Germline BMP9 mutation causes idiopathic pulmonary arterial hypertension: a perspective exome sequencing based study.

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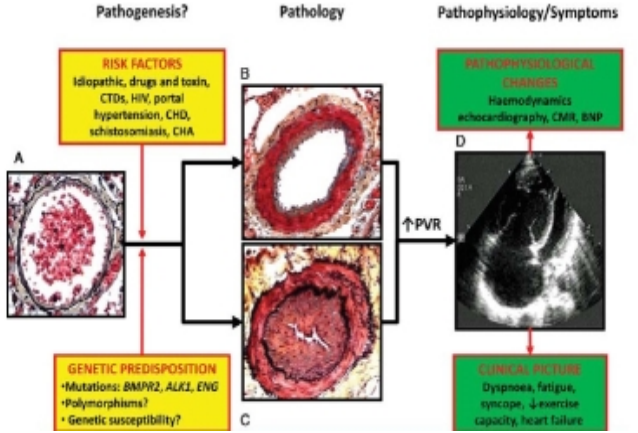
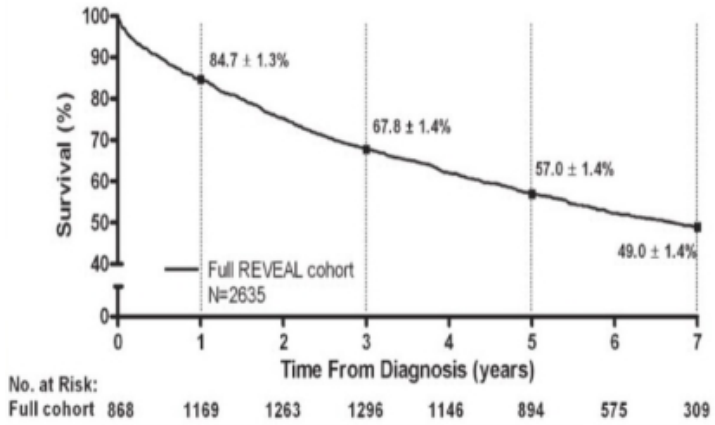
Declaration of interest

- I have nothing to declare

Idiopathic pulmonary arterial hypertension

- **Idiopathic pulmonary arterial hypertension (IPAH)**: a subgroup of pulmonary arterial hypertension, referring to individuals without any family history or associated conditions.
- **Prevalence**: Prevalence, 15–50/million; Incidence, 6/million/year.
- **The pathogenic mechanism remains largely unknown.**
- **The prognosis is poor**: 5-year survival rate for IPAH patients was still around 50%.



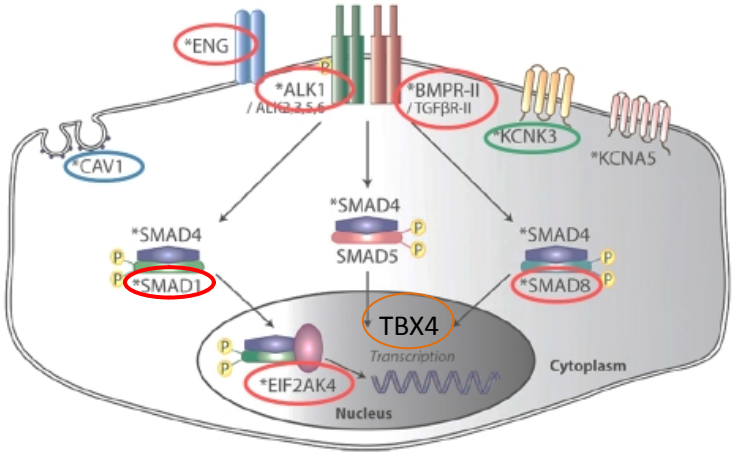


Jing ZC, et al. *Chest.* 2011;140:301-309. *Ann Internal Med.* 1991;115:343-349. *Am J Respir Crit Care Med.* 2006;173:1023-1030. *Chest.* 2010;137:376-387.

