Hannah Goulart, B.S.
Massachusetts General Hospital
Boston, Massachusetts

Andrew M. Tager, M.D.
Melissa J. Suter, Ph.D.
Massachusetts General Hospital
Boston, Massachusetts
and
Harvard Medical School
Boston, Massachusetts

References

1. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, *et al.*; ATS/ERS/JRS/ALAT Committee on Idiopathic Pulmonary Fibrosis. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788–824.
2. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ; Idiopathic Pulmonary Fibrosis Clinical Research Network. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *N Engl J Med* 2012;366:1968–1977.
3. Richeldi L, du Bois RM, Raghu G, Azuma A, Brown KK, Costabel U, *et al.*; INPULSIS Trial Investigators. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2071–2082.
4. King TE Jr, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, *et al.*; ASCEND Study Group. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2083–2092.
5. Gotway MB, Freemer MM, King TE Jr. Challenges in pulmonary fibrosis. 1: Use of high resolution CT scanning of the lung for the evaluation of patients with idiopathic interstitial pneumonias. *Thorax* 2007;62:546–553.
6. Watadani T, Sakai F, Johkoh T, Noma S, Akira M, Fujimoto K, *et al.* Interobserver variability in the CT assessment of honeycombing in the lungs. *Radiology* 2013;266:936–944.
7. Utz JP, Ryu JH, Douglas WW, Hartman TE, Tazelaar HD, Myers JL. High short-term mortality following lung biopsy for usual interstitial pneumonia. *Eur Respir J* 2001;17:175–179.
8. Fibla JJ, Brunelli A, Cassivi SD, Deschamps C. Aggregate risk score for predicting mortality after surgical biopsy for interstitial lung disease. *Interact Cardiovasc Thorac Surg* 2012;15:276–279.
9. Kreider ME, Hansen-Flaschen J, Ahmad NN, Rossman MD, Kaiser LR, Kucharczuk JC, *et al.* Complications of video-assisted thoracoscopic lung biopsy in patients with interstitial lung disease. *Ann Thorac Surg* 2007;83:1140–1144.
10. Yun SH, Tearney GJ, Vakoc BJ, Shishkov M, Oh WY, Desjardins AE, *et al.* Comprehensive volumetric optical microscopy in vivo. *Nat Med* 2006;12:1429–1433.
11. Hariri LP, Adams DC, Colby TV, Tager AM, Suter MJ. Assessing idiopathic pulmonary fibrosis (IPF) with bronchoscopic OCT (conference presentation). *Proc SPIE* 2016;9697:969710.
12. Hariri LP, Adams DC, Lanuti M, Wain JC, Tager AM, Suter MJ. Bronchoscopic optical coherence tomography as a low risk method for microscopic diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016;193:A2608.
13. Hariri LP, Adams DC, Lanuti M, Wain JC, Muniappan A, Goulart H, *et al.* In vivo endobronchial optical imaging for microscopic assessment and diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2017;195:A6782.

The Y Chromosome Plays a Protective Role in Experimental Hypoxic Pulmonary Hypertension

To the Editor:

Pulmonary arterial hypertension (PAH) is a chronic pulmonary vascular disease characterized by elevated pulmonary arterial pressure and vascular remodeling leading to right ventricular (RV) failure and death (1). PAH in general, and its idiopathic form in particular, has long been considered to be a disease of young women, with female sex itself being a risk factor (1). Surprisingly, being female is protective against the development of pulmonary hypertension (PH) in various animal models (2). Because this effect has largely been attributed to the protective effects of estradiol, the phenomenon is known as the “estrogen paradox” (3, 4). One way to resolve this paradox is to consider that sex hormones alone are not sufficient to explain the marked sex bias observed in PH in humans.

Here, we report that the type of sex chromosome (XX vs. XY) contributes to sex differences in PH mouse models. Sex chromosomes and sex hormones are both known to act directly on tissues to produce sex differences in physiology and disease (5). Mouse studies in which the number and type of sex chromosomes were independent of gonadal sex indicated that sex chromosomes contribute to sex differences in autoimmune disease, metabolic dysfunction, neurodegeneration, and cardiovascular disease (6, 7). Therefore, genes encoded on the sex chromosomes have already been shown to have strong effects that contribute to sex differences in various phenotypes, including disease susceptibility and progression. We investigated, for the first time, the role of sex chromosomes (in the absence of gonadal hormones) in the development of hypoxia-induced PH, using the informative “four core genotypes” (FCG) and XY* mouse models.

