Female XX sex chromosomes increase survival and extend lifespan in aging mice

Emily J. Davis1 | Iryna Lobach2 | Dena B. Dubal1

1Department of Neurology, Biomedical Sciences Graduate Program, and Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, California
2Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, California

Correspondence
Dena B. Dubal, Department of Neurology, Biomedical Sciences Graduate Program, and Weill Institute for Neurosciences, University of California, San Francisco, San Francisco, CA.
Email: Dena.Dubal@ucsf.edu

Funding information
Coulter-Weeks Foundation; Bakar Foundation; American Federation for Aging Research; Glenn Foundation for Medical Research; National Science Foundation, Grant/Award Number: 1650113; National Institute of Health, Grant/Award Number: AG034531, NS092918

Abstract
Female longevity is observed in humans and much of the animal kingdom, but its causes remain elusive. Using a genetic manipulation that generates XX and XY mice, each with either ovaries or testes, we show that the female XX sex chromosome complement increases survival during aging in male and female mice. In combination with ovaries, it also extends lifespan. Understanding causes of sex-based differences in aging could lead to new pathways to counter age-induced decline in both sexes.

KEYWORDS
aging, four core genotype, life-span studies, mortality, mouse models, sex differences, sex hormones

1 | INTRODUCTION

Women live longer than men around the world, regardless of culture or socioeconomic status (UnitedNations, 2015; Zarulii et al., 2018). Female longevity is also observed in the animal kingdom (Barrett & Richardson, 2011; Bronikowski et al., 2011; Clutton-Brock & Isvaran, 2007) due to causes that may be extrinsic, intrinsic, or both. Extrinsic causes of sex difference in invertebrates can signal antagonistic survival strategies: female pheromones reduce male lifespan in Drosophila (Gendron et al., 2014), and male secretions shorten hermaphrodite lifespan in C. elegans (Maures et al., 2014). Intrinsic effects—operating within the organism—underlie longer life in organisms following removal of reproductive cells or organs in C. elegans hermaphrodites (Berman & Kenyon, 2006), male and female dogs (Hoffman, Creevy, & Promislow, 2013), and possibly men as suggested by a study of eunuchs (Min, Lee, & Park, 2012). Nonetheless, causes of intrinsic sex difference in lifespan remain largely unknown. The pervasive nature of female longevity in humans, even in early death during severe epidemics and famine (Zarulii et al., 2018), suggests a role for innate biology in the survival gap between the sexes. Here, we sought to identify intrinsic causes of female longevity in mammalian lifespan.

Sex chromosomes or gonads cause intrinsic sex differences in mammals, but whether they directly contribute to increased female lifespan is unknown in mammalian aging. To dissect these etiologies, we used four core genotypes (FCG) mice (Arnold, 2004). In mice and humans, the Sry gene normally resides on the Y chromosome and codes for a protein (testicular determining Y factor) that induces development of testes and perinatal masculinization. In FCG mice, Sry resides instead on an autosome, enabling inheritance of Sry—and

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2018 The Authors. Aging Cell published by the Anatomical Society and John Wiley & Sons Ltd.

Aging Cell. 2019;18:e12871.
https://doi.org/10.1111/acel.12871
thus male