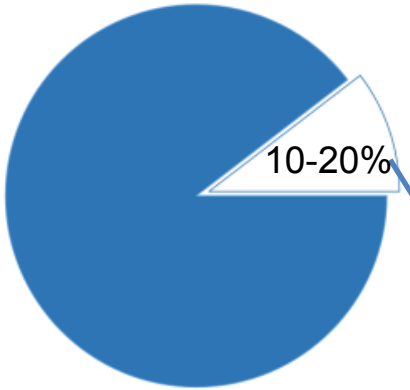
Genetic mutation in IPAH

- Genetic mutation plays a crucial role in the initiation and development of PAH.
- At least 10 PAH-predisposing genes have been identified as family-based genetic approaches.

The PAH disease-causing genes

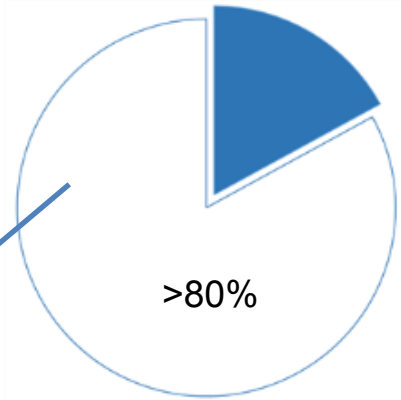


Familial PAH



could not find mutations in the 10 known genes

Idiopathic PAH



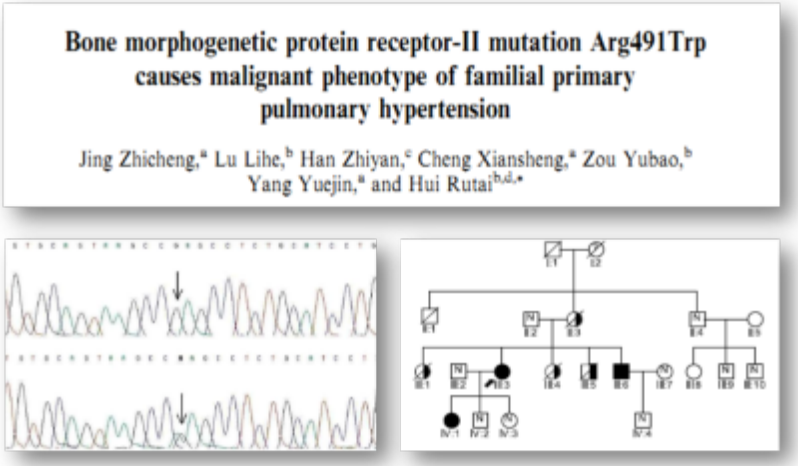
The big question: What are the novel IPAH disease-causing genes?

Eur Respir J. 2016;47:388-389. *Hum Mutat.* 2015;36:1113-1127. Liu D. Jing ZC et al. *Eur Respir J.* 2012;39:597-603.

The 1st HPAH pedigree in China

The 1ST HPAH Pedigree - 2002

The 1st International peer reviewed publication on PAH genetic study from China-2004



Bone morphogenetic protein receptor-II mutation Arg491Trp causes malignant phenotype of familial primary pulmonary hypertension

Jing Zhicheng,[a] Lu Lihe,[b] Han Zhiyan,[c] Cheng Xiansheng,[a] Zou Yubao,[b] Yang Yuejin,[a] and Hui Rutai[b,d,*]

Jing ZC et.al. *Biochem Biophys Res Commun*. 2004;315:1033-1038.

Study cohort and biobank (2002-2018)

- Patients with pulmonary vascular disease： > 4000
- DNA samples：> 8,500.
- Plasma/ Serum samples: > 200,000 (Longitudinal visit: one patient, multiple samples).
- Immortalized lymphocyte: > 400
- Induced pluripotent stem cells (iPSCs) bank: 10 patients.

