The concept that men do worse with cardiovascular diseases is both broadly relevant and wholly inadequate to capture the complexity of the situation and, perhaps more important, to inform the development of productive treatment strategies that integrate the nuances imposed by sex. Although perhaps most comprehensively scrutinized in the context of ischemic heart disease and heart failure, this remains an issue of paramount importance when considering the predisposition, therapeutic protocols, and outcomes in individuals with aortic aneurysm. Abdominal aortic aneurysm (AAA) is (by far) the most common presentation of aortic aneurysm, the largest contributor to morbidity and mortality, and the least experimentally tractable form to study mechanistically and to reliably model because of low heritability, complex genetics, and a myriad of environmental influences. Notably, sex is the most prevalent yet least modifiable determinant of AAA. Even this is not simple, with a far greater incidence of AAA in men, but compelling evidence for earlier aneurysm rupture and worse surgical outcomes in women. Likewise, although the association between bicuspid aortic valve and distal ascending aortic aneurysm is far more prevalent in men, and both component phenotypes are more penetrant in men than women in extended pedigrees segregating bicuspid aortic valve and distal ascending aortic aneurysm as an autosomal dominant trait, women can show worse outcomes, including earlier or more extensive dissections. Complicating factors in the precise elucidation of the contribution of sex to more common but causatively complex presentations of aortic aneurysm, such as AAA and bicuspid aortic valve with distal ascending aortic aneurysm, include extreme locus and/or allelic heterogeneity that has an overt influence on natural history and outcome and poor control for environmental variables. Strategies to manage this complexity include a focus on mendelian presentations of aortic aneurysm with a single major gene effect imposing predisposition, and use of animal models that allow rigorous control of the underlying mutation, the genetic background (and hence the repertoire of other genetic modifiers), and environment.

In this issue of the Journal of the American Heart Association (JAHA), Tashima and colleagues report sexual dimorphism in proximal aortic growth patterns in a genetically defined and previously validated mouse model of Marfan syndrome (MFS), an autosomal dominant connective tissue disorder caused by deficiency of the extracellular matrix protein fibrillin-1 that is encoded by the FBN1 gene. The Fbn1C1039G/+ model of MFS used in this study harbors a heterozygous cysteine substitution in an epidermal growth factor–like domain, the most common class of mutation seen in patients with MFS, and faithfully recapitulates progressive fusiform dilatation of the aortic root with subsequent aortic dissection and premature death. When stratified by sex, the authors observed more rapid aortic root growth between 6 and 16 weeks of age in male mice with MFS compared with corresponding female mice; no sex difference was observed in wild-type animals. Although the authors also describe
accelerated growth of the more distal ascending aorta in male mice with MFS, visual images suggest that this reflects diffuse prominence of the aorta beyond the aortic root, extending at least to proximal descending segments, without evidence for fusiform aneurysm. Sex-specific differences in the size of proximal aortic segments were observed at the earliest recorded time point (6 weeks), with the greatest deviation in growth rate between males and females occurring between 6 and 8 weeks of age; thereafter, it appears that the absolute size difference was largely maintained over time. It would be interesting to determine if sex-specific differences are maintained throughout adult life in this model, as one might expect if driven by exposure to androgens, or if this fully plateaus, as one might expect if driven by puberty and sex-biased events, such as attainment of adult size (which was controlled for in this study) or differences in adult hemodynamic parameters (which was not).

