Journal of the American Heart Association

EDITORIAL

Mendelian Randomization in Pulmonary Arterial Hypertension: Unveiling Early Insights With Promise for Future Discoveries

Steeve Provencher, MD, MSc; Benoit J. Arsenault, PhD; Sebastien Bonnet, PhD

Pulmonary arterial hypertension (PAH) is a complex condition characterized by the gradual restructuring of the distal pulmonary arteries, culminating in right ventricular failure and premature mortality.[1] Substantial progress has been achieved in unraveling the risk factors and mechanisms underpinning the onset and progression of this disease. The landmark discovery of germline mutations associated with heritable PAH marked a significant milestone. However, these mutations are only detected in ≈80% of familial PAH cases, a minority of seemingly sporadic idiopathic PAH cases, and are generally absent in other forms of PAH. Moreover, the puzzling question of why only a fraction of individuals sharing similar risk factors develop PAH remains enigmatic, despite numerous efforts to forecast PAH risk by exploring various facets of an individual's phenome (eg, specific autoantibodies linked to scleroderma).

See article by Alhathli et al.

Several biomarkers have been identified either independently or in combination that predict the long-term prognosis of patients with PAH. Nevertheless, the molecular processes guiding the shift from adaptive to maladaptive remodeling in the right ventricle and elucidating the diverse prognoses observed among patients with PAH remain largely elusive.[2] It is crucial to note that although they have significantly contributed to identify pivotal risk factors for PAH development and prognostic, these correlational studies have primarily relied on observational epidemiology. Despite using sophisticated statistical methods and stringent epidemiologic strategies to rectify or diminish confounding factors, the potential for reverse causality and unmeasured confounding, which are frequently overlooked limitations in these study designs, remains unverifiable.

In recent years, Mendelian randomization (MR) has emerged as a valuable epidemiologic tool, offering insights into causal relationships. In essence, MR investigates whether the presence of ≥1 common genetic variants influences targeted risk factors, such as circulating metabolites. It aims to discern whether carriers of these genetic variants exhibit different disease risks or outcomes compared with noncarriers. More important, genetic variants are randomly inherited at conception and remain stable throughout an individual's lifespan. This characteristic ostensibly allows MR to explore the association between genetically predicted exposures and outcomes without succumbing to reverse causality. Because genetic variants used as instrumental variables to genetically predict exposures are presumed to

Key Words: Editorials ■ biomarkers ■ Mendelian randomization ■ prognosis ■ pulmonary hypertension

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

Correspondence to: Steeve Provencher, MD, MSc, Pulmonary Hypertension Research Group, IUCPQ Research Centre, 2725, Chemin Sainte-Foy, Québec, QC, Canada. Email: steeve.provencher@criucpq.ulaval.ca

This article was sent to Ferhaan Ahmad, MD, PhD, Senior Associate Editor, for editorial decision and final disposition.

For Disclosures, see page 3.

JAHA is available at: www.ahajournals.org/journal/jaha

be randomly distributed, MR is less susceptible to the influence of unmeasured confounding.

These advantages hinge on 3 crucial assumptions: first, the genetic variants used as instrumental variables for predicting risk factors must be strongly associated with those risk factors (relevance assumption); second, there should be no unmeasured confounders influencing the associations between genetic variants and outcomes (independence assumption); and third, the genetic variants should impact the outcome solely through their effects on the risk factor of interest (exclusion restriction).[3]

When these assumptions are met, MR becomes a potent tool for identifying risk factors, prognostic biomarkers, and targetable pathways. Noteworthy examples include MR studies revealing that individuals carrying genetic variants in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene consistently had lower low-density lipoprotein cholesterol levels throughout their lives, leading to the development of PCSK9 inhibitors that subsequently reduced low-density lipoprotein cholesterol levels and lowered the risk of cardiovascular events.[4,5] Equally significant, MR can provide evidence negating causal relationships, potentially averting the initiation of unnecessary trials and thereby conserving time and resources for more promising research areas. However, it is crucial to acknowledge that single genetic variants typically explain only a minimal proportion of phenotype variation, limiting study power and introducing the risk of false negatives.