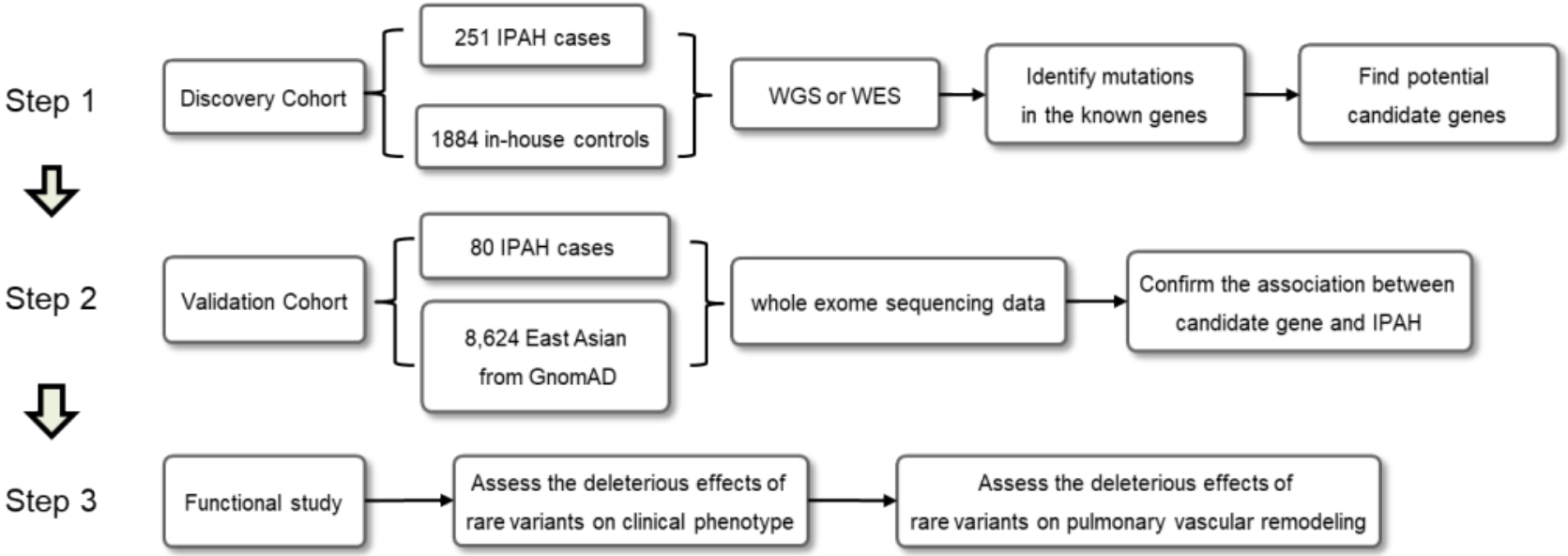
Ultra Cold Freezer

Liquid Nitrogen Tank

Clinical Data

Study design

Aim: Since 2014, we started to explore the novel IPAH disease-causing gene.



The discovery cohort

Characteristics	Shanghai Pulmonary Hospital (N = 122)	FuWai Hospital (N = 129)
Age at diagnosis, years	27.4 ± 9.0	27.0 ± 12.7
Female sex, no. (%)	94 (77.0)	103 (79.8)
Right atrium pressures, mm Hg	9.3 ± 6.3	8.1 ± 4.5
Mean pulmonary artery pressure, mm Hg	64.6 ± 13.7	62.5 ± 15.8
Pulmonary artery wedge pressure, mm Hg	8.5 ± 3.1	9.2 ± 3.0
Cardiac index, L/min/m^2	2.6 ± 1.2	2.3 ± 0.7
Pulmonary vascular resistance, Wood units	17.4 ± 9.4	15.7 ± 6.5
Mixed venous oxygen saturation, %	61.5 ± 12.1	64.4 ± 9.2

