Sirtuin 3 and Uncoupling Protein 2, the Missing Link Between Genetics, Metabolism, and Pulmonary Arterial Hypertension

Annie C. Lajoie, MD; François Potus, PhD

SIRT3, UCP2, metabolism, and pulmonary hypertension, the saga continues. SIRT3 (Sirtuin 3) and UCP2 (uncoupling protein 2) are 2 genes located in chromosome 11 coding for a major mitochondrial deacetylase and a mitochondrial calcium uniporter facilitating the entry of calcium into the mitochondria. Almost a decade ago, the Michelakis group demonstrated that the lack of UCP2 contributes to mitochondrial dysfunction, metabolic defects, and a pro-proliferative and antiapoptotic phenotype of pulmonary arterial hypertension (PAH) pulmonary arterial smooth muscle cells. In a prospective cohort of 60 patients with PAH (32 with IPAH and 28 with associated PAH), the authors investigate the occurrence of SIRT3 (rs11246020) and UCP2 (rs659366) loss of function SNP. Nearly 70% of their cohort, irrespective of the PAH subtype, carried an SNP on at least 1 of the SIRT3 or UCP2 alleles. Moreover, they report that SIRT3 and UCP2 SNP is clinically associated with disease severity and propose SIRT3 (rs11246020) and UCP2 (rs659366) as potential novel biomarkers of PAH severity. Interestingly, Zhang et al’s findings suggest an additive effect of the SNP on disease severity, as patients carrying homozygous or heterozygous SNP in both genes displayed worse hemodynamics and event-free survival compared with patients with a single SIRT3 or UCP2 allele. Whether this observation reflects an incremental SIRT3/UCP2 loss of function/expression in patients remains unknown and needs to be further clarified. Thus, SIRT3 and UCP2 were added

Key Words: Editorial ■ genetic ■ preclinical model ■ pulmonary arterial hypertension

See Article by Zhang et al.
to the constantly growing list of potential genetic determinants of PAH.4–6

Although the genetic association reported by Zhang et al needs to be replicated in an independent cohort, their findings are supported by complementary analyses. They conducted an impressive in vivo study investigating the development of pulmonary hypertension in Sirt3/Ucp2 heterozygous (Sirt3+/−/Ucp2+/−), Sirt3 homozygous Ucp2 heterozygous (Sirt3+/−/Ucp2+/−), Sirt3 heterozygous Ucp2 homozygous (Sirt3+/−/Ucp2+/−), and Sirt3/Ucp2 double homozygous (Sirt3+/−/Ucp2+/−) knockout mice (KO). They observed that all the mice exhibited adverse pulmonary vascular lesions and developed pulmonary hypertension in a gene dosedependent manner, with the Sirt3+/−/Ucp2+/− mice being the most affected. Consistently, at the cellular level, Sirt3+/−/Ucp2+/− pulmonary arterial smooth muscle cells had more mitochondrial defects and displayed a greater pro-proliferative/antiapoptosis phenotype. Thus, using a combination of Sirt3/Ucp2 double KO mice the authors elegantly demonstrated that mitochondrial dysfunction mediated by Sirt3 and Ucp2 depletion contributes to PAH etiology in a gene dosedependent manner.

The characterization and the development of a novel genetically engineered mouse model mimicking an advanced stage of human PAH is one of the main strengths of the study. Indeed, mice models of the disease traditionally develop mild pulmonary hypertension and harbor few features of severe PAH.7 However, the double KO mice (Sirt3−/−/Ucp2−/−) in this study displayed features of right ventricular failure and plexogenic lesions and had overall increased mortality. Plexiform lesions, the hallmark feature of severe PAH, were never observed in mice so far.8 Interestingly, only the mice carrying the double homozygote Sirt3 and Ucp2 KO developed these complex lesions, suggesting that the disruption of both Sirt3 and Ucp2 gene expression is required for the development of plexiform lesions. Whether impaired SIRT3/UCP2 expression and mitochondrial dysfunction are observed in human plexiform lesions is unknown and the exact mechanism connecting Sirt3/Ucp2 and the formation of plexiform lesions remains to be pinpointed.

