

**EDITORIAL**

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

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**S**IRT3, 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.<sup>1</sup> Moreover, they reported that genetically engineered mice lacking *Ucp2* spontaneously developed pulmonary hypertension associated with adverse pulmonary vascular remodeling and mitochondrial and metabolic dysfunction. In separate work, the same group also uncovered the critical role of *SIRT3* in PAH etiology and metabolic dysfunction.<sup>2</sup> They reported that heterozygous and homozygous *Sirt3* depleted mice spontaneously developed pulmonary vascular lesions and pulmonary hypertension in a dose-dependent manner. They were also the first to propose that *SIRT3* loss of function single nucleotide polymorphism (SNP rs11246020) was associated with decreased *SIRT3* expression and idiopathic pulmonary arterial hypertension (IPAH) in humans.

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**See Article by Zhang et al.**

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In this issue of the *Journal of the American Heart Association (JAHA)*, Zhang and colleagues further investigate the connection between *SIRT3*, *UCP2* genes, metabolic dysfunction, and PAH.<sup>3</sup> 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* heterozygous SNP. 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

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to the constantly growing list of potential genetic determinants of PAH.<sup>4–6</sup>

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<sup>+/-</sup>/Ucp2<sup>+/-</sup>*), *Sirt3* homozygous *Ucp2* heterozygous (*Sirt3<sup>-/-</sup>/Ucp2<sup>+/-</sup>*), *Sirt3* heterozygous *Ucp2* homozygous (*Sirt3<sup>+/-</sup>/Ucp2<sup>-/-</sup>*), and *Sirt3/Ucp2* double homozygous (*Sirt3<sup>-/-</sup>/Ucp2<sup>-/-</sup>*) knockout mice (KO). They observed that all the mice exhibited adverse pulmonary vascular lesions and developed pulmonary hypertension in a gene dose-dependent manner, with the *Sirt3<sup>-/-</sup>/Ucp2<sup>-/-</sup>* mice being the most affected. Consistently, at the cellular level, *Sirt3<sup>-/-</sup>/Ucp2<sup>-/-</sup>* 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 dose-dependent manner.

The characterization and the development of a novel genetically engineered mice 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.<sup>7</sup> However, the double KO mice (*Sirt3<sup>-/-</sup>/Ucp2<sup>-/-</sup>*) in this study displayed features of right ventricular failure and plexiform lesions and had overall increased mortality. Plexiform lesions, the hallmark feature of severe PAH, were never observed in mice so far.<sup>8</sup> 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) results in a change of valine to isoleucine at residue 208 of the *SIRT3* polypeptide affecting the enzyme's catalytic activity.<sup>9</sup> 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.<sup>2</sup> Both *SIRT3* (rs11246020) (homozygote and heterozygote) carrier 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.<sup>10</sup> 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.<sup>11</sup> Conversely, rs659366 SNP has also been associated with increased *UCP2* expression in adipose cells, pancreatic  $\beta$ -cells, inflammatory cell lines, and human blood samples.<sup>12–15</sup> 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.<sup>16</sup>

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<sup>-/-</sup>/Ucp2<sup>-/-</sup>*) exhibited marked glucose intolerance and insulin resistance. This observation supports clinical data where insulin resistance has been associated with PAH development and disease severity.<sup>17</sup> 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.<sup>9,18,19</sup> Metabolic syndrome has been linked with a plethora of cardiovascular diseases including pulmonary hypertension.<sup>20,21</sup> However, the association between

*SIRT3* and *UCP2* SNP and gene expression deficiency with other features of metabolic syndrome (eg, central obesity and dyslipidemia) in patients with PAH and *Sirt3*<sup>-/-</sup>/*Ucp2*<sup>-/-</sup> 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.<sup>14</sup> 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.<sup>2</sup> 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.<sup>22</sup> Whether the same resistance to dichloroacetate can be found in the *Sirt3*<sup>-/-</sup>/*Ucp2*<sup>-/-</sup> 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*<sup>-/-</sup>/*Ucp2*<sup>-/-</sup> mice model proposed by this team is a powerful preclinical model that will, without a doubt, benefit and stimulate future translational research.

## ARTICLE INFORMATION

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