Analyze the known PAH-predisposing genes

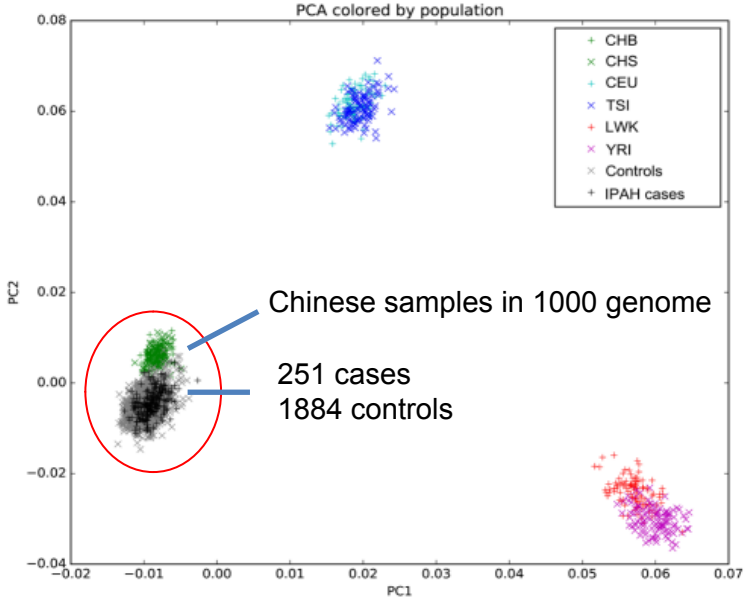
Mono-gene mutation	Discovery cohort (n = 251)
BMPR2, no. (%)	49 (19.5)
ACVRL1, no. (%)	15 (6.0)
TBX4, no. (%)	10 (4.0)
BMPR1B, no. (%)	1 (0.4)
KCNK3, no. (%)	1 (0.4)
SMAD9, no. (%)	1 (0.4)
SMAD1, no. (%)	2 (0.8)
EIF2AK4, no. (%)	4 (1.6)

The pathogenic mutations of 10 PAH-predisposing genes was identified in 91 patients, corresponding to 36% of all cases.

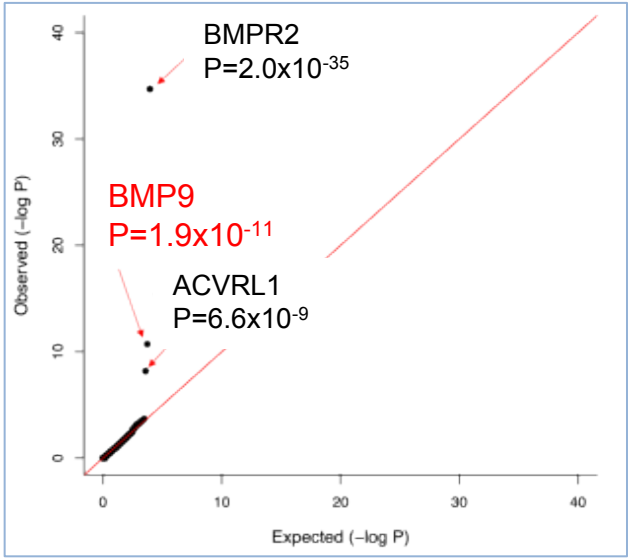
Double-gene mutations	Discovery cohort (n = 251)
BMPR2 & ACVRL1, no. (%)	1 (0.4)
BMPR2 & BMPR1B, no. (%)	3 (1.2)
BMPR2 & SMAD9, no. (%)	2 (0.8)
BMPR2 & TBX4, no. (%)	1 (0.4)
ACVRL1& TBX4, no. (%)	1 (0.4)

Identify *BMP9* as an IPAH causative gene in the discovery cohort

Analysis for ancestry of cases and controls in the discovery cohort



Quantile-quantile Plot of Discovery Cohort for Dominant Model.

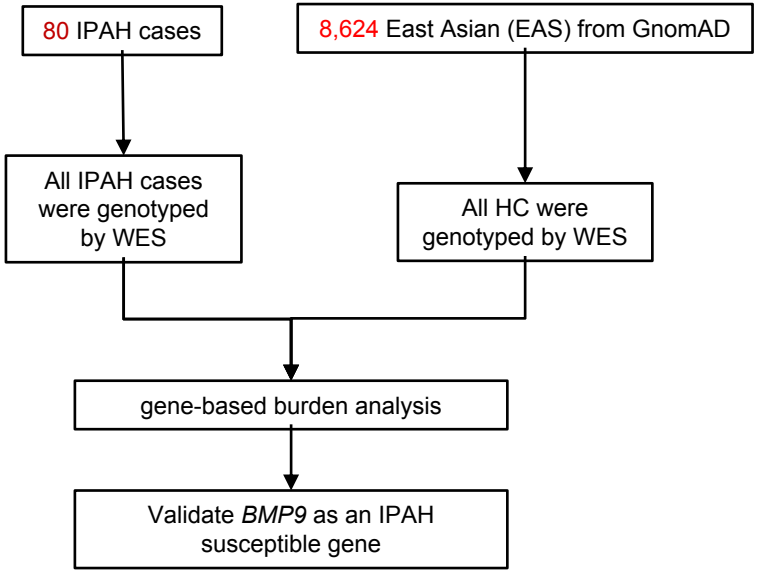


Rare deleterious variants found in BMP9：6.8% (17/251) in cases and 0.4% (7/1884) in controls.

