X Marks the Spot

The Profound Impact of Sex on Aortic Disease

Siddharth K. Prakash, Dianna M. Milewicz

Biological sex and gender exert profound effects on all aspects of cardiovascular disease, from susceptibility to disease to therapeutic outcomes to access to care. Women typically develop coronary heart disease when they are older than men but experience disproportionate harm from risk factors, such as smoking and diabetes mellitus, and have a higher mortality after myocardial infarction.[1,2] Similar sex differences are observed for both thoracic and abdominal aortic aneurysms (AAAs). Male sex is the most potent nonmodifiable risk factor for AAAs, with estimates ranging from a 4- to 10-fold higher incidence in men than in women.[3,4]

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Thoracic aortic aneurysms and acute aortic dissections also primarily affect men but to a lesser extent than AAA. Individuals presenting for surgical repair of either a thoracic aneurysm or acute dissection are 65% men.[5] Similarly, the International Registry of Acute Aortic Dissection reported a male predominance of 65% among individuals presenting with an acute aortic dissection.[6] Furthermore, individuals with a single autosomal gene mutation predisposing them to thoracic aortic aneurysms are predominantly men. Recent data on the largest cohort to date of patients with Marfan syndrome in the National Registry of Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions demonstrate that male sex is associated with more severe and earlier symptoms.[7–9] The age of onset of aortic events was even more dramatically different based on sex in patients with *TGFBR1* (TGF-β [transforming growth factor-β] receptor type 1) mutations, with 90% of men having an event by age of 60 years compared with only half the women.[10] The proportion of men presenting with aortic dissections is also higher than women. In contrast, there is no dramatic difference based on sex in patients with either *TGFBR2* (TGF-β receptor type II) or smooth muscle *ACTA2* (α-actin) mutations.[10,11] Finally, an alteration in a sex chromosome can increase the risk for thoracic aortic disease. Turner syndrome is a disorder that affects only women and results from partial or complete loss of 1 X chromosome. The prevalence of bicuspid aortic valves and aortic coarctation are both increased in women with Turner syndrome and are associated with premature deaths from thoracic aortic aneurysms and acute aortic dissections.[12]

Therefore, aortic aneurysm disease, in particular AAA, is a vascular disease for which human clinical data indicate a strong male predominance. A mouse model of AAA that recapitulates the male predominance observed in humans, angiotensin II (Ang II) infusion in a hyperlipidemic (*Ldlr*$^{-/-}$) strain, has been used extensively to investigate the molecular basis of sex differences in vascular diseases. These studies have demonstrated a clear role of testosterone in increasing AAA in male mice. In fact, exposing female fetuses to testosterone increased aortic aneurysms in the adult *Lldr*$^{-/-}$ mice after Ang II infusion. In addition, an XY sex chromosome complement also promotes AAA susceptibility in female *Ldlr*$^{-/-}$ mice after Ang II infusion. Thus, both gonadal sex hormones and sex chromosomes contribute to the increased risk for AAA with Ang II in this hyperlipidemic mouse.

Alsiraj et al[13] continue the focus of the Cassis group on sex differences in their investigation of AAA.[14] These investigators cleverly created a unique mouse model to dissect the role of sex chromosomes and hormones in AAA biology. To evaluate the separate effects of gonadal sex hormones and sex chromosomes, they utilized an inbred mouse strain with a natural mutation in the sex-determining *Sry* gene, which was substituted with an autosomal wild-type *Sry* transgene. *Sry* is a gene on the Y chromosome that initiates male development starting in the embryo and leads to a male phenotype, including testes development. Matings, therefore, generated phenotypic males with either XX or XY sex chromosomes (Figure). After they crossed these mice with LDLR (low-density lipoprotein receptor)-deficient hyperlipidemic mice, they found that an XY chromosome complement and intact testes are both necessary to explain regional differences in aortic pathology in response to Ang II infusion. XY male mice primarily developed diffuse adventitial thickening throughout the thorax and abdomen, whereas XX male mice tended to develop thinner-walled aneurysms that were concentrated in the suprarenal abdominal aorta. These striking differences in regional aortic pathology were abolished by castration. Their findings suggest that genes on the Y chromosome, or X chromosome genes that escape X inactivation, in part, determine these significant sex differences in regional aortic remodeling in response to Ang II infusion.

