CBLN2 rs2217560 was Associated with Pulmonary Arterial Hypertension in Systemic Lupus Erythematosus

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To the Editor: Pulmonary arterial hypertension (PAH) is a hemodynamic disorder with elevated pressure of pulmonary circulation. Genetic studies in familial PAH (fPAH) and idiopathic PAH (iPAH) have discovered that transforming growth factor-β (TGF-β) superfamily plays an important role, and the identified mutations occur in bone morphogenetic protein type 2 receptor (*BMPR2*), activin receptor-like kinase type 1 (*ALK1*), *Endoglin,* and *SMAD9*.[1] A genome-wide association study (GWAS) in patients without *BMPR2* mutations discovered that one single-nucleotide polymorphism (SNP) rs2217560 had a significant association with i/fPAH, which located 52-kb downstream of the *CBLN2* gene.[2] A major cause of PAH is connective tissue diseases (CTDs), including systemic sclerosis (SSc) and systemic lupus erythematosus (SLE). Even though CTD-associated PAH (CTD-PAH) constitutes 25% of PAH cases, little has been done on related genetic study. SSc-associated PAH (SSc-PAH) has higher occurance in Western countries, while in China, SLE-PAH consists over 50% of CTD-PAH.[3] Nearly 4% of lupus patients suffer from PAH, and PAH is one of the leading causes of death for SLE.[4] We conducted this research to explore the genetic susceptibility of PAH in SLE.

Totally 87 SLE-PAH patients were included based on a clinical registry in Peking Union Medical College Hospital, China.[5] PAH was diagnosed by right heart catheterization. About 96.3% of the patients are female. The average age is 34.4 ± 8.1 years. SLE-PAH patients present with mean pulmonary arterial pressure, 46 mmHg; pulmonary vascular resistance, 10 wood units; and cardiac index, 2.7 L/min·m^2. The control group was selected by choosing admitted SLE-non-PAH patients simultaneously, and PAH was ruled out by echocardiogram (estimated PAP <40 mmHg). The SLE-non-PAH group consisted of 166 patients, with a female percentage of 90.6% and average age of 30.9 ± 10.8 years. SNP selection was based on a review of literature, allelic frequency, and functional position. *CBLN2* (rs2217560), *BMPR2* (rs34135567, rs140683387, and rs2228545), *ALK1* (rs2277382), *Endoglin* (rs45608833 and rs35400405), and *SMAD9* (rs141647648) were genotyped by matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry.

Four out of eight SNPs (rs2217560, rs34135567, rs2277382, and rs141647648) showed heterogeneity. The association between SNP allelic/genotypic frequencies and disease onset was listed in Table 1.

Rs2217560 (G>A) lies 52-kb downstream of the *CBLN2* gene, within the transcriptional regulatory region. Rs2217560 G-allele was more frequent in SLE-PAH group than in SLE-non-PAH group (27% vs. 20%); genotypic frequency of GG/GA/AA was 4/36/41 versus 10/37/94 and was statistically significant (χ^2 = 7.742, P = 0.021). Dominant and additive hereditary models were further tested for rs2217560, and G-allele was found associated with PAH onset with odds ratio (*OR*) of 1.951 in dominant model (95% confidence interval [*CI*] = 1.116–3.412, P = 0.019).

Rs2277382 (C>T) is located in 5'-UTR of *ALK1* gene. In a previous small-scale study in SSc-PAH patients, rs2277382 was detected only in SSc-PAH patients, but replication set did not prove association with SSc-PAH.[6] In our study, there was a difference between the frequency of allele in SLE-PAH patients compared with SLE-non-PAH patients (9% vs. 6.1%); however, it was not statistically significant (*OR* = 1.542, 95% *CI* = 0.762–3.121, P = 0.228).

Rs34135567 is a deletion/CAA variation in 3'-UTR of *BMPR2*. There was no statistical association with PAH onset (*OR* = 3.815, 95% *CI* = 0.343–42.388, P = 0.276; χ^2 = 1.381, P = 0.275).

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Table 1: Association between SNP allelic/genotypic frequencies and disease onset

Rs	Group	Allele		*OR*	*P*	Genotype			χ^2	*P*
CBLN2		**G**	**A**			**GG**	**GA**	**AA**		
rs2217560	PAH	44 (27.2%)	118 (72.8%)	1.472 (0.937–2.313)	0.093	4 (4.9%)	36 (44.4%)	41 (50.6%)	7.742	0.021
	Non-PAH	57 (20.2%)	225 (79.8%)			10 (7.1%)	37 (26.2%)	94 (66.7%)		
ALK1		**T**	**C**			**TT**	**TC**	**CC**		
rs2277382	PAH	15 (9.0%)	151 (91.0%)	1.542 (0.762–3.121)	0.228	1 (1.2%)	13 (15.7%)	69 (83.1%)	2.551	0.279
	Non-PAH	19 (6.1%)	295 (93.9%)			0 (0.0%)	19 (12.1%)	138 (87.9%)		
BMPR2		**D**	**CAA**			**D/CAA**	**CAA/CAA**			
rs34135567	PAH	2 (1.2%)	162 (98.8%)	3.815 (0.343–42.388)	0.276	2 (2.4%)	80 (97.6%)		1.381	0.275
	Non-PAH	1 (0.3%)	309 (99.7%)			1 (0.6%)	154 (99.4%)			
SMAD9		**D**	**AGATTA**			**D/AGATTA**	**AGATTA/AGATTA**			
rs141647648	PAH	3 (1.8%)	165 (98.2%)	0.813 (0.207–3.185)	0.766	3 (3.6%)	81 (96.4%)		0.090	0.529
	Non-PAH	7 (2.2%)	313 (97.8%)			7 (4.4%)	153 (95.6%)			