Odds ratios, 18.8; 95% CI, 7.3-53.8; raw *P* = 1.9 x 10^{-11}.

Validate the association of *BMP9* with IPAH in the replication cohort

- In the replication cohort, *BMPR2*, *BMP9*, and *ACVRL1* were again identified as the top three disease-associated genes.
- We identified five additional (6.3%) BMP9 heterozygous mutations.
- The prevalence of BMP9 variants were significantly higher than the reference controls of GnomAD-EAS (raw $P=1.0 \times 10^{-5}$).



Combined analysis

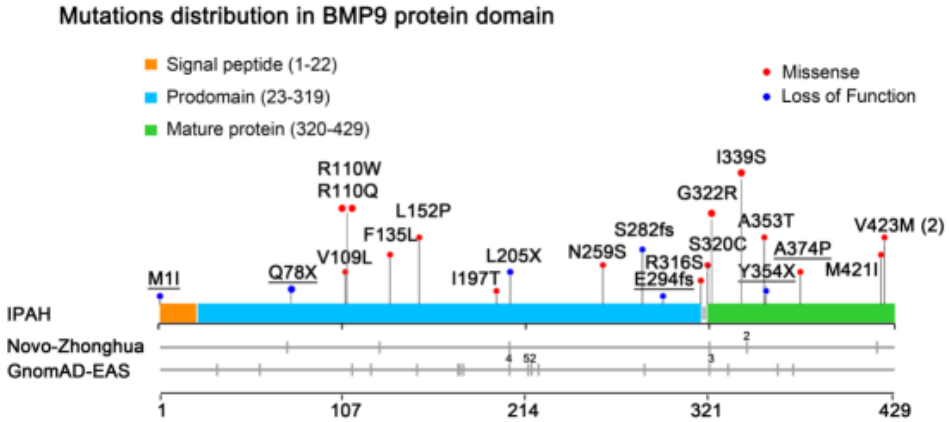
- In the combined discovery and replication datasets, rare deleterious variants in the *BMP9* were 6.7% (22/331) and 0.3% (34/10,508) for cases and controls, comprising a combined significance of $P=2.7 \times 10^{-19}$ (odds ratio: 21.2; 95% CI, 11.7 - 37.6).
- The prevalence for BMP9 rare variants was comparable between the discovery and replication cohorts (6.8% vs 6.3%).

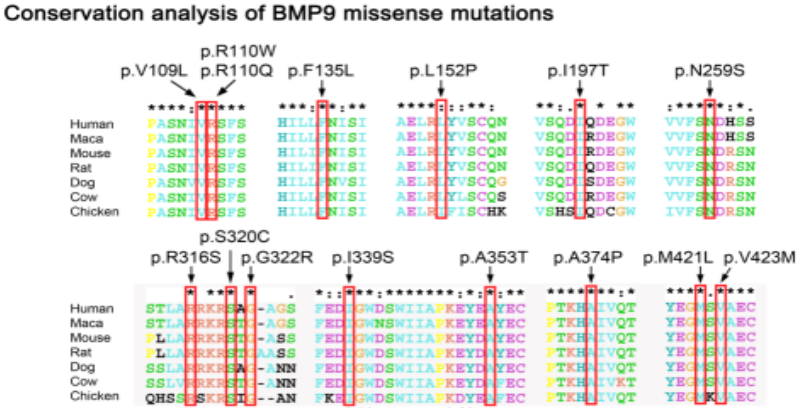
Table 2. Association of Three Genes with IPAH in Two Independent Case-control Studies. *

