Association between a Single Nucleotide Polymorphism in the 3′-UTR of ARHGEF18 and the Risk of Nonidiopathic Pulmonary Arterial Hypertension in Chinese Population

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1. Introduction

Pulmonary arterial hypertension is a progressive and lethal disease which occurs at the small pulmonary arteries and is characterized by increased pulmonary vascular resistance due to vascular proliferation and remodeling [1]. Although growing studies revealed that multiple mechanisms contributed to disease development [2], the exact pathogenesis of PAH remains unclear. However, several experimental models have been used to understand the mechanism underlying the pathogenesis. The most commonly used model is the monocrotaline (MCT) rat model. In this model, MCT is injected subcutaneously and becomes metabolically activated, as a pyrrolizidine alkaloid, by hepatic cytochrome P450 3A [3, 4]. The active MCT pyrrole is pneumotoxic and damages the pulmonary artery endothelial cells (PAECs), which leads to a disturbed barrier function [5]. MCT could lead to pulmonary vascular remodeling by inducing arterial medial hyperplasia of the axial arteries, interstitial oedema, adventitial inflammation, haemorrhage, and fibrosis [6, 7]. Eventually, pulmonary vascular resistance increases and the right ventricle compensates by hypertrophy [8, 9]. In addition, the MCT PAH rat model, hypoxia is also widely used to study experimental PAH [10]. MCT- or hypoxia-treated animals can be induced to develop PAH suggesting that the expression level of certain genes could be altered.
These altered genes may be involved in the development of PAH.

PAH and coronary heart disease (CHD) are circulatory system diseases that may simultaneously emerge in a patient and they are often treated together in clinical practices. CHD is common among patients with PAH [11]. A previous study has identified 29 overlapping genes between the two diseases which demonstrated that genetic similarity existed [12]. However, the occurrence and impact of CHD in patients with PAH are still unknown.

In the year 2000, ARHGEF18 (p114RhoGEF), a novel guanine nucleotide exchange factor (GEF) for Rho GTPases which is widely expressed in human tissues, was identified [13]. ARHGEF18 regulates the activity of RhoA and Rac1, and Gβγ subunits of heterotrimeric G proteins are activators of ARHGEF18. The results demonstrated the role played by ARHGEF18 in actin stress fiber formation, cell shape change, and reactive oxygen species (ROS) production [14]. ARHGEF18 is a component of a junction-associated Rho signaling module that drives spatially restricted activation of RhoA to regulate junction formation and epithelial morphogenesis [15]. ARHGEF18 controls RhoA activity in the human bronchial epithelial cell line 16HBE14o- (16HBE) to promote apical junction assembly. The abnormal ARHGEF18 expression level and DNA variants in the gene have been associated with different diseases, including adult-onset retinal degeneration, systemic capillary leak syndrome, and squamous-cell lung carcinoma [16–18].

In the present study, 293 participants were recruited, including 117 niPAH patients and 176 healthy control subjects. A genetic epidemiological study is cost-effective for exploring the association between genetic variation and diseases. This study is aimed at investigating the influence of ARHGEF18 polymorphism on the prevalence of niPAH.

2. Materials and Methods

2.1. Subjects. The present study was composed of 117 cases of niPAH and 176 controls. Patients were consecutively recruited from the Affiliated Hospital of Jiangsu University between May 2014 and July 2017. Clinical information was obtained from pathological records, including gender, age, drinking habits, smoking, and CHD history. Baseline profiles of the study populations are summarized in Table 1. The control subjects were collected from healthy volunteers who visited the Sir Run Run Hospital Nanjing Medical University for medical examination during the same period. Informed consent was obtained from each participant.

2.2. SNP Selection. The SNPinfo web tool was employed to screen the potential functional SNPs within the ARHGEF18 gene. We mainly focused on the 3′-UTR region, which could be a potential functional region. Among the 10 potential functional SNPs, rs3745357 and rs1043412 have a minor allele frequency >0.1 in the Han Chinese population. The two SNPs are also in complete linkage disequilibrium. To the best of our knowledge, no studies have examined the role of rs3745357 or rs1043412 polymorphisms within ARHGEF18 in the development of PAH so far.

2.3. Genotyping. Genomic DNA was extracted from 200 μl of EDTA-anticoagulated peripheral blood using a commercial extraction kit (Tiangen Biotech Corporation, Beijing, China) according to the instruction manual. We performed a polymerase chain reaction-restriction fragment length polymorphism assay to detect the genotype of the SNP. Primer sequence was forward: \(5′-AGAGAACATTCCCAGACCC \) and reverse: \(5′-AGAGAACATTCCCAGACCC \). The restriction enzyme used was Hpy188I, and its C allele is cuttable, yielding two fragments of 98 bp and 59 bp (Figure 1). About 10% of the samples were randomly selected to perform repeated assays and acquire the results.