Despite its strengths (and inherent assumptions), MR has rarely been used in the PAH field to date. Observational studies documented that red cell distribution width correlated with survival in PAH.[6] Because red cell distribution width increases with iron deficiency and preclinical works linked iron deficiency and pulmonary hypertension, small open-label trials were launched and showed slightly improved exercise capacity and quality of life with iron supplementation in patients with PAH.[7] Conversely, a MR study failed to show evidence of a causal association between genetic variants associated with increased red cell distribution width and the occurrence of PAH,[8] suggesting that increased red cell distribution width may not be mechanistically linked to the development of PAH and that iron supplementation may provide benefit through mechanisms unrelated to those driving the development of PAH. More recently, after the identification of 507 genes with differential RNA expression and the validation of an RNA model score distinguishing patients with PAH from controls, Rhodes et al[9] observed that a genetic variant associated with a lower *SMAD5* (mothers against decapentaplegic homolog-5) RNA level was associated with an increased risk of PAH independently of the *BMPR2* (bone morphogenetic protein receptor type 2) mutational status, providing novel insights on the mechanisms of PAH development. Finally, MR confirmed that a genetic variant associated with differential expression of the interleukin-6 receptor was not associated with the risk of PAH development or outcomes,[10] suggesting that modulation of interleukin-6 signaling may not be of benefit for most patients with PAH, and perhaps explaining the absence of an efficacy signal after 6 months of intravenous tocilizumab in an open-label PAH study. This supplemental analysis was, however, unpowered for small effects and not replicated in a more recent MR study.[11]

In this issue of the *Journal of the American Heart Association* (*JAHA*), Alhathli et al[12] complement the growing evidence supporting the role of MR in PAH. Using genome-wide association study summary statistics, they used 2-sample MR analyses to indirectly assess the genetically determined association between serum concentration of 575 metabolites and the development of PAH in a cohort of 125 patients with PAH and >160 000 controls. Genetic variants associated with 5 metabolites were associated with the risk of PAH development. In a confirmatory (although overlapping) cohort, the expected levels of acetylphosphate and serine remained protective, whereas homostachydrine expected levels were associated with an increased risk of PAH. This association remained significant after stringent multiple testing correction, sensitivity analyses, and reassuring F statistic supporting the robustness of their findings and minimizing the risk that genetic variants affect the outcome through pathways other than through the risk factor of interest (ie, horizontal pleiotropy). In a parallel analysis of rare variants in a cohort of 578 patients with PAH and 361 675 controls, loss-of-function mutations within ATF4 (activating transcription factor 4), a transcription factor that promotes the expression of enzymes involved in the endogenous synthesis of serine, were more prevalent in PAH cases (0.53% versus 0.14%). This observation further reinforces the notion of the protective role of serine in the development of PAH. In addition, the authors identified noteworthy, albeit limited, correlations between direct measurements of serine and homostachydrine plasma concentrations and the hemodynamic severity in a cohort of 446 patients with PAH. Elevated homostachydrine plasma concentrations were also associated with a reduction in survival. Finally, the authors used a genome-wide association study for questionnaire-reported coffee consumption and multivariable MR and found no associations between coffee consumption and no evidence for serine-mediated effects on immunomodulation to explain the differential risk of PAH related to serine and homostachydrine levels, respectively.

Although the identified genetic variants were significantly associated with changes in metabolites based on publicly available genome-wide association studies of the serum metabolome, the authors did not