Nevertheless, one must exert caution when translating these experimental findings to humans. Indeed, the mutation strategy performed in mice results in the total abrogation of the Sirt3a and Ucp2 gene expression. The SIRT3 (rs11246020) and UCP2 (rs659366) SNP reported in humans does not necessarily lead to impaired gene expression. SIRT3 (rs11246020) SNP was observed in valine to isoleucine at residue 208 of the SIRT3 polypeptide affecting the enzyme’s catalytic activity.9 Experimentally, the SIRT3-V208I variant has been associated with a 34% reduction in the SIRT3 catalytic efficiency. There is no effect of SIRT3 (rs11246020) on gene expression reported in the literature. In previous work, Paulin et al showed that SIRT3 expression is decreased in patients with IPAH independently of the SIRT3 (rs11246020) SNP.2 Both SIRT3 (rs11246020) (homozygote and heterozygote) carriers and patients with noncarrier IPAH exhibit a similar decreased SIRT3 expression compared with controls. This observation suggests that impaired SIRT3 expression observed in patients with PAH occurs independently of the rs11246020 SNP. UCP2 (rs659366) SNP is associated with a -866G>A nucleotide change in the promoter of the gene.10 The functional consequence of UCP2 (rs659366) SNP on gene expression remains conflictual in the literature. On one hand, Wang and colleagues reported that the -866 A allele is associated with decreased UCP2 expression in adipose tissue.11 Conversely, rs659366 SNP has also been associated with increased UCP2 expression in adipose cells, pancreatic β-cells, inflammatory cell lines, and human blood samples.12–15 The differences in effect on mRNA levels might suggest that other factors, either independently or in interaction with the -866 variant, influence the UCP2 mRNA levels. Although the authors successfully demonstrated the association between SIRT3 (rs11246020) SNP, UCP2 (rs659366) SNP, and PAH severity, the contribution of those specific SNPs to PAH etiology in humans remains to be experimentally investigated.

Other important findings of this work merit discussion and offer potential areas for future research. First, Zhang et al also reported that a greater proportion of male patients (77%) carried a SIRT3/UCP2 SNP compared with female patients (64%). If replicated in larger independent patient cohorts, this observation might provide some insight on the sexual dimorphism observed in PAH, where women are more affected than men but have a greater survival.16

Another exciting observation is the association between SIRT3, UCP2, and diabetes mellitus in patients with PAH. In the patient cohort, they observed that homozygous or heterozygous SNP in both genes was associated with a 75% increased risk of type 2 diabetes mellitus, whereas heterozygous SNP in SIRT3 or UCP2 had a 58% risk. In addition to pulmonary hypertension, Sirt3/Ucp2 double homozygous KO mice (Sirt3−/−/Ucp2−/−) exhibited marked glucose intolerance and insulin resistance. This observation supports clinical data where insulin resistance has been associated with PAH development and disease severity.17 Interestingly both SIRT3 (rs11246020) and UCP2 (rs659366) SNPs as well as impaired SIRT3 and UCP2 gene expression have been widely associated with diabetes mellitus and metabolic syndrome.9,18,19 Metabolic syndrome has been linked with a plethora of cardiovascular diseases including pulmonary hypertension.20,21 However, the association between
SIRT3 and UCP2 SNP and gene expression deficiency with other features of metabolic syndrome (e.g., central obesity and dyslipidemia) in patients with PAH and Sirt3−/−/Ucp2−/− mice remains to be explored. Thus, their observations provide strong evidence of the role of SIRT3 and UCP2 in the so-far unexplained association between insulin resistance and PAH.