2.4. Statistical Analysis. All data were analyzed using SPSS 13 (SPSS Inc., Chicago, IL). Genotype frequencies of the SNP were obtained by directed computing. Genotypic association analysis in a case-control pattern assuming codominant, dominant, recessive, and overdominant genetic models was performed using SNPstats. Odds ratio (OR) and respective 95% confidence intervals were reported to evaluate the effects
of any differences between allele and genotype frequencies. A probability of 0.05 or less was regarded as statistically significant in patients with PAH compared to healthy controls.

3. Results

To confirm the expression level of ARHGEF18, we screened the expression profiling result published on the GEO database, which was produced by using Agilent-028282 Whole Rat Genome Microarray (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE72707). The dataset profiled the lungs of adult male Sprague-Dawley rats kept for 4 weeks in normal atmospheric conditions or in hypobaric hypoxia (380 mmHg), or injected with 40 mg/kg MCT. The ARHGEF18 expression value was increased both when treated with hypoxia (logFC = 6.958, \( p = 2.96 \times 10^{-6} \)) or MCT (logFC = 4.67, \( p = 1.59 \times 10^{-4} \)), respectively (Figure 2).

The ARHGEF18 3′-UTR variant rs3745357 was successfully genotyped in 117 niPAH patients and 176 healthy controls. When the niPAH patients were divided as those with or without the history of CHD, significant differences were observed between the patients and controls. In niPAH patients without CHD history, the frequency of C allele carriers was significantly higher than the controls. Conversely, in the niPAH patients with CHD history, the frequency of C allele carriers was significantly lower than the controls (Table 2). As shown in Table 3, a significantly increased niPAH risk was associated with the CC genotype (OR = 3.94, 95% CI = 1.58–9.80, \( p = 0.0053 \)) when compared to the TT genotype. In the dominant model, CT/CC genotype carriers had a 2.34-fold decreased niPAH susceptibility compared to TT genotype carriers. In the recessive model, CC genotype carriers also had a 2.93-fold increased risk to develop PAH compared to TT/CT genotype carriers.

Table 4 lists the differences between the PAH patients with CHD history and controls. In contrast to the patients without CHD history, CT or CT/CC genotype carriers were observed having significantly decreased niPAH risk when compared to TT or TT/CC genotypes in codominant (CT versus TT: OR = 0.38, 95% CI = 0.20–0.72, \( p = 0.0092 \)), dominant (CT/CC versus TT: OR = 0.43, 95% CI = 0.24–0.75, \( p = 0.0031 \)), and overdominant (CT versus TT/CC: OR = 0.47, 95% CI = 0.26–0.85, \( p = 0.011 \)) models, respectively. When we further adjusted the results by sex and age, significant differences were also observed between patients and controls in codominant (CC versus TT: OR = 0.11, 95% CI = 0.01–0.93, \( p = 0.031 \)) models, respectively.

4. Discussion

ARHGEF18 encodes ARHGEF18 (also known as p114Rho-GEF), the Rho/Rac guanine nucleotide exchange factor 18. The gene is essential for podocyte cytoskeletons and plays diverse roles in regulating collective cell migration [19, 20]. Kim et al. has reported that ARHGEF18 governs cell motility and lumen formation during tubulogenesis through a ROCK-myosin-II pathway [21]. Other studies revealed that ARHGEF18 could regulate the Rho signaling pathway [15, 22–25]. Abnormal ARHGEF18 expression levels and DNA variants in the gene have been associated with different diseases, including adult-onset retinal degeneration, systemic capillary leak syndrome, and squamous-cell lung carcinoma [16–18]. Evidence that the Rho kinase pathway contributes to vasoconstriction in PAH is demonstrated by the effects of Rho kinase inhibitors to acutely reduce PAH in both chronically hypoxic rats and neonatal rats with PAH [26–29]. Rho kinase-mediated Ca\(^{2+}\) sensitization also plays an important role in mediating enhanced basal pulmonary arterial tone as well as agonist- and depolarization-dependent vasoconstriction in small pulmonary arteries from animal models.