confirm through direct measurements whether predicted changes in metabolite levels truly differed between patients with PAH and controls. Similarly, the authors did not explore whether the identified genetic variants directly predicted outcomes of patients with PAH. This is relevant because an individual biomarker may specifically predict the risk of disease development, outcomes, or both. Perhaps the most obvious example of this potential paradox in PAH is sex. Female subjects are more susceptible to the disease but have a better right ventricular function and prognosis than male subjects.[13] Similarly, BMPR2 mutations tremendously increase the risk of PAH development, whereas their effects on survival are much less impressive.[14] More important, the associations estimated by MR in patients with PAH and controls, versus plasma concentrations among prevalent patients, may not be the same. The MR estimate likely reflects the effects of lifelong perturbations in the risk factor, whereas Cox regression analyses evaluating the association of direct measurement of a risk factor and survival may reflect more acute effects. Despite triangulating the evidence, the association between serine and homostachydrine levels and outcomes thus remains prone to limitations inherently associated with observational epidemiology, including reverse causality and unmeasured confounding. It is noteworthy, however, that the present study would have had sufficient power to confirm the association between genetic variants presumably associated with metabolite levels and outcomes because of weak instrument bias. Indeed, the power of an MR study is determined by sample size and strength of the association between the proposed instrument and risk factor. As a result, MR estimates are almost always less precise and have wider CIs than regression analysis because of genetic variants that explain only a small proportion of the variation in the risk factor/outcome prediction, thus requiring significant sample sizes.

The authors should be commended for providing compelling evidence about the association between common genetic variants and the risk of PAH development, as well as the identification of serine and homostachydrine plasma levels as possible prognostic biomarkers.

There remains a substantial body of work essential to advancing our understanding, including the following:

1. Confirming these findings through broader and independent study populations. The crucial importance of sample size for MR studies mandates intense collaborative efforts by the PAH community to leverage large-scale genetic data and advance the field;
2. Unraveling the underlying mechanisms responsible for these identified associations;
3. Evaluating the additional discriminative value of serine and homostachydrine plasma levels when added to the existing array of prognostic biomarkers, including validated risk assessment tools;
4. Establishing that tailoring disease management based on these new biomarkers leads to improved outcomes (ie, conducting utility studies). Although modulating therapy according to risk stratification, as suggested by current treatment algorithms, is enticing, its practical implementation is not always straightforward. For instance, B-type natriuretic peptide blood levels consistently predicted outcomes in patients with heart failure,[15] yet utility studies failed to demonstrate a clinical benefit of B-type natriuretic peptide–guided therapy in these patients[16]; and finally
5. Confirming that interventions targeting these pertinent components of the metabolome can either prevent the onset of PAH or effectively treat established disease.

Collectively, this study underscores the valuable contribution of MR investigations, even in the context of a rare disease like PAH. The incorporation of MR studies may prove instrumental in pinpointing novel risk factors and harnessing the potential of extensive genetic data sets to reveal promising targetable pathways. This approach not only enhances our understanding of PAH but also strategically minimizes the initiation of unwarranted trials, optimizing research efforts for more fruitful avenues.

ARTICLE INFORMATION

Affiliations

Pulmonary Hypertension Research Group, Institut Universitaire de Cardiologie et de Pneumologie de Québec Research Centre, Québec, Canada (S.P., S.B.); Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Québec City, Québec, Canada (S.P., B.J.A., S.B.); and Department of Medicine (S.P., B.J.A., S.B.)., Université Laval, Québec City, Québec, Canada.

Disclosures

None.

REFERENCES

1. Pullamsetti SS, Savai R, Seeger W, Goncharova EA. Translational advances in the field of pulmonary hypertension. From cancer biology to new pulmonary arterial hypertension therapeutics. Targeting cell growth and proliferation signaling hubs. *Am J Respir Crit Care Med*. 2017;195:425–437. doi: 10.1164/rccm.201606-1226PP
2. Khassafi F, Chelladurai P, Valasarajan C, Nayakanti SR, Martineau S, Sommer N, Yokokawa T, Boucherat O, Kamal A, Kiely DG, et al. Transcriptional profiling unveils molecular subgroups of adaptive and maladaptive right ventricular remodeling in pulmonary hypertension. *Nat Cardiovasc Res*. 2023;2:917–936. doi: 10.1038/s44161-023-00338-3