The FCG model produces mice in which sex chromosomes are independent of gonadal sex (XX and XY mice with testes [males], and XX and XY mice with ovaries [females]), allowing the effects of sex chromosomes to be assessed independently of gonadal sex (5). To eliminate the effect of gonadal hormones in adulthood, FCG mice were gonadectomized (GDX) at 75 days after birth, and 30 days later were placed in hypoxia (10% oxygen) or normoxia for 3 weeks. We found that hypoxic XY mice, irrespective of gonadal sex, developed less severe PH than XX mice. RV systolic pressures (RVSPs) were significantly lower in XY mice than in XX mice (RVSP: mean $\pm$ SEM, 43.31 ± 5.72 mm Hg in XX females and 46.51 ± 4.52 in XX males vs. 33.17 ± 2.36 in XY females and 40.4 ± 2.31 in XY males). Lower RVSPs in XY mice were accompanied by the presence of less severe pulmonary vascular remodeling (percent wall thickness: 23.84 ± 3.14 in XY females

Supported by an American Thoracic Society Proof of Concept grant (S.U.), American Heart Association grant 17PRE33420159 (C.M.C.), and NIH grants R01HL129051 (M.E.), R01HL131182 (M.E. and A.P.A.), and R01HD076125 (A.P.A.).

Author Contributions: Conception and design: S.U., C.M.C., Y.I., S.M., M.V., S.S., A.C., A.P.A., and M.E. Acquisition, analysis, or interpretation of data: S.U., C.M.C., Y.I., S.M., M.V., S.S., A.C., A.P.A., and M.E. Drafting the work or revising it critically for important intellectual content: S.U., C.M.C., Y.I., S.M., M.V., S.S., A.C., A.P.A., and M.E. Final approval of the version to be published: S.U., C.M.C., Y.I., S.M., M.V., S.S., A.C., A.P.A., and M.E. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: S.U., C.M.C., Y.I., S.M., M.V., S.S., A.C., A.P.A., and M.E.

Originally Published in Press as DOI: 10.1164/rccm.201707-1345LE on September 21, 2017

Figure 1. Gonadectomized mice with a Y chromosome develop less severe pulmonary hypertension than mice without a Y chromosome in hypoxia. (A) Bar graphs show right ventricular systolic pressure (RVSP; mm Hg) in normoxia or hypoxia for gonadectomized “four core genotypes” mice. Female XX and XY mice (with ovaries, red bars) are plotted against male XX and XY mice (with testes, black bars), $n = 4$ –5. Two-way ANOVA, with factors of sex chromosome complement and sex, revealed a main effect of sex chromosomes, shown by the asterisk ($*P < 0.05$), with no significant main effect of sex or interaction. (B) Representative Masson trichrome (upper panel) and immunofluorescence (lower panel) staining for α -smooth muscle actin (green), von Willebrand factor (red), and DAPI (blue) of lung sections of gonadectomized “four core genotypes” mice in hypoxia shows thicker pulmonary arterioles in XX mice than in XY mice, regardless of gonadal type. Scale bar in XYM for the upper panel applies to all images in the upper panel, and scale bar in XXF for the lower panel applies to all images in the lower panel. (C) Quantification of the percent wall thickness of the arterioles in lung sections from XX and XY male and female mice. Two-way ANOVA comparing sex chromosomes and sex revealed that wall thickness was much lower in XY mice than in XX mice (main effect of sex chromosomes, $***P < 0.000001$; $n = 4$ –6), but XX females had greater wall thickness than XX males (main effect of sex, $P < 0.05$; interaction trend $P = 0.083$). Thus, factors other than the Y chromosome also contribute to protection, such as the presence of *Sry* in males and/or the difference in effects of testicular and ovarian hormones acting before gonadectomy. (D) Bar graphs show RVSPs in hypoxic gonadectomized XY* mice. Female XX and XO mice (red bars) are plotted against male XXY and XY mice (black bars). $*P < 0.05$; $n = 5$. Two-way ANOVA, with factors of Y chromosome number and X chromosomes number, showed a main effect of the Y chromosome ($*P < 0.05$) but no significant effect of X chromosome number. Values were expressed as mean $\pm$ SEM. XXF = XX-female; XXM = XX-male; XYF = XY-female; XYM = XY-male.

and 22.47 ± 3.15 XY males vs. 58.8 ± 7.95 in XX females and 44.56 ± 11.68 XX males; Figures 1B and 1C).