The authors identified a set of potential target genes that are differentially expressed in XX and XY male aortic tissues and thus are candidates to mediate divergent AAA pathologies. The essential downstream components of this pathway may, in part, be modulated by the hypertensive response to Ang II. XY males were significantly more hypertensive than XX males, and this blood pressure difference may have

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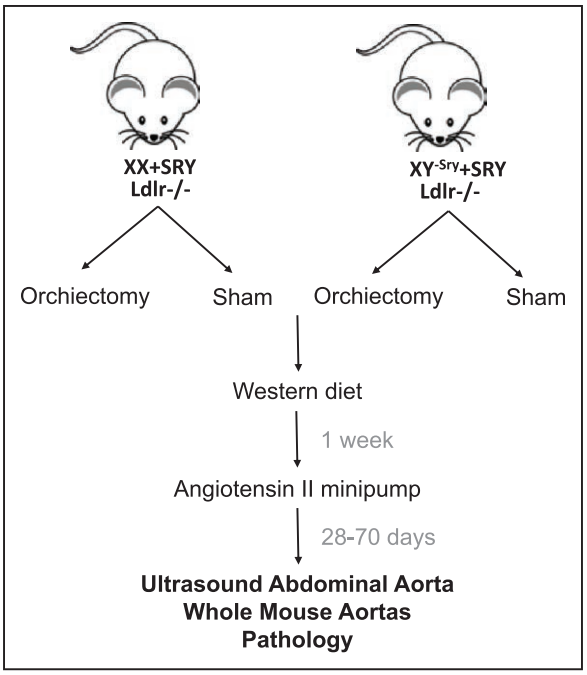

Figure. Schematic illustrating the design of the study by Alsiraj et al.[13] XX+SRY: masculinized XX mice that express an autosomal *Sry* transgene; XY^{-SRY}+SRY: XY mice with a natural mutation of the native *Sry* gene on the Y chromosome that express an autosomal *Sry* transgene; *Ldlr*$^{-/-}$: low-density lipoprotein receptor-deficient mouse strain; mice either underwent orchiectomy or a sham operation, followed by a high-fat Western diet and angiotensin II infusion. Aortas were imaged at intervals until mice were euthanized for pathological analysis.

contributed to more pronounced adventitial hypertrophy in XY males. It will also be important to evaluate the respective contributions of gonadal hormones besides testosterone, such as estrogen and AMH (anti-Müllerian hormone), which may interact with sex chromosome genes to regulate this gene network and modulate susceptibility to AAA.[15] Some differences between XY males, with normal testes, and XX males, with small testes, may be caused by reduced concentrations of AMH, which was inversely associated with aortic diameter in AAA cohorts.[16] Finally, these experiments should be repeated in older animals to determine whether sex-related differences persist because the general decline of male sex hormones with age may itself be associated with increased cardiovascular morbidity and AAA.[17,18]

Interestingly, sex differences in the location of aortic atherosclerotic plaques in hyperlipidemic mice have also been reported; female Ldlr$^{-/-}$ mice have larger aortic root lesions than male mice.[19,20] Furthermore, the beneficial effect of a PPARγ (peroxisome proliferator-activated receptor gamma) agonist on atherosclerosis in C57BL/6 *Ldlr*$^{-/-}$ mice was only observed in male mice.[21] Thus, both atherosclerotic plaques and aortic aneurysm formation demonstrated regional differences based on sex, and potential explanations are becoming clear. Epidemiological and animal studies showed that serum estrogen protects against AAA formation and rupture. Infusion of 17-β estradiol or hormone replacement therapy is associated with reduced AAA growth rates, but oophorectomy or menopause is associated with increased AAA growth and rupture.[22] In the abdominal aorta, estrogens suppress the expression of matrix metalloproteinases, which are increased after estrogen depletion; in contrast, the relationship between estrogen levels and thoracic aortic disease is less clear.[23,24] Regional differences in the embryological origins of aortic smooth muscle cells (SMCs) may also predispose to aneurysm formation. SMCs in the ascending aorta and arch arise primarily from the neural crest (ectoderm), whereas the thoracoabdominal aorta is derived from the somites (mesoderm).[25] SMCs of different origins secrete different levels of extracellular matrix proteins, and cultured SMCs from women were found to express significantly higher levels of extracellular matrix components than SMCs from men.[26] In addition, explanted aortic SMCs from male and female rats show significant sex-specific differences in survival after oxidative stress, which are implicated in AAA and thoracic aortic disease pathogenesis.[27,28] Taken together, these structural and functional differences could account for profound differences in regional susceptibility to dilation or dissection, and could it be that these factors also predict differences in response to therapeutics for aortic aneurysms based on sex?