3. Davies NM, Holmes MV, Davey SG. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi: 10.1136/bmj.k601
4. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354:1264–1272. doi: 10.1056/NEJMoa054013
5. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med*. 2017;376:1713–1722. doi: 10.1056/NEJMoa1615664
6. Rhodes CJ, Howard LS, Busbridge M, Ashby D, Kondili E, Gibbs JS, Wharton J, Wilkins MR. Iron deficiency and raised hepcidin in idiopathic pulmonary arterial hypertension: clinical prevalence, outcomes, and mechanistic insights. *J Am Coll Cardiol*. 2011;58:300–309. doi: 10.1016/j.jacc.2011.02.057
7. Ruiter G, Manders E, Happe CM, Schalij I, Groepenhoff H, Howard LS, Wilkins MR, Bogaard HJ, Westerhof N, van der Laarse WJ, et al. Intravenous iron therapy in patients with idiopathic pulmonary arterial hypertension and iron deficiency. *Pulm Circ*. 2015;5:466–472. doi: 10.1086/682217
8. Ulrich A, Wharton J, Thayer TE, Swietlik EM, Assad TR, Desai AA, Graf S, Harbaum L, Humbert M, Morrell NW, et al. Mendelian randomisation analysis of red cell distribution width in pulmonary arterial hypertension. *Eur Respir J*. 2020;55:1901486. doi: 10.1183/13993003.01486-2019
9. Rhodes CJ, Otero-Nunez P, Wharton J, Swietlik EM, Kariotis S, Harbaum L, Dunning MJ, Elinoff JM, Errington N, Thompson AAR, et al. Whole-blood RNA profiles associated with pulmonary arterial hypertension and clinical outcome. *Am J Respir Crit Care Med*. 2020;202:586–594. doi: 10.1164/rccm.202003-0510OC
10. Toshner M, Church C, Harbaum L, Rhodes C, Villar Moreschi SS, Liley J, Jones R, Arora A, Batai K, Desai AA, et al. Mendelian randomisation and experimental medicine approaches to interleukin-6 as a drug target in pulmonary arterial hypertension. *Eur Respir J*. 2022;59:2002463. doi: 10.1183/13993003.02463-2020
11. Zhang M, Zeng Q, Zhou S, Zhu G, Xu Y, Gao R, Su W, Wang R. Mendelian randomization study on causal association of IL-6 signaling with pulmonary arterial hypertension. *Clin Exp Hypertens*. 2023;45:2183963. doi: 10.1080/10641963.2023.2183963
12. Alhathli E, Julian T, Girach ZUA, Thompson AAR, Rhodes C, Gräf S, Errington N, Wilkins MR, Lawrie A, Wang D, et al. A Mendelian randomization study with clinical follow-up links metabolites to risk and severity of pulmonary arterial hypertension. *J Am Heart Assoc*. 2024;12:e032256. doi: 10.1161/JAHA.123.032256
13. Dignam JP, Sharma S, Stasinopoulos I, MacLean MR. Pulmonary arterial hypertension: sex matters. *Br J Pharmacol*. [published online November 8, 2023]. doi: 10.1111/bph.16277
14. Evans JD, Girerd B, Montani D, Wang XJ, Galie N, Austin ED, Elliott G, Asano K, Grunig E, Yan Y, et al. BMPR2 mutations and survival in pulmonary arterial hypertension: an individual participant data meta-analysis. *Lancet Respir Med*. 2016;4:129–137. doi: 10.1016/S2213-2600(15)00544-5
15. van Veldhuisen DJ, Linssen GC, Jaarsma T, van Gilst WH, Hoes AW, Tijssen JG, Paulus WJ, Voors AA, Hillege HL. B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. *J Am Coll Cardiol*. 2013;61:1498–1506. doi: 10.1016/j.jacc.2012.12.044
16. Felker GM, Anstrom KJ, Adams KF, Ezekowitz JA, Fiuzat M, Houston-Miller N, Januzzi JL Jr, Mark DB, Pina IL, Passmore G, et al. Effect of natriuretic peptide-guided therapy on hospitalization or cardiovascular mortality in high-risk patients with heart failure and reduced ejection fraction: a randomized clinical trial. *JAMA*. 2017;318:713–720. doi: 10.1001/jama.2017.10565