Next, we used the XY* mouse model to determine whether the XX-XY difference found in the FCG model is caused by the presence or absence of a Y chromosome or the number of X chromosomes. The XY* model consists of female mice with XX or XO chromosomes and male mice with XY or XXY chromosomes, which allowed us to compare mice with or without a Y chromosome (XY vs. XO, or XXY vs. XX) and mice with different numbers of X chromosomes (XX vs. XO, or XXY vs. XY) (5). We found that the less severe PH in XY mice was due to the presence of a Y chromosome, but not the number of X chromosomes. RVSPs in GDX XY* mice were significantly lower in XXY (32.03 ± 2.17 mm Hg) and XY (33.49 ± 0.60 mm Hg) mice than in XX (41.56 ± 3.84 mm Hg; $P < 0.05$) and XO (38.72 ± 2.44 mm Hg; Figure 1D) mice.

These findings in GDX FCG and XY* mice support a protective role for the Y chromosome in PH. A few Y chromosome genes are expressed widely in the body, including in the lungs, and could explain the protective effect of the Y chromosome on PH. Some of these Y genes are related to counterbalancing genes on the X chromosome, but in some cases the Y partner gene has evolved novel functions (8). To discover which Y genes are expressed in lungs, we analyzed several RNA-sequencing datasets in the GEO (Gene Expression Omnibus) database that report the expression of Y genes in lungs. In mice, four protein-coding Y genes are expressed in lungs (*Ddx3y* [Y-linked DEAD-box helicase 3], *Eif2s3y* [eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked], *Kdm5d* [lysine demethylase 5D], and *Uty* [ubiquitously transcribed tetratricopeptide repeat containing, Y-linked]), and therefore are candidates to explain the protective effect of the Y chromosome in PH (Table 1). The same genes are also expressed in human lungs, except for *Eif2s3y*, which is not encoded by the Y chromosome in humans. We also analyzed a microarray dataset from the GEO database and compared the expression of Y chromosome genes in the lungs of patients with PAH versus controls. We found that expression of *KDM5D* and *UTY* was downregulated in PAH lungs ($P < 0.05$), whereas *DDX3Y* was not significantly altered (Table 1).

Our study adds dimension to the current literature by exploring the influence of sex chromosomes on the development of PH. A protective effect of the Y chromosome might help explain why male sex is the single best deterrent against the development of PAH. Y chromosome genes expressed in the lungs could serve as novel therapeutic targets for PH treatment. Although information is limited, a growing body of evidence suggests that *Ddx3y*, *Eif2s3y*, *Kdm5d*, and *Uty* are interesting candidate genes with a potential impact on fundamental cellular processes affecting proliferation, apoptosis, and epigenetic regulation (8). Dysregulation of proliferation and apoptosis is a hallmark of PAH pathogenesis, and epigenetic regulation has recently emerged as a promising direction for PAH research (9).

We conclude that the Y chromosome is protective against the development of hypoxia-induced PH in GDX mice. This study identifies the effects of sex chromosomes in the absence of gonadal hormones. Because gonadal hormones also contribute to sex differences in PH (2, 3), we expect that they interact with Y chromosome factors to affect PH development. Subsequent studies investigating these genes in different animal models may uncover a much-needed novel strategy for the treatment of PAH in patients. ■

Table 1. Chromosome Y Genes Expressed in Mouse and Human Lungs and Their Changes in the Lungs of Patients with Pulmonary Arterial Hypertension

Chromosome Y Genes Expressed in Lung			
Mouse Lung		Human Lung	
Gene	RPKM	Gene	RPKM
<i>Ddx3y</i>	9.8	<i>CD24P4*</i>	94.4
<i>Eif2s3y</i>	9.6	<i>PSMA6P1*</i>	61.2
<i>Kdm5d</i>	4.6	<i>EIF4A1P2*</i>	38.3
<i>Uty</i>	2.4	<i>DDX3Y</i>	32.4
		<i>RPS4Y1</i>	6
		<i>RNU6-941P*</i>	4.7
		<i>EIF1AY</i>	4
		<i>RPL26P37*</i>	3.3
		<i>KDM5D</i>	3.2
		<i>USP9Y</i>	2.8
		<i>TXLNGY*</i>	2.6
		<i>ZFY</i>	2.6
		<i>PRKY*</i>	2.3
		<i>TTY15†</i>	2
		<i>UTY</i>	1.8
		<i>VDAC1P6*</i>	1.5
		<i>KRT18P10*</i>	1.2