Correlates to the accelerated proximal aortic growth that was observed in male mice with MFS included an increase in elastic fiber breaks in the aortic media, increased activation of the transforming growth factor-β (TGF-β) and extracellular signal-regulated kinase (ERK) signaling cascades, as monitored by phosphorylation of SMAD2 or ERK, respectively, and enhanced activity of matrix metalloproteinase (MMP)-2 activity within the aortic wall. Although implication of these events in aneurysm progression in this study was strictly correlative, prior evidence for their pathogenic significance in this mouse model of MFS is substantial, including enhanced activity in the natural history of disease that is accentuated with provocations that worsen disease (eg, administration of calcium channel blockers or lactation-associated oxytocin signaling) but attenuated with interventions that are protective against aneurysm progression (eg, treatment with angiotensin receptor blockers).\textsuperscript{5–6} Most convincingly, the natural and/or provoked progression of vascular disease in \textit{Fbn}\textsuperscript{1}\textit{C1039G/+} mice can be attenuated using selective direct antagonists of TGF-β, ERK, or MMP activity, such as TGF-β neutralizing antibodies, inhibitors of MAPK/ERK kinase (MEK) (the kinase that activates ERK), or doxycycline, respectively, often in a context-specific manner.\textsuperscript{5–10} This prior work has suggested that activation of ERK is, at least in part, TGF-β dependent and that MMP expression is likely a distal mediator of disease progression. In this light, the proposal by Tashima and colleagues that the TGF-β-ERK-MMP axis contributes to their observed sex differences in proximal aortic growth in mice with MFS seems likely, but remains worthy of definitive testing using specific antagonists.\textsuperscript{3}

Prior studies have attempted to define the basis for sex-specific differences in aneurysm predisposition or progression in mouse models. Although the results have been interesting, they have not provided evidence for a generalizable, or even predominant, mechanism. In a model of aneurysm based on targeting of the type I TGF-β receptor subunit, Schmit and colleagues observed that aortic aneurysm or rupture was only observed in males; although orchietomy showed minimal benefit, ovariectomy caused overt disease expression in females, suggesting a dominant role for protection afforded by female hormones.\textsuperscript{11} Interestingly, penetrance in females could also be induced by hypertension, and disease severity in both sexes correlated with the extent of ERK activation. In a model of AAA induced by both chemical injury and angiotensin II (AngII) infusion, Son and colleagues observed that the disease was enhanced on castration or treatment of males with an androgen receptor (AR) antagonist, but prevented by administration of testosterone.\textsuperscript{12} The apparent protection afforded by androgens correlated with suppression of inflammation, a hyperacute and overt contributor to aneurysm progression in chemical injury models. In contrast, multiple studies of AAA induced by AngII infusion in apolipoprotein E–deficient mice have suggested that suppression of androgen signaling on castration or pharmacologic or genetic interventions can prevent aneurysm formation in association with a reduction in inflammation or suppression of expression of the AngII type 1a receptor that is upregulated by testosterone in the abdominal (but not thoracic) aorta.\textsuperscript{13–16} Ovariectomy was not protective in the AngII/apolipoprotein E–deficient model of AAA.\textsuperscript{16} The bottom line when it comes to sex and aneurysm models appears to be that context matters, with critical determinants potentially including cause, extent of inflammation, aortic segment under consideration, and stage of disease. Any effort to propose a general principle, or to extrapolate to human disease, seems risky, at best.

In the \textit{Fbn}\textsuperscript{1}\textit{C1039G/+} mouse model of MFS, Tashima and colleagues provide compelling evidence for a deleterious effect of androgens.\textsuperscript{3} The authors convincingly show that treatment with an AR antagonist reduces proximal aortic growth rate and elastic fiber fragmentation in male mice with MFS. There was not assessment for protection in female mice with MFS, limiting determination if most of the sexual dimorphism in aortic size and growth is attributable to androgen signaling or assessment for a potential role for female androgens in disease progression.

In an attempt to further explore mechanism, the authors perform in vitro analyses of cultured vascular smooth muscle cells (VSMCs) derived from the aortas of mice with MFS.\textsuperscript{3} Male VSMCs show the expected activation of Smad2, ERK, and MMP2 in response to TGF-β, with an exaggerated response when dihydrotestosterone is also added, an effect that can be blocked by the addition of AR antagonist. Similar, albeit somewhat blunted, results were
obtained for female VSMCs derived from mice with MFS. Both dihydrotestosterone and TGF-β induced the expression of AR in cultured VSMCs, but the latter effect was marginal. The authors observed increased expression and activation of TGF-β1 in the aortic wall of male (versus female) mice with MFS, and on this basis propose that androgen stimulation of cultured aortic VSMCs might enhance the TGF-β signaling response through increased expression of active TGF-β ligand. This hypothesis was not directly tested in the cell culture system and seems unlikely given that dihydrotestosterone alone did not lead to autocrine TGF-β signaling (ie, an increase in pSmad2 in their assay). Unfortunately, VSMCs from wild-type mice were not assessed in parallel. This is important given the lack of sexual dimorphism for aortic root growth in wild-type animals, suggesting the necessity of a gene (ie, FBN1 mutation) by environment (ie, androgen exposure) interaction for phenotypic expression. If wild-type and MFS VSMCs behave identically in these assays, we would question the ability of the results to inform disease pathogenesis. Historically, it has been difficult to model pathogenic events in MFS using cell culture, likely because cellular phenotypes are both matrix and context dependent. The proposition that a simple culture system can recapitulate critical in vivo variables, including matrix deficiency, cellular composition, and cross talk, relevant effector molecule repertoire and concentrations, and chronicity is fraught with challenges and needs to be rigorously proved.