To address disparities in basic and preclinical research, the National Institutes of Health recently mandated that sex must be factored into the design and methods of grant applications.[29] Inclusion of sex and gender is not only integral to ethical research but is also required for rigorous science because sex is a preeminent biological variable for vascular diseases.[30,31] Additionally, response to therapeutics for aortic disease may also have sex differences, information that is important in the design of clinical trials and ultimately in precision medicine for the treatment of disease. The study by Alsiraj et al shows how sex and sex differences can be exploited to develop new insights into an old disease.

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Disclosures

None.

References

1. Mosca L, Barrett-Connor E, Wenger NK. Sex/gender differences in cardiovascular disease prevention: what a difference a decade makes. *Circulation*. 2011;124:2145–2154. doi: 10.1161/CIRCULATIONAHA.110.968792.
2. Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E, Peters SA. Sex differences in cardiovascular risk factors and disease prevention. *Atherosclerosis*. 2015;241:211–218. doi: 10.1016/j.atherosclerosis.2015.01.027.
3. Pockley AG, Georgiades A, Thulin T, de Faire U, Frostegård J. Serum heat shock protein 70 levels predict the development of atherosclerosis in subjects with established hypertension. *Hypertension*. 2003;42:235–238. doi: 10.1161/01.HYP.0000086522.13672.23.
4. Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, Mohan A, Yan C, Abe J, Illig KA, Berk BC. Cyclophilin a enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. *Nat Med*. 2009;15:649–656. doi: 10.1038/nm.1958.
5. LeMaire SA, Russell L. Epidemiology of thoracic aortic dissection. *Nat Rev Cardiol*. 2011;8:103–113. doi: 10.1038/nrcardio.2010.187.
6. Hagan PG, Nienaber CA, Isselbacher EM, et al. The international registry of acute aortic dissection (IRAD): new insights into an old disease. *JAMA*. 2000;283:897–903.