Previous studies identified numerous biological characteristics of PAH and CHD. There were 29 overlapping genes with genetic similarity between PAH and CHD. The existing data strongly suggested that CHD might have an impact on the patients with PAH. Rs3745357 is located in the 3′-UTR region of ARHGEF18. In many genes, the 3′-UTR plays
an important role in regulating transcription and protein expression. SNPs in the 3′-UTR region may be able to influence either of these processes in protein expression. In the present study, we have provided primary evidence that the C allele of the rs3745357 polymorphism is more frequent in PAH patients without CHD history, while it is less frequent in PAH patients with CHD history, compared with control subjects. Moreover, the frequencies of the CT/CC genotypes of the rs3745357 polymorphism were significant lower in patients with CHD history after adjustment for sex and age. The present results suggested that the T allele of rs3745357 in ARHGEF18 is a protective factor for the initiation of PAH when the patients do not have CHD history. As for the patients who have CHD history, the T allele turned out to be a risk factor for the PAH susceptibility.

In summary, it is biologically plausible that the rs3745357 variant in ARHGEF18 may have an effect on individual susceptibility to pulmonary hypertension. The effect was contrary to that of the patients with or without CHD history. The results obtained in the study suggest that the ARHGEF18 rs3745357 variant may be useful as a marker to reflect the genetic susceptibility to nPAH.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

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**Table 2: Allele frequencies of rs3745357 in the ARHGEF18 gene among PAH patients and controls.**

| Allele | PAH patients<sup>a</sup> Allele numbers (%) | PAH patients<sup>b</sup> Allele numbers (%) | PAH patients<sup>all</sup> Allele numbers (%) | Controls Allele numbers (%) | Overall patients versus controls | P value PAH<sup>a</sup> versus controls | P value PAH<sup>b</sup> versus controls |
|--------|------------------------------------------|------------------------------------------|------------------------------------------|----------------------------|--------------------------------|--------------------------------|--------------------------------|
| C      | 106 (45.3)                               | 60 (63.8)                                | 46 (32.9)                                | 157 (45.9)                | 0.932                          | **0.001**                     | **0.017**                      |
| T      | 128 (54.7)                               | 34 (36.2)                                | 94 (67.1)                                | 195 (54.1)                |                                 |                                |                                |

<sup>a</sup>PAH patients without history of coronary heart disease; <sup>b</sup>PAH patients with history of coronary heart disease.

**Table 3: Association between the ARHGEF18 rs3745357 polymorphism and risk of pulmonary hypertension without history of coronary heart disease.**

| Genetic model | Genotype | Patients<sup>a</sup> N = 47 | Control N = 176 | Logistic regression OR (95% CI) | P value | Logistic regression (adjusted)<sup>c</sup> OR (95% CI) | P value |
|---------------|----------|-----------------------------|-----------------|-------------------------------|---------|--------------------------------------------------------|---------|
| Codominant    | TT       | 8 (17)                      | 57 (32.4)       | 1.00                          |         | 1.00                                                   |         |
|               | CT       | 18 (38.3)                   | 81 (46)         | 1.58 (0.64–3.89)              | **0.0053** | 1.65 (0.38–7.17)                                       | 0.13    |
|               | CC       | 21 (44.7)                   | 38 (21.6)       | **3.94 (1.58–9.80)**          | **0.0003** | 4.10 (0.90–18.68)                                      |         |
| Dominant      | CT/CC    | 26 (55.3)                   | 119 (78.5)      | **2.34 (1.02–5.32)**          | **0.032** | 2.48 (0.64–9.60)                                       | 0.18    |
| Recessive     | TT/CT    | 39 (83)                     | 138 (70.2)      | 1.00                          |         | 1.00                                                   |         |
|               | CC       | 8 (17)                      | 38 (29.8)       | **2.93 (1.49–5.78)**          | **0.0021** | 2.90 (0.97–8.73)                                       | 0.055   |
| Overdominant  | TT/CC    | 29 (61.7)                   | 62 (51.2)       | 1.00                          |         | 1.00                                                   |         |
|               | CT       | 18 (38.3)                   | 59 (48.8)       | 0.73 (0.38–1.41)              | 0.34    | 0.67 (0.23–1.94)                                       | 0.46    |

<sup>a</sup>PAH patients without history of coronary heart disease; <sup>c</sup>adjusted by sex and age.