However, some aspects of the relationship between those genes and PAH remain to be elucidated. It is unclear whether UCP2 (rs659366) is specific to PAH or associated with the proinflammatory status of PAH remains. Indeed, UCP2 (rs659366) has been associated with the development of numerous chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, primary sclerosing cholangitis, inflammatory bowel diseases, and vasculitis. Moreover, additional details about the association of SIRT3 and UCP2 SNP and PAH subtype are lacking in the present publication. Former work by the Michelakis group reported that the association between SIRT3 (rs11246020) SNP and PAH was limited to patients with IPAH. Patients with associated PAH and healthy controls had the same frequency of SIRT3 (rs11246020) SNP. Furthermore, Michelakis and colleagues previously reported that SIRT3 (rs11246020) and/or UCP2 (rs659366) SNP in one or both alleles were associated with resistance to dichloroacetate in patients with IPAH. Whether the same resistance to dichloroacetate can be found in the Sirt3−/−/Ucp2−/− mice is unknown and would motivate further characterization.

In conclusion, the work by Zhang et al provides additional striking evidence in favor of the role of SIRT3 and UCP2 in the development of PAH, leveraging yet again another biomarker for the diagnosis and prognosis of the disease. They suggest that SIRT3 and UCP2 SNP have an additive effect on disease severity but could also explain the sexual dimorphism observed in PAH, as well as the relationship between PAH and impaired glucose metabolism. The Sirt3−/−/Ucp2−/− mice model proposed by this team is a powerful preclinical model that will, without a doubt, benefit and stimulate future translational research.

ARTICLE INFORMATION

Affiliation
Centre de Recherche de l’Institut Universitaire de Cardiologie et de Pneumologie de Québec (CRIUCPQ), Québec, Quebec, Canada.

REFERENCES
1. Dromparis P, Paulin R, Sutendra G, Qi AC, Bonnet S, Michelakis ED. Uncoupling protein 2 deficiency mimics the effects of hypoxia and endoplasmic reticulum stress on mitochondria and triggers pseudohypoxic pulmonary vascular remodeling and pulmonary hypertension. Circ Res. 2013;113:126–136. doi: 10.1161/CIRCRESAHA.112.300699
2. Paulin R, Dromparis P, Sutendra G, Gurtu V, Zervopoulos S, Bowers L, Haromy A, Webster L, Provencer S, Bonnet S, et al. Sirtuin 3 deficiency is associated with inhibited mitochondrial function and pulmonary arterial hypertension in rodents and humans. Cell Metab. 2014;20:827–839. doi: 10.1016/j.cmet.2014.08.011
3. Zhang Y, Zervopoulos SD, Boukouris AE, Lorenzana-Carrillo MA, Saleme B, Webster L, Liu Y, Haromy A, Tabatabaei-Dakhili SA, Ussher JR, et al. SNPs for genes encoding the mitochondrial proteins Sirt3 and UCP2 are associated with disease severity, type 2 diabetes and outcomes in pulmonary arterial hypertension (PAH) patients and this is recapitulated in a new PAH mouse model lacking both genes. J Am Heart Assoc. 2021;10:e020451. doi: 10.1161/JAHA.120.020451
4. Morrell NW, Alvord MA, Chung WK, Elliott CG, Nichols WC, Soubrier F, Trembath RC, Loyd JE. Genetics and genomics of pulmonary arterial hypertension. Eur Respir J. 2019;53:1801899. doi: 10.1183/13993900.001899-2018
5. Potus F, Pauculo MW, Cook EK, Zhu N, Hsieh A, Welch CL, Shen Y, Tian L, Lima P, Mewburn J, et al. Novel mutations and decreased expression of the epigenetic regulator TET2 in pulmonary arterial hypertension. Circulation. 2020;141:1986–2000. doi: 10.1161/CIRCULATIONAHA.119.044320
6. Rhodes CJ, Batai K, Bleda M, Haimel M, Southgate L, Germain M, Pauculo MW, Hadimnapola C, Aman J, Sirer B, et al. Genetic determinants of risk in pulmonary arterial hypertension: international genome-wide association studies and meta-analysis. Lancet Respir Med. 2019;7:227–238. doi: 10.1016/S2213-2600(18)30409-9
7. Gomez-Arroyo J, Saleem SJ, Mizuno S, Syed AA, Bogaard HJ, Abbate A, Tarasevicie-Stewart L, Sung Y, Kraskauskas D, Farkas D, et al. A brief overview of mouse models of pulmonary arterial hypertension: problems and prospects. Am J Physiol Lung Cell Mol Physiol. 2012;302:L977–L991. doi: 10.1152/ajplung.00362.2011
8. Jonigk D, Golpon H, Bockmeyer CL, Maegel L, Hoepf MM, Gottlieb J, Nickel N, Hussein K, Maus U, Lehmann U, et al. Plexiform lesions in pulmonary arterial hypertension composition, architecture, and microenvironment. Am J Pathol. 2011;179:167–179. doi: 10.1016/j.ajpath.2011.03.040
9. Hirschey M, Shimazu T, JIng E, Grueter C, Collins A, Aouizerat B, Stančáková A, Goetzman E, Lam M, Schwer B, et al. Sirt3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. Mol Cell. 2011;44:177–190. doi: 10.1016/j.molcel.2011.07.019
10. Oktavianthi S, Trimarsanto H, Febinia CA, Suastika K, Saraswati MR, Rieckmann P, Epplen JT, Ibrahim SM, et al. Association of a common polymorphism in the promoter of UCP2 with susceptibility to multiple sclerosis. J Mol Med (Berl). 2005;83:806–811. doi: 10.1007/s00109-005-0661-5
11. Wang H, Chu WS, Lu T, Hasstedt SJ, Kern PA, Elbein SC. Uncoupling protein 2 polymorphism in the development of PAH, leveraging yet another biomarker for the diagnosis and prognosis of the disease. They suggest that SIRT3 and UCP2 SNP have an additive effect on disease severity but could also explain the sexual dimorphism observed in PAH, as well as the relationship between PAH and impaired glucose metabolism. The Sirt3−/−/Ucp2−/− mice model proposed by this team is a powerful preclinical model that will, without a doubt, benefit and stimulate future translational research.