Sexual dimorphism in disease severity has been observed in both people and mouse models of vascular Ehlers-Danlos syndrome, caused by a deficiency of type III collagen, with a worse outcome in males that becomes apparent around the time of sexual maturity. Informatively, vascular rupture in mice with vascular Ehlers-Danlos syndrome is also driven by ERK activation and can be prevented via administration of a selective MEK antagonist. ERK activation is seen in both sexes of mice with vascular Ehlers-Danlos syndrome, but is accentuated in postpubertal males. Treatment with an AR antagonist reduces ERK activation and prevents aortic rupture in both male and female mice with vascular Ehlers-Danlos syndrome. As in the study by Tashima and colleagues, this experience highlights the pathogenic importance of an as of yet undefined mechanism of cross talk between androgen signaling and ERK activation in vascular connective tissue disorders, and perhaps more broadly in disorders of arterial homeostasis.

There is no clear consensus in the literature on a single mechanism by which the TGF-β and mitogen-activated protein kinases pathways engage in cross talk with the AR or even whether the AR acts upstream or downstream of mitogen-activated protein kinases and TGF-β. Additional work needs to be performed in the critical cell types that drive aneurysm progression in MFS and related conditions, potentially including VSMC lineages that populate proximal aortic segments, endothelial cells, and constituents of inflammatory infiltrates that may contribute to late events, such as aortic dissection or rupture.

The therapeutic opportunities related to a better understanding of the mechanism of sexual dimorphism in mouse models of MFS remain speculative. Indeed, population-based evidence for sex-determined differences in outcome in people with MFS remains inconsistent, with at most modest effects. This issue is further complicated by the lack of standardization for body size and other clinical variables, and by the extreme allelic heterogeneity in MFS. Nevertheless, studies such as the one described herein highlight the importance of integrating sex and other sex-biased variables into future studies aimed at the development and testing of precision medicine treatment algorithms.

ARTICLE INFORMATION

Affiliations
From the McKusick‐Nathans Department of Genetic Medicine and Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD.

Disclosures
None.

REFERENCES

1. Villard C, Hultgren R. Abdominal aortic aneurysm: sex differences. Maturitas. 2018;109:63–69.
2. Holmes KW, Maslen CL, Kindem M, Kroner BL, Song HK, Ravakees W, Dietz HC, Weinsait JW, Roman MJ, Devereux RB, et al. GenTAC Registry Report: gender differences among individuals with genetically triggered thoracic aortic aneurysm and dissection. Am J Med Genet Part A. 2013;161:779–786.
3. Tashima Y, He H, Cui JZ, Pedroza AJ, Nakamura K, Yokoyama N, Iosef C, Burdon G, Koyano T, Yamaguchi A, et al. Androgens accentuate TGF-β dependent Erk/Smad activation during thoracic aortic aneurysm formation in Marfan syndrome male mice. J Am Heart Assoc. 2020;9:e015773. DOI: 10.1161/JAHA.119.015773.
4. Judge DP, Biery NJ, Keene DR, Geubner J, Myers L, Huso DL, Sakai LY, Dietz HC. Evidence for a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. J Clin Invest. 2004;114:172–181.
5. Doyle JJ, Doyle AJ, Wilson NK, Habashi JP, Bedja D, Whitworth RE, Lindsay ME, Schoenhoff F, Myers L, Huso N, et al. A deleterious gene-by-environment interaction imposed by calcium channel blockers in Marfan syndrome. Elife. 2015;4:e08648.
6. Habashi JP, Huso N, Manning M, Chen Y, Bedja D, Creamer TJ, Rykiel G, Huso D, Dietz HC. Oxytocin antagonism prevents pregnancy-associated aortic dissection in a mouse model of Marfan syndrome. Sci Transl Med. 2019;11:eaat4822.
7. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeyes BL, Cooper TK, Myers L, Klein EC, Liu G, Calvi G, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006;312:117–121.
8. Holm TM, Habashi JP, Doyle JJ, Bedja D, Chen Y, van Erp C, Lindsay ME, Kim D, Schoenhoff F, Cohn RD, et al. Noncanonical TGFβ signaling contributes to aortic aneurysm progression in marfan syndrome mice. Science. 2011;795:358–361.