7. Roman MJ, Devereux RB, Preiss LR, et al; GenTAC Investigators. Associations of age and sex with marfan phenotype: the national heart, lung, and blood institute GenTAC (Genetically Triggered Thoracic Aortic Aneurysms and Cardiovascular Conditions) Registry. *Circ Cardiovasc Genet*. 2017;10:e001647. doi: 10.1161/CIRCGENETICS.116.001647.
8. Groth KA, Stochholm K, Hove H, Kyhl K, Gregersen PA, Vejlstrup N, Østergaard JR, Gravholt CH, Andersen NH. Aortic events in a nationwide Marfan syndrome cohort. *Clin Res Cardiol*. 2017;106:105–112. doi: 10.1007/s00392-016-1028-3.
9. Détaint D, Faivre L, Collod-Beroud G, et al. Cardiovascular manifestations in men and women carrying a FBN1 mutation. *Eur Heart J*. 2010;31:2223–2229. doi: 10.1093/eurheartj/ehq258.
10. Jondeau G, Ropers J, Regalado E, et al; Montalcino Aortic Consortium. International registry of patients carrying TGFBR1 or TGFBR2 mutations: results of the MAC (Montalcino Aortic Consortium). *Circ Cardiovasc Genet*. 2016;9:548–558. doi: 10.1161/CIRCGENETICS.116.001485.
11. Regalado ES, Guo DC, Prakash S, et al; Montalcino Aortic Consortium. Aortic disease presentation and outcome associated with ACTA2 mutations. *Circ Cardiovasc Genet*. 2015;8:457–464. doi: 10.1161/CIRCGENETICS.114.000943.
12. Carlson M, Airhart N, Lopez L, Silberbach M. Moderate aortic enlargement and bicuspid aortic valve are associated with aortic dissection in turner syndrome: report of the international turner syndrome aortic dissection registry. *Circulation*. 2012;126:2220–2226. doi: 10.1161/CIRCULATIONAHA.111.088633.
13. Alsiraj Y, Thatcher SE, Blalock E, Fleenor B, Daugherty A, Cassis LA. Sex chromosome complement defines diffuse versus focal angiotensin II–induced aortic pathology. *Arterioscler Thromb Vasc Biol*. 2018;38:143–153. doi: 10.1161/ATVBAHA.117.310035.
14. Grundy SM; National Cholesterol Education Program (NCEP)-The National Cholesterol Guidelines in 2001, (ATP)III. Approach to lipoprotein management in 2001 National Cholesterol Guidelines. *Am J Cardiol*. 2002;90:11i–21i.
15. Johnston WF, Salmon M, Su G, Lu G, Ailawadi G, Upchurch GR Jr. Aromatase is required for female abdominal aortic aneurysm protection. *J Vasc Surg*. 2015;61:1565–1574.e1. doi: 10.1016/j.jvs.2014.01.032.
16. Dennis NA, Jones GT, Chong YH, van Rij AM, McLennan IS. Serum antimullerian hormone (AMH) levels correlate with infrarenal aortic diameter in healthy older men: is AMH a cardiovascular hormone? *J Endocrinol*. 2013;219:13–20. doi: 10.1530/JOE-13-0125.
17. Kloner RA, Carson C III, Dobs A, Kopecky S, Mohler ER III. Testosterone and cardiovascular disease. *J Am Coll Cardiol*. 2016;67:545–557. doi: 10.1016/j.jacc.2015.12.005.
18. Yeap BB, Hyde Z, Norman PE, Chubb SA, Golledge J. Associations of total testosterone, sex hormone-binding globulin, calculated free testosterone, and luteinizing hormone with prevalence of abdominal aortic aneurysm in older men. *J Clin Endocrinol Metab*. 2010;95:1123–1130. doi: 10.1210/jc.2009-1696.
19. Maeda N, Johnson L, Kim S, Hagaman J, Friedman M, Reddick R. Anatomical differences and atherosclerosis in apolipoprotein E-deficient mice with 129/SvEv and C57BL/6 genetic backgrounds. *Atherosclerosis*. 2007;195:75–82. doi: 10.1016/j.atherosclerosis.2006.12.006.
20. Surra JC, Guillén N, Arbonés-Mainar JM, Barranquero C, Navarro MA, Arnal C, Orman I, Segovia JC, Osada J. Sex as a profound modifier of atherosclerotic lesion development in apolipoprotein E-deficient mice with different genetic backgrounds. *J Atheroscler Thromb*. 2010;17:712–721.
21. Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK. Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest*. 2000;106:523–531. doi: 10.1172/JCI10370.
22. Starr JE, Halpern V. Abdominal aortic aneurysms in women. *J Vasc Surg*. 2013;57(suppl 4):3S–10S. doi: 10.1016/j.jvs.2012.08.125.
23. Schuster V, Eggersmann TK, Eifert S, Ueberfuhr P, Zugenmaier B, Kolben TM, Thaler CJ, Kublickiene K, Rieger A, Reichart B, Hagl C, Pichlmaier MA, Guethoff S. Ascending aortic disease is associated with earlier menopause and shorter reproductive life span. *J Womens Health (Larchmt)*. 2016;25:912–919. doi: 10.1089/jwh.2015.5559.
24. Thalmann M, Sodeck GH, Domanovits H, Grassberger M, Loewe C, Grimm M, Czerny M. Acute type A aortic dissection and pregnancy: a population-based study. *Eur J Cardiothorac Surg*. 2011;39:e159–e163. doi: 10.1016/j.ejcts.2010.12.070.
25. Sinha S, Iyer D, Granata A. Embryonic origins of human vascular smooth muscle cells: implications for in vitro modeling and clinical application. *Cell Mol Life Sci*. 2014;71:2271–2288. doi: 10.1007/s00018-013-1554-3.
26. Li Y, Wen Y, Green M, Cabral EK, Wani P, Zhang F, Wei Y, Baer TM, Chen B. Cell sex affects extracellular matrix protein expression and proliferation of smooth muscle progenitor cells derived from human pluripotent stem cells. *Stem Cell Res Ther*. 2017;8:156. doi: 10.1186/s13287-017-0606-2.
27. Malorni W, Straface E, Matarrese P, Ascione B, Coinu R, Canu S, Galluzzo P, Marino M, Franconi F. Redox state and gender differences in vascular smooth muscle cells. *FEBS Lett*. 2008;582:635–642. doi: 10.1016/j.febslet.2008.01.034.
28. Straface E, Vona R, Campesi I, Franconi F. Mitochondria can orchestrate sex differences in cell fate of vascular smooth muscle cells from rats. *Biol Sex Differ*. 2015;6:34. doi: 10.1186/s13293-015-0051-9.
29. Miller VM, Rocca WA, Faubion SS. Sex differences research, precision medicine, and the future of women's health. *J Womens Health (Larchmt)*. 2015;24:969–971. doi: 10.1089/jwh.2015.5498.
30. Department of Health, Education, and Welfare; National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. The Belmont Report. Ethical principles and guidelines for the protection of human subjects of research. *J Am Coll Dent*. 2014;81:4–13.
31. Robinet P, Milewicz DM, LA C, Leeper NJ, JD S. Consideration of sex differences in design and reporting of experimental arterial pathology studies: a statement from the ATVB Council. *Arterioscler Thromb Vasc Biol*. 2018. Under review.

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