**Table 4: Association between the ARHGEF18 rs3745357 polymorphism and risk of pulmonary hypertension with history of coronary heart disease.**

| Genetic model | Genotype | Patients<sup>b</sup> N = 70 | Control N = 176 | Logistic regression OR (95% CI) | P value | Logistic regression (adjusted)<sup>c</sup> OR (95% CI) | P value |
|---------------|----------|-----------------------------|-----------------|-------------------------------|---------|--------------------------------------------------------|---------|
| Codominant    | TT       | 37 (52.9)                   | 57 (32.4)       | 1.00                          |         | 1.00                                                   |         |
|               | CT       | 20 (28.6)                   | 81 (46)         | **0.38 (0.20–0.72)**          | **0.0092** | 0.14 (0.02–1.02)                                       | 0.011   |
|               | CC       | 13 (18.6)                   | 38 (21.6)       | 0.53 (0.25–1.12)              |         | **0.03 (0.00–0.46)**                                   |         |
| Dominant      | CT/CC    | 33 (47.1)                   | 119 (67.6)      | **0.43 (0.24–0.75)**          | **0.0031** | 0.10 (0.02–0.69)                                       | 0.009   |
| Recessive     | TT/CT    | 33 (47.1)                   | 119 (78.4)      | 1.00                          |         | 1.00                                                   |         |
|               | CC       | 13 (18.6)                   | 38 (21.6)       | 0.83 (0.41–1.67)              | 0.6     | **0.11 (0.01–0.93)**                                   | 0.031   |
| Overdominant  | TT/CC    | 50 (71.4)                   | 95 (54)         | 1.00                          |         | 1.00                                                   |         |
|               | CT       | 20 (28.6)                   | 81 (46)         | **0.47 (0.26–0.85)**          | **0.011** | 0.54 (0.12–2.44)                                       | 0.42    |

<sup>b</sup>PAH patients with history of coronary heart disease; <sup>c</sup>adjusted by sex and age.
References

[1] S. A. Barman, F. Chen, X. Li et al., “Galectin-3 promotes vascular remodeling and contributes to pulmonary hypertension,” American Journal of Respiratory and Critical Care Medicine, vol. 197, no. 11, pp. 1488–1492, 2018.

[2] Y. C. Lai, K. C. Potoka, H. C. Champion, A. L. Mora, and M. T. Gladwin, “Pulmonary arterial hypertension: the clinical syndrome,” Circulation Research, vol. 115, no. 1, pp. 115–130, 2014.

[3] R. J. Huxtable, “Activation and pulmonary toxicity of pyrrolizidine alkaloids,” Pharmacology & Therapeutics, vol. 47, no. 3, pp. 371–389, 1990.

[4] M. E. Campian, M. Hardzienka, M. C. Michel, and H. L. Tan, “How valid are animal models to evaluate treatments for pulmonary hypertension?,” Naunyn-Schmiedeberg’s Archives of Pharmacology, vol. 373, no. 6, pp. 391–400, 2006.

[5] J. G. Gomez-Arroyo, L. Farkas, A. A. Alhussaini et al., “The monocrotaline model of pulmonary hypertension in perspective,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 302, no. 4, pp. L363–L369, 2012.

[6] Y.-S. Lee, J. Byun, J.-A. Kim et al., “Monocrotaline-induced pulmonary hypertension correlates with upregulation of connective tissue growth factor expression in the lung,” Experimental & Molecular Medicine, vol. 37, no. 1, pp. 27–35, 2005.

[7] C. Jardim, M. Humbert, and R. Souza, “Idiopathic pulmonary arterial hypertension,” Seminars in Respiratory and Critical Care Medicine, vol. 34, no. 5, pp. 560–567, 2013.

[8] M. H. M. Hessel, P. Steendijk, B. den Adel, C. I. Schutte, and A. van der Laarse, “Characterization of right ventricular function after monocrotaline-induced pulmonary hypertension in the intact rat,” American Journal of Physiology-Heart and Circulatory Physiology, vol. 291, no. 5, pp. H2424–H2430, 2006.

[9] M. L. Handoko, F. S. de Man, C. M. Happe et al., “Opposite effects of training in rats with stable and progressive pulmonary hypertension,” Circulation, vol. 120, no. 1, pp. 42–49, 2009.

[10] K. R. Stenmark, B. Meyrick, N. Galie, W. J. Mooi, and I. F. McMurtry, “Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 297, no. 6, pp. L1013–L1032, 2009.

[11] A. Shimony, M. J. Eisenberg, L. G. Rudski et al., “Prevalence and impact of coronary artery disease in patients with pulmonary arterial hypertension,” The American Journal of Cardiology, vol. 108, no. 3, pp. 460–464, 2011.

[12] Y. Yuan, Y. Zhang, X. Zhang et al., “Deciphering the genetic and modular connections between coronary heart disease, idiopathic pulmonary arterial hypertension and pulmonary heart disease,” Molecular Medicine Reports, vol. 14, no. 1, pp. 661–670, 2016.