	Discovery					Replication					Combined				
Gene	IPAH (N = 251)	Novo-Zhonghua WES (N = 1884)	P Value##	OR (95% CI)	BONF###	IPAH (N = 80)	GnomAD-EAS (N = 8624)	P Value	OR (95% CI)	BONF	IPAH (N = 331)	Control (N = 10508)	P Value	OR (95% CI)	BONF
BMPR2	56 (22.3%)	23 (1.2%)	2.0×10^{-35}	20.4 (12.2-35.1)	2.6×10^{-31}	25 (31.3%)	68 (0.8%)	3.1×10^{-30}	40.7 (27.4-77.5)	2.5×10^{-26}	81 (24.5%)	91 (0.9%)	3.1×10^{-76}	32.1 (23.2-44.2)	4.4×10^{-72}
BMP9	17 (6.8%)	7 (0.4%)	1.9×10^{-11}	18.8 (7.3-53.8)	2.6×10^{-7}	5 (6.3%)	27 (0.3%)	1.0×10^{-5}	20.5 (6.1 – 55.1)	8.1×10^{-2}	22 (6.7%)	34 (0.3%)	2.7×10^{-19}	21.2 (11.7-37.6)	3.7×10^{-15}
ACVRL1#	17 (6.4%)	14 (0.7%)	6.6×10^{-9}	9.4 (4.3-20.7)	8.8×10^{-5}	6 (7.5%)	48 (0.6%)	1.0×10^{-5}	13.9 (4.7 – 33.1)	8.0×10^{-2}	23 (6.7%)	62 (0.6%)	6.2×10^{-16}	12.1 (7.1-20.0)	8.6×10^{-12}

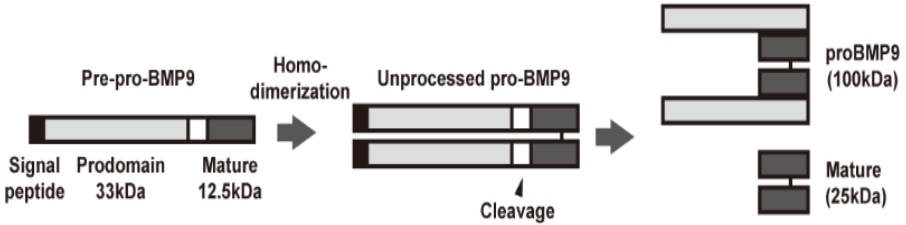
The characteristics of *BMP9* mutations in IPAH

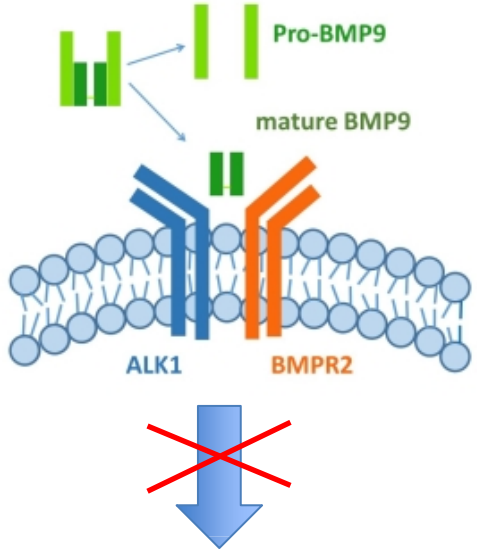
- Among the 331 IPAH patients, we identified 22 cases carrying 21 distinct rare heterozygous variants in *BMP9*.
  - Missense mutation:15.
  - Loss of Function mutation: 6. None of these mutations was found In the population-based genome databases.