SIRT3, UCP2, Metabolism, and PAH
17. Zamanian RT, Hansmann G, Snook S, Lilienfeld D, Rappaport KM, Reaven GM, Rabinovitch M, Doyle RL. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J*. 2009;33:318–324. doi: 10.1183/09031936.00000508

18. Dalgaard LT. Genetic variance in uncoupling protein 2 in relation to obesity, type 2 diabetes, and related metabolic traits: focus on the functional -866G>A promoter variant (rs659366). *J Obes*. 2011;2011:340241. doi: 10.1155/2011/340241

19. Andersen G, Dalgaard LT, Justesen JM, Anthonsen S, Nielsen T, Thørner LW, Witte D, Jørgensen T, Clausen JO, Lauritzen T, et al. The frequent UCP2 -866G>A polymorphism protects against insulin resistance and is associated with obesity: a study of obesity and related metabolic traits among 17 636 Danes. *Int J Obes (Lond)*. 2013;37:175–181.

20. Jackson EA, McLaughlin V. The metabolic syndrome and pulmonary vascular disease. *Chest*. 2009;136:3–4. doi: 10.1378/chest.09-0366

21. Ranchoux B, Nadeau V, Bourgeois A, Provencher S, Tremblay É, Omura J, Coté N, Abu-Alhayja’a R, Dumais V, Nachbar RT, et al. Metabolic syndrome exacerbates pulmonary hypertension due to left heart disease. *Circ Res*. 2019;125:449–466. doi: 10.1161/CIRCRESAHA.118.314555

22. Michelakis ED, Gurtu V, Webster L, Barnes G, Watson G, Howard L, Cupitt J, Paterson I, Thompson RB, Chow K, et al. Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients. *Sci Transl Med*. 2017;9:eaa04583. doi: 10.1126/scitranslmed.aao4583