[13] A. Blomquist, G. Schwörer, H. Schabloksi et al., “Identification and characterization of a novel Rho-specific guanine nucleotide exchange factor,” Biochemical Journal, vol. 352, no. 2, pp. 319–325, 2000.

[14] J. Niu, J. Profirovic, H. Pan, R. Vaiskunaita, and T. Voyno-Yasenetskaya, “G protein β subunits stimulate p114RhoGEF, a guanine nucleotide exchange factor for RhoA and Rac1,” Circulation Research, vol. 93, no. 9, pp. 848–856, 2003.

[15] S. J. Terry, C. Zihni, A. Elbediwy et al., “Spatially restricted activation of RhoA signalling at epithelial junctions by p114RhoGEF drives junction formation and morphogenesis,” Nature Cell Biology, vol. 13, no. 2, pp. 159–166, 2011.

[16] C. Song, Y. Gao, Y. Tian, X. Han, Y. Chen, and D. L. Tian, “Expression of p114RhoGEF predicts lymph node metastasis and poor survival of squamous-cell lung carcinoma patients,” Tumor Biology, vol. 34, no. 3, pp. 1925–1933, 2013.

[17] Z. Xie, V. Nagarajan, D. E. Sturdevant et al., “Genome-wide SNP analysis of the systemic capillary leak syndrome (Clarkson disease),” Rare Diseases, vol. 1, no. 1, 2013.

[18] G. Arno, K. J. Carss, S. Hull et al., “Biallelic mutation of ARHGEF18, involved in the determination of epithelial apicolabasal polarity, causes adult-onset retinal degeneration,” The American Journal of Human Genetics, vol. 100, no. 2, pp. 334–342, 2017.

[19] Y. Lu, Y. Ye, W. Bao et al., “Genome-wide identification of genes essential for podocyte cytoskeletons based on single-cell RNA sequencing,” Kidney International, vol. 92, no. 5, pp. 1119–1129, 2017.

[20] A. Zaritsky, Y.-Y. Tseng, M. Angeles Rabadán et al., “Diverse roles of guanine nucleotide exchange factors in regulating collective cell migration,” The Journal of Cell Biology, vol. 216, no. 6, pp. 1543–1556, 2017.

[21] M. Kim, A. M. Shewan, A. J. Ewald, Z. Werb, and K. E. Mostov, “p114RhoGEF governs cell motility and lumen formation during tubulogenesis through a ROCK–myosin-II pathway,” Journal of Cell Science, vol. 128, no. 23, pp. 4317–4327, 2015.

[22] F. Loosli, “ArhGEF18 regulated Rho signaling in vertebrate retina development,” Small GTPases, vol. 4, no. 4, pp. 242–246, 2013.

[23] C. Herder, J. M. Swierz, C. Müller et al., “ArhGEF18 regulates RhoA-Rock2 signaling to maintain neuro-epithelial apicobasal polarity and proliferation,” Development, vol. 140, no. 13, pp. 2787–2797, 2013.

[24] X. Xu, D. Jin, J. Durgan, and A. Hall, “LKB1 controls human bronchial epithelial morphogenesis through p114RhoGEF-dependent RhoA activation,” Molecular and Cellular Biology, vol. 33, no. 14, pp. 2671–2682, 2013.

[25] K.-I. Nagata and M. Inagaki, “Cytoskeletal modification of Rho guanine nucleotide exchange factor activity: identification of a Rho guanine nucleotide exchange factor as a binding
partner for Sept9b, a mammalian septin,” Oncogene, vol. 24, no. 1, pp. 65–76, 2005.

[26] K. A. Fagan, M. Oka, N. R. Bauer et al., “Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of rho-kinase,” American Journal of Physiology. Lung Cellular and Molecular Physiology, vol. 287, no. 4, pp. L656–L664, 2004.

[27] J. M. Hyvelin, K. Howell, A. Nichol, C. M. Costello, R. J. Preston, and P. McLoughlin, “Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation,” Circulation Research, vol. 97, no. 2, pp. 185–191, 2005.

[28] P. J. McNamara, P. Murthy, C. Kantores et al., “Acute vasodilator effects of Rho-kinase inhibitors in neonatal rats with pulmonary hypertension unresponsive to nitric oxide,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 294, no. 2, pp. L205–L213, 2008.

[29] T. Nagaoka, Y. Morio, N. Casanova et al., “Rho/Rho kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats,” American Journal of Physiology-Lung Cellular and Molecular Physiology, vol. 287, no. 4, pp. L665–L672, 2004.