BMP9-BMPR2-ACVRL1 axis



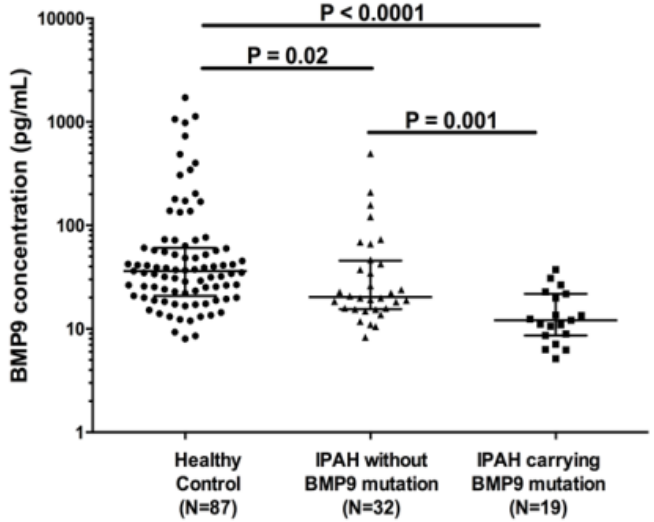


maintain the quiescence of pulmonary vasculature

Pulmonary arterial hypertension

The *BMP9* mutations were associated with decreased protein expression

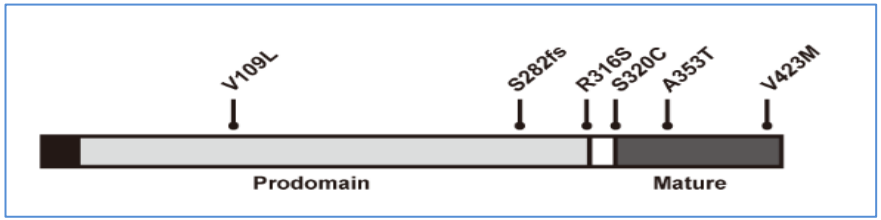
- There were no significant differences in age and hemodynamic characters between individuals with or without *BMP9* mutations.
- The patients with *BMP9* mutations had lower plasma level of BMP9 than those without (*P* = 0.001).



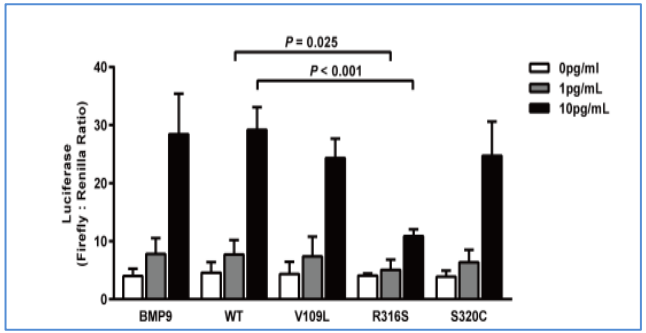
Levels of BMP9 were depicted with median, top, and bottom quartile. The circulating BMP9 level was decreased in the patients without BMP9 mutations and lowest in the BMP9 mutation carriers. IPAH denotes idiopathic pulmonary arterial hypertension.

Functional studies

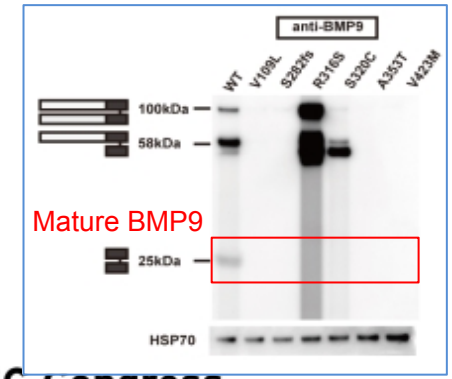
Six *BMP9* mutations were selected for functional study

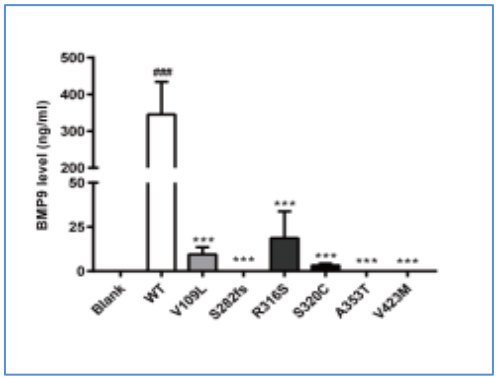


Mutation caused significantly reduced BMP activity.

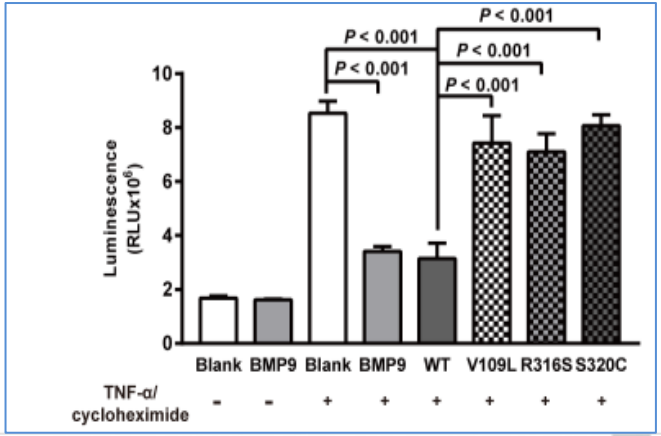


Mutations impaired the biosynthesis and secretion.