Chromosome Y Gene Expression Changes in PAH Lungs			
Gene	P Value	FDR	PAH/Ctrl
<i>KDM5D</i>	0.00001	0.04	0.52
<i>USP9Y</i>	0.001	0.11	0.13
<i>TXLNGY*</i>	0.002	0.13	0.16
<i>ZFY</i>	0.005	0.14	0.64
<i>UTY</i>	0.018	0.19	0.4
<i>EIF1AY</i>	0.11	0.35	0.55
<i>DDX3Y</i>	0.151	0.4	0.7
<i>RPS4Y1</i>	0.779	0.89	1.07

Definition of abbreviations: Ctrl = healthy control; FDR = false discovery rate; PAH = pulmonary arterial hypertension; RPKM = reads per kilobase of transcript per million mapped reads.

RNA-sequencing analysis of chromosome Y gene expression (GSE29278: male mouse lung; GSE49417: male human lung). RPKM values were used for the comparison. The R package "QuasR" was used for the read alignment to the human (hg19) or mouse (mm10) genome, followed by the counting of reads at the gene level and calculation of RPKM (10). Only genes with RPKM > 1 are listed. Microarray analysis showed changes in Y gene expression between PAH and Ctrl lungs (GSE15197: Ctrl and PAH lungs). Gene expression levels were normalized and compared using one-way ANOVA followed by FDR analysis. Genes found in both mouse and human tissues are set in bold.

*Pseudogene.

†Noncoding RNA.

Soban Umar, M.D., Ph.D.
Christine M. Cunningham, B.S.
Yuichiro Itoh, Ph.D.
Shayan Moazeni, B.S.
Mylene Vaillancourt, M.Sc.
Shervin Sarji, B.S.
Alex Centala, B.S.
Arthur P. Arnold, Ph.D.
Mansoureh Eghbali, Ph.D.
University of California, Los Angeles
Los Angeles, California

Author disclosures are available with the text of this letter at www.atsjournals.org.

References

1. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest* 2012;122:4306–4313.
2. Rabinovitch M, Gamble WJ, Miettinen OS, Reid L. Age and sex influence on pulmonary hypertension of chronic hypoxia and on recovery. *Am J Physiol* 1981;240:H62–H72.
3. Umar S, Rabinovitch M, Eghbali M. Estrogen paradox in pulmonary hypertension: current controversies and future perspectives. *Am J Respir Crit Care Med* 2012;186:125–131.
4. White RJ. Estrogen: friend or foe in pulmonary hypertension? *Am J Respir Crit Care Med* 2016;193:1084–1086.
5. Burgoyne PS, Arnold AP. A primer on the use of mouse models for identifying direct sex chromosome effects that cause sex differences in non-gonadal tissues. *Biol Sex Differ* 2016;7:68.
6. Arnold AP, Reue K, Eghbali M, Vilain E, Chen X, Ghahramani N, et al. The importance of having two X chromosomes. *Philos Trans R Soc Lond B Biol Sci* 2016;371:20150113.
7. Du S, Itoh N, Askarinam S, Hill H, Arnold AP, Voskuhl RR. XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 2014;111:2806–2811.
8. Bellott DW, Hughes JF, Skaletsky H, Brown LG, Pyntikova T, Cho T-J, et al. Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature* 2014;508:494–499.
9. Soubrier F, Chung WK, Machado R, Grünig E, Aldred M, Geraci M, et al. Genetics and genomics of pulmonary arterial hypertension. *J Am Coll Cardiol* 2013;62(25, Suppl):D13–D21.
10. Gaidatzis D, Lerch A, Hahne F, Stadler MB. QuasR: quantification and annotation of short reads in R. *Bioinformatics* 2015;31:1130–1132.

Copyright © 2018 by the American Thoracic Society

Histopathology of Interstitial Lung Abnormalities in the Context of Lung Nodule Resections

To the Editor:

Differentiating among the various interstitial lung diseases (ILDs) involves a summation of clinical, physiologic, radiologic, and, when available, histopathologic information (1). Although studies have demonstrated that subclinical stages of ILD (termed interstitial lung abnormalities [ILAs]) can be detected by chest computed tomography (CT) in research participants with no diagnosis of ILD (2–8), little is known about the histopathologic correlates of ILAs. We hypothesized that ILAs could represent an early and/or mild stage of idiopathic pulmonary fibrosis (IPF), and would therefore yield similar histopathologic

findings. To test this hypothesis, we evaluated paired chest CT scans and histopathologic samples obtained during lung nodule resections.

