of colorectal cancer in the Chinese Han population. *Mol Genet Genomic Med*, **7**, e667 https://doi.org/10.1002/mgg3.667 (2019).
15. Wu, H. *et al.* Correlation of MicroRNA 17–92 Cluster Host Gene (MIR17HG) Polymorphisms With Susceptibility and Prognosis for Multiple Myeloma. *Clin Lymphoma Myeloma Leuk*, **19**, e359–e366 https://doi.org/10.1016/j.clml.2019.03.018 (2019).
16. Gabry, A. L., Ledoux, X., Mozziconacci, M. & Martin, C. High-altitude pulmonary edema at moderate altitude (< 2,400 m; 7,870 feet): a series of 52 patients., **123**, 49–53 https://doi.org/10.1378/chest.123.1.49 (2003).
17. He, X. *et al.* Long Non-coding RNA LINC-PINT and LINC00599 Polymorphisms are Associated With High-altitude Pulmonary Edema in Chinese. *Arch Bronconeumol*, https://doi.org/10.1016/j.arbres.2019.09.021 (2019).
18. Ji, M. *et al.* The miR-17-92 microRNA cluster is regulated by multiple mechanisms in B-cell malignancies. *Am J Pathol*, **179**, 1645–1656 https://doi.org/10.1016/j.ajpath.2011.06.008 (2011).
19. Yan, H. L. *et al.* Repression of the miR-17-92 cluster by p53 has an important function in hypoxia-induced apoptosis. *EMBO J*, **28**, 2719–2732 https://doi.org/10.1038/emboj.2009.214 (2009).
20. Tsai, S. H. *et al.* Roles of the hypoximir microRNA-424/322 in acute hypoxia and hypoxia-induced pulmonary vascular leakage. *FASEB J*, **33**, 12565–12575 https://doi.org/10.1096/fj.201900564RR (2019).
21. Chamorro-Jorganes, A. *et al.* VEGF-Induced Expression of miR-17-92 Cluster in Endothelial Cells Is Mediated by ERK/ELK1 Activation and Regulates Angiogenesis. *Circ Res*, **118**, 38–47 https://doi.org/10.1161/CIRCRESAHA.115.307408 (2016).