Mutations attenuated the anti-apoptosis effect in the PAECs



Discussion

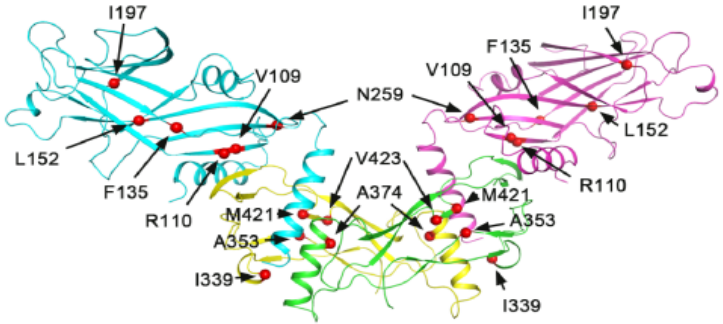
Chinese cohort

Cases：331

Mean age：27 years

BMP9 mutation rate：6.7% (22/331)

21 distinctive mutations



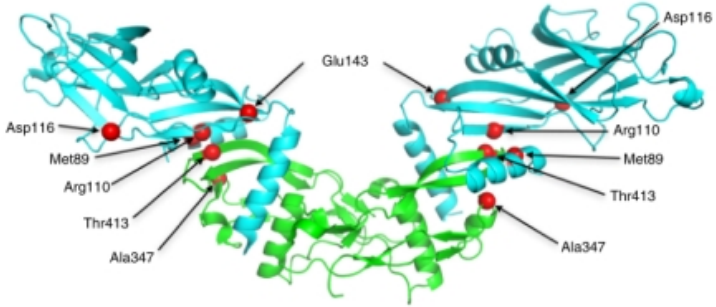
European cohort

Cases：1084

Mean age：50 years

BMP9 mutation rate：1.1% (12/1084)

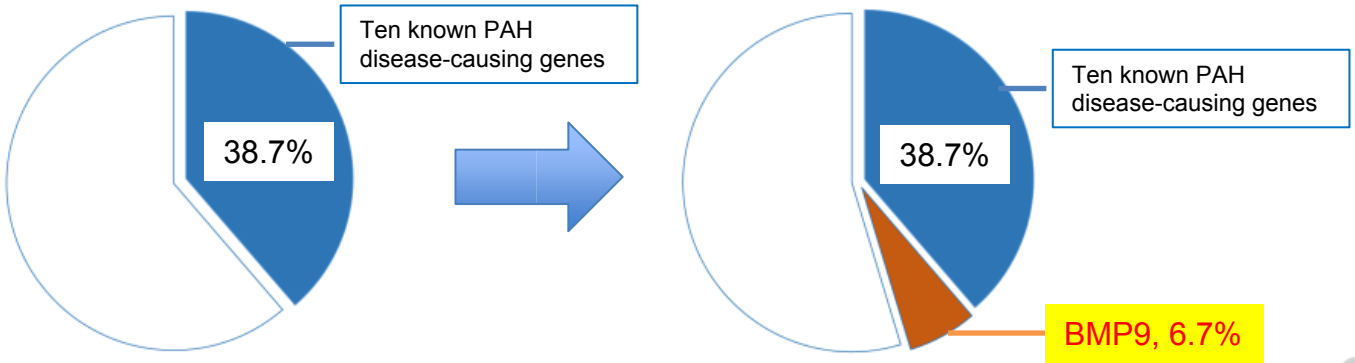
8 distinctive mutations



Nat Commun 2018;9:1416.

Conclusion

- To the best of our knowledge, this is the first time to identify novel IPAH causative genes in East Asia using next-generation sequencing on a large population of IPAH patients.
- The rare mutations of *BMP9* occurred in 6.7% of cases, conferring a more than 22-fold greater risk of IPAH, ranking this gene second to *BMPR2*.
- IPAH patients carrying *BMP9* mutants may receive a BMP9 supplemental therapy as part of their personalized treatment.



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