Methods

We retrospectively identified a cohort of 424 patients who had undergone lung nodule resection, had a chest CT scan within 3 months before surgery, and had no history of ILD, at Brigham and Women's Hospital (BWH) between January 2001 and July 2015. ILA and ILA subtype characterizations were performed as previously described (2, 3, 6–8). In all cases, at least one section of grossly uninvolved, nonneoplastic lung was submitted for histopathologic evaluation. The slides were scored by two pulmonary pathologists blind to radiologic and clinical data. A diagnosis of usual interstitial pneumonia (UIP) or other ILDs (e.g., smoking-related ILD) was made based on published criteria (1, 9), and the slides were scored for additional histopathologic findings as noted. This study was approved by the institutional review board at BWH.

Bivariate analyses were conducted using Fisher's exact tests (for categorical variables) and two-tailed *t* tests or Wilcoxon rank-sum tests (for continuous variables) when appropriate. A multivariable logistic regression analysis was performed to identify associations between ILA and histopathologic patterns, adjusting for age, sex, and pack-years smoked based on prior work (3, 7). Analyses were conducted using SAS software (version 9.4). The reported *P* values are two-sided, and values less than 0.05 were considered statistically significant.

Results

Of the 424 patients, 26 (6%) had ILA, 257 (61%) did not have ILA, and 141 (33%) had indeterminate ILA status (baseline characteristics and histopathologic patterns stratified by ILA status are presented in Table 1). There was evidence that compared with patients without ILA (or those judged to be indeterminate for ILA), patients with ILA had increased rates of pulmonary fibrosis in general and subpleural interstitial fibrosis (Figure 1A1–A3), fibroblastic foci (Figure 1C1–C4), honeycombing/UIP (Figure 1C1–C4), and atypical adenomatous hyperplasia (AAH) (Figure 1B1–B3) in particular. After adjustment for covariates, patients with ILA were more likely to have subpleural fibrosis (odds ratio [OR], 2.1; 95% confidence interval [95% CI], 1.3–3.1; *P* = 0.0009), fibroblastic foci (OR, 4.2; 95% CI, 2.2–8.1; *P* < 0.0001), and AAH (OR, 1.7; 95% CI, 1.1–2.6; *P* = 0.03) compared with those without ILA. Similar results were obtained after an additional adjustment for a history of congestive heart failure.

Of the 26 cases of ILA, 3 (12%) were centrilobular, 17 (65%) were subpleural, and 6 (23%) were mixed. There was some evidence that associations between ILA and histopathologic findings were influenced by the imaging subtype. For example, no case of fibrosis, fibroblastic foci, or honeycombing/UIP was identified in patients with centrilobular predominant ILA. In contrast, the association between AAH and ILA appeared to be limited to centrilobular predominant ILA (OR, 3.2; 95% CI, 1.5–6.4; *P* = 0.002). There was no evidence that the distance between the lung nodule and the nearest imaging findings, or the malignant status of the nodule, affected our findings of association. Of note, three patients had subpleural ILA with no pathologic evidence of fibrosis, and all of these

Supported by NIH grants T32 HL007633 (E.R.M. and R.K.P.); National Cancer Institute R01 CA203636 (M.N.); R01 HL116473 and R01 HL122424 (G.R.W.); R01 HL130974, R01 HL129920, and P01 HL114501 (I.O.R.); and R01 HL111024, R01 HL130974, R01 HL135142, and P01 HL114501 (G.M.H.).

Author Contributions: Study design: L.M.S., H.H., and G.M.H. Acquisition, analysis, or interpretation of the data: E.R.M., R.K.P., M.V., Y.H., T.A., M.N., G.R.W., I.O.R., L.M.S., H.H., and G.M.H. Critical revision of the manuscript for important intellectual content: E.R.M., R.K.P., M.V., Y.H., T.A., M.N., G.R.W., I.O.R., L.M.S., H.H., and G.M.H. Statistical analysis: E.R.M., R.K.P., and G.M.H. Obtained funding: G.M.H.

Originally Published in Press as DOI: 10.1164/rccm.201708-1679LE on September 21, 2017