All values were expressed as *n* (%). SNP: Single-nucleotide polymorphism; *OR*: Odds ratio; PAH: Pulmonary arterial hypertension.

Rs141647648 is a deletion/AGATTA in 3'-UTR of *SMAD9*. Allelic frequency and genotypic frequency in SLE-PAH and SLE-non-PAH group were 1.8% versus 2.2% (*OR* = 0.813, 95% *CI* = 0.207–3.185, *P* = 0.766) and 3.6% versus 4.4% (χ^2 = 0.09, *P* = 0.529), respectively.

This study attempted to explore the genetic susceptibility of PAH development in SLE. *CBLN2* rs2217560 G allele was associated with an increased risk of 1.97 in a GWAS for iPAH and fPAH,[2] which is in consistent with our result (*OR* = 1.951, 95% *CI* = 1.116–3.412, *P* = 0.019). *CBLN2* gene encodes neuronal glycoprotein, which mainly expresses in the brain; it is also expressed in the lung, particularly in pulmonary vascular endothelial cells. *CBLN2* peptide acts on vascular smooth muscle cell proliferation in a paracrine fashion and participates in pulmonary hypertension.[2] We do not yet know the role of *CBLN2* in lupus patients who developed PAH, but our results suggested that different etiologies lead to PAH in the same mechanism and *CBLN2* might be a component of the common pathway.

Even though TGF-β signaling pathway has been proved to play an important role in fPAH and iPAH onset, previous studies on SSc-PAH did not report an association. The research on the Asian SLE-PAH population has regional characteristics; nevertheless, we could not find correlation of selected SNPs with PAH onset either. Considering that SSc and SLE both belong to the CTD spectrum, it is likely that PAH onset in CTD patients is distinct from IPAH patients and might have other genetic polymorphisms.

However, the sample number of SLE-PAH patients is still a limitation for the research to be more conclusive. According to the Genetic Power Calculator, over 560 samples were needed statistically. Further study with large database would be needed in order to obtain more genetic information on SLE-PAH patients.

In conclusion, the SNP discovered in GWAS in fPAH and iPAH was proved to be associated with SLE-PAH, which revealed an important role of *CBLN2* in PAH onset despite different etiologies. So far, we have not identified a correlation between the eight tested SNPs in TGF-β pathway and SLE-PAH onset. Further studies would be needed to reveal mechanism of PAH onset, and hopefully, more genetic study could provide a possibility for CTD-PAH early diagnosis and treatment.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

References

1. Soubrier F, Chung WK, Machado R, Grünig E, Aldred M, Geraci M, *et al.* Genetics and genomics of pulmonary arterial hypertension. J Am Coll Cardiol 2013;62:D13-21. doi: 10.1016/j.jacc.2013.10.035.
2. Germain M, Eyries M, Montani D, Poirier O, Girerd B, Dorfmüller P, *et al.* Genome-wide association analysis identifies a susceptibility locus for pulmonary arterial hypertension. Nat Genet 2013;45:518-21. doi: 10.1038/ng.2581.
3. Hao YJ, Jiang X, Zhou W, Wang Y, Gao L, Wang Y, *et al.* Connective tissue disease-associated pulmonary arterial hypertension in Chinese patients. Eur Respir J 2014;44:963-72. doi: 10.1183/09031936.00182813.
4. Li M, Wang Q, Zhao J, Li Z, Ye Z, Li C, *et al.* Chinese SLE treatment and research group (CSTAR) registry: II. Prevalence and risk factors of pulmonary arterial hypertension in Chinese patients with systemic lupus erythematosus. Lupus 2014;23:1085-91. doi: 10.1177/0961203314527366.
5. Huang C, Li M, Liu Y, Wang Q, Guo X, Zhao J, *et al.* Baseline characteristics and risk factors of pulmonary arterial hypertension in systemic lupus erythematosus patients. Medicine (Baltimore) 2016;95:e2761. doi: 10.1097/md.0000000000002761.
6. Koumakis E, Wipff J, Dieudé P, Ruiz B, Bouaziz M, Revillod L, *et al.* TGFβ receptor gene variants in systemic sclerosis-related pulmonary arterial hypertension: Results from a multicentre EUSTAR study of European Caucasian patients. Ann Rheum Dis 2012;71:1900-3. doi: 10.1136/annrheumdis-2012-201755.