9. Cook JR, Clayton NP, Carta L, Galatioto J, Chiu E, Smaldone S, Nelson CA, Cheng SH, Wentworth BM, Ramirez F. Dimorphic effects of transforming growth factor-β signaling during aortic aneurysm progression in mice suggest a combinatorial therapy for Marfan syndrome. Arterioscler Thromb Vasc Biol. 2015;35:911–917.

10. Cui JZ, Lee L, Sheng X, Chu F, Gibson OP, Aydinian T, Walker DC, Sandor GGS, Bernatchez P, Tibbits GF, et al. In vivo characterization of doxycycline-mediated protection of aortic function and structure in a mouse model of Marfan syndrome-associated aortic aneurysm. Sci Rep. 2019;9:1–17.

11. Schmit BM, Yang P, Fu C, DeSart K, Berceli SA, Jiang Z. Hypertension overrides the protective effect of female hormones on the development of aortic aneurysm secondary to Alk5 deficiency via ERK activation. Am J Physiol Heart Circ Physiol. 2015;308:H115–H125.

12. Son B-K, Kojima T, Ogawa S, Akishita M. Testosterone inhibits aneurysm formation and vascular inflammation in male mice. J Endocrinol. 2019;241:307–317.

13. Huang C-K, Luo J, Lai K-P, Wang R, Pang H, Chang E, Yan C, Sparks J, Lee SO, Choa J, et al. Androgen receptor (AR) promotes abdominal aortic aneurysm (AAA) development via modulating inflammatory IL1α and TGFβ1 expression. Hypertension. 2015;66:881–891.

14. Henriques T, Zhang X, Yiannikouris FB, Daughters A, Cassis A. Androgen increases AT1a receptor expression in abdominal aortas to promote angiotensin II-induced AAAs in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2008;28:1251–1256.

15. Zhang X, Thatcher SE, Rateri DL, Bruemmer D, Charnigo R, Daughters A, Cassis LA. Transition between neonatal female mice to testosterone abrogates the sexual dimorphism of abdominal aortic aneurysms. Circ Res. 2012;110:73–85.

16. Henriques TA, Huang JD, Souza SS, Daughters A, Cassis LA. Orchidectomy, but not ovariectomy, regulates angiotensin II-induced vascular diseases in apolipoprotein E-deficient mice. Endocrinology. 2004;145:3866–3872.

17. Bowen CJ, Calderon-Gladiosic JF, Burger Z, Rykiel G, Davis EC, Helmers MR, Gallo MacFarlane E, Dietz HC. Targetable cellular signaling events mediate vascular pathologies in vascular Ehlers-Danlos syndrome. J Clin Invest. 2020;130:686–698.

18. Mueller GC, Stark V, Steiner K, Von Kodolitsch Y, Rybczynski M, Weil J, Mir TS. Impact of age and gender on cardiac pathologies in children and adolescents with Marfan syndrome. Pediatr Cardiol. 2013;34:991–998.

19. Meijboom LJ, Timmermans J, Zwinderman AH, Engelfriet PM, Mulder BJ M. Aortic root growth in men and women with the Marfan's syndrome. Am J Cardiol. 2005;96:1441–1444.

20. Detaint D, Faivre L, Collod-Beroud G, Child AH, Loeyls BL, Binquet C, Gautier E, Arbustini E, Mayer K, Arslan-Kirchner M, et al. Cardiovascular manifestations in men and women carrying a FBN1 mutation. Eur Heart J. 2010;31:2223–2229.