Sex differences in pulmonary hypertension: are we cleaning up the mess?

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Genomic and non-genomic factors determine disparate right ventricular and pulmonary vascular effects of sex steroids http://ow.ly/WdBNQ

When I was looking for a research project to pursue as a fellow in 2006, I became interested in sex differences in pulmonary arterial hypertension (PAH). When I proposed the idea of pursuing this topic further to a well-regarded expert in the field, his sobering answer was "That area is a mess"; and what a mess it was! We knew at that time that oestrogens exert protective effects in acute and chronic hypoxia-induced pulmonary hypertension (PH) [1–3], as well as in monocrotaline-induced PH (MCT–PH) [4]. However, we also knew that women are more prone to PAH [5], a finding at odds with the data derived from animal studies. Soon after that, we learned that women, despite being more prone to PAH development, exhibit better survival and better right ventricular function than their male counterparts [6, 7]. The superior survival in female patients ultimately was linked at least in part to improved right ventricular function [8, 9]. Epidemiological studies in healthy subjects implicated oestrogens and dehydroepiandrosterone as mediators of superior right ventricular function, whereas testosterone was associated with higher right ventricular mass, higher volumes and lower right ventricular ejection fraction (RVEF) [10].

At the same time, studies emerged that linked oestrogens and their metabolites to PAH development [11–13]. However, other studies demonstrated protective effects of oestrogens in various animal models of PAH [14–16]. This leaves us with several conundrums: First, disparate data exist from animal models with regards to beneficial *versus* detrimental effects of oestrogens. Second, oestrogen's protective effects noted in some animal models are at odds with the human disease, for which women are more commonly affected. Third, even if we accept a paradigm that implicates oestrogens as mediators of PAH development, this does not explain why women have superior right ventricular function and survival rates. So, almost 10 years after my conversion with our PAH expert, the field is still a mess. Or is it? Despite the conundrums listed above, I propose that we have come a long way, and that we have identified several key concepts.

Oestrogens enhance right ventricular function, while testosterone may exert negative effects on the right ventricle

It has long been known that oestrogens exert protective effects in various forms of left ventricular injury. Women of reproductive age tolerate acute and chronic left ventricular injuries better than men, but this effect is abolished with the onset of menopause [17]. In models of both acute and chronic left ventricular injury, the presence of oestrogens is associated with substantial functional, structural and molecular benefits (reviewed in [18, 19]). The latter include anti-inflammatory, anti-apoptotic, anti-oxidant and anti-fibrotic effects, as well as beneficial effects on pro-contractile signalling and mitochondrial function [18, 19]. Oestrogen's effects on the right ventricle have been studied only recently. In healthy postmenopausal

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hormone replacement therapy users, higher oestrogen levels correlate with higher RVEF [10]. In a model of severe, angioproliferative PAH with significant right ventricular dysfunction, our laboratory demonstrated that both endogenous as well as exogenous oestrogen improves right ventricular function, attenuates right ventricular hypertrophy and decreases PAH-induced pro-inflammatory and pro-apoptotic signalling as well as PAH-induced mitochondrial dysfunction [20]. Anti-inflammatory, as well as anti-fibrotic effects were also demonstrated in MCT–PH [15]. A study in mice with sugen/hypoxia-induced PH revealed that oestrogen exerts both direct as well as indirect right ventricular protection, with the latter being mediated by increased compliance of the proximal pulmonary artery (PA) [21]. While data for testosterone are less abundant, studies demonstrated pro-hypertrophy and pro-fibrotic effects in the left ventricle [22, 23]. In a pulmonary artery banding model of right ventricular pressure overload, the presence of testosterone was associated with more right ventricular fibrosis and right ventricular dysfunction [24].

Sex steroids exhibit context-dependent effects on pulmonary vascular function

Progesterone, testosterone, dehydroepiandrosterone, as well as oestrogens and their metabolites have all been shown to affect pulmonary vascular tone and cellular proliferation [25]. However, these effects are complex and often contradictory. For example, oestrogen appears to exert protective (*e.g.* vasodilator and anti-proliferative) effects in hypoxia-induced PH and MCT–PH [14, 15], while it promotes pulmonary artery remodelling in the setting of BMPR2 mutations or increases in serotonin signalling [11, 26]. Our laboratory demonstrated that oestrogen exerts anti-proliferative effects in the setting of hypoxia, but not during normoxia [14]. The conglomerate of these studies suggests that oestrogen's effects on the pulmonary vasculature are highly context-specific. In light of the complexities of oestrogen signalling (which includes three receptors with several splice forms, multiple co-regulators, and genomic as well as non-genomic effects [18, 19, 25]), this is not surprising. Similarly, disparate data exist for testosterone signalling [27]. However, the question that then emerges is what determines the net effect (*e.g.* protective *versus* disease-promoting) of a sex steroid?

The study by Ventetuolo *et al.* [28] in this issue of the *European Respiratory Journal* makes important contributions to both key concepts reviewed above. In a cohort of >2700 genotyped adults without obvious cardiovascular disease, the authors demonstrate that genetic variations in oestradiol metabolism and androgen receptor signalling are associated with right ventricular morphology (measured by cardiac MRI) in a sex-specific manner. Specifically, the authors report that in African-American women, a single nucleotide polymorphism (SNP) in the gene for the oestrogen-metabolizing enzyme CYP1B1 was associated with increased RVEF. Interestingly, this SNP was in tight linkage disequilibrium with two SNPs associated with PAH as well as with the development of breast and lung cancer, invoking E2 metabolism as a potentially important modifier of right ventricular function in both health and disease. While this CYP1B1 SNP did not have any effects on the oestrogen metabolites measured, the authors also found that, in whites, higher levels of the oestrogen metabolites 2-hydroxyoestradiol and 16α-hydroxyoestrone were associated with higher RVEF. In white men, two SNPs in the androgen receptor gene were associated with higher right ventricular mass and end-diastolic volumes. These SNPs modified the previously reported relationship between testosterone and RVEF (higher testosterone levels were associated with lower RVEF in those with two copies of the G risk allele, while there was no association with RVEF in men homozygous for the A allele).

How do these findings advance the field? First, this study shows that genetic alterations in oestradiol metabolism and androgen receptor signalling are significant modifiers of right ventricular function. The finding that an SNP in the androgen receptor gene exerts relevant effects on the right ventricular–pulmonary artery axis is of particular interest, as most prior studies in PAH have focused on oestrogen, thus indicating that we need to pay attention to other sex hormones as well. Second, the observation that SNPs can modify how sex hormones affect right ventricular function exemplifies the complexities of sex steroid signalling and further supports a paradigm of context-dependent sex steroid effects. Genetic alterations may be a major contributor to the sex hormone conundrum in PAH. Third, the authors demonstrate that the genotype–phenotype relationships they observed are affected by sex and race, providing a correlate for the sex- and race-specific differences in right ventricular morphology previously reported in this population [29], and reminding us that sex and race can be important modifiers of treatment responses. Finally, and maybe most importantly, while this study focused on a healthy cohort, it generates interesting hypotheses relevant to patients with right ventricular dysfunction from PAH or other cardiopulmonary diseases: Do the reported genetic alterations affect the susceptibility of these individuals to development of right ventricular dysfunction? Are these subjects more resilient to right ventricular injury from such common insults as obstructive sleep apnoea, diastolic dysfunction or pulmonary embolism? Granted, these were healthy individuals without obvious cardiopulmonary disease, but given the high incidence and prevalence of sleep-disordered breathing, diastolic dysfunction and venous thromboembolism, these subjects certainly are at risk for cardiopulmonary disease development in their lifetime. And what happens in the hypothetical situation in which a subject with one of these SNPs

develops PAH? Will these individuals have clinically relevant differences in their right ventricular adaptation to the disease? It therefore would be extremely interesting to identify the prevalence of the reported SNPs and to dissect the resulting genotype–right ventricular phenotype relationships in patients with PAH as well as in patients with non-PAH PH (World Health Organization groups 2–5).

In systemic cardiovascular diseases, there already is precedence of SNPs in oestrogen signalling affecting outcomes. For example, SNPs in ESR1 (the gene encoding oestrogen receptor α) are associated with a higher risk for the development of myocardial infarction and stroke [30, 31], whereas SNPs in ESR2 (the gene encoding oestrogen receptor β) are associated with an increase in left ventricular hypertrophy [32]. Two examples for a linkage between genetic alterations in oestrogen signalling and disease susceptibility in PAH already exist: in patients with liver cirrhosis, SNPs in aromatase and ESR1 are associated with a higher risk for the development of portopulmonary hypertension [33]. And, as previously mentioned, SNPs in CYP1B1 have been linked to PAH development in female BMPR2 mutation carriers [11]. While the current study did not evaluate all relevant enzymes and receptors involved in oestrogen and testosterone synthesis, signalling and metabolism and, while it did not apply the gold standard of liquid chromatography–mass spectrometry to quantify urinary oestrogen metabolites [34], it is an important first step in identifying genetically determined variations in sex steroid signalling as modifiers of right ventricular function.

Does the study by Ventetuolo *et al.* [28] suggest that we should put more efforts in explaining sex differences by genetic screening methods? Should we even continue studies in animal models if their results are so difficult to translate to the clinical situation? The answer to both questions should be "yes". While population-based studies are a powerful tool to identify genetic variations and epidemiological concepts, mechanistic animal studies provide a more controlled environment and provide opportunities to dissect and manipulate new pathways and to rigorously test new interventions. However, studies in rats with hypoxia-induced PH and MCT–PH are limited models for human PAH [35]. Similarly, studies in mice are limited by absence of profound vascular remodelling or right ventricular failure [35]. Rather, mechanistic animal studies should be performed in animal models that closely recapitulate the human PAH phenotype (*e.g.* the sugen/hypoxia rat model) [25, 35]. In addition, in light of the female preponderance for PAH development, these studies should include female animals [25, 36]. For example, the results by Ventetuolo *et al.* [28] should trigger rigorous mechanistic studies of CYP1B1 and androgen receptor signalling in right ventricles and cardiomyocytes of female and male animals with right ventricular dysfunction. Only the combination of mechanistic animal studies (if performed in appropriate models) and population-based studies such as the one by Ventetuolo *et al.* [28] will help us clean up the mess of sex differences in PAH and reach the ultimate goal of establishing successful clinical trials improving outcomes in both male and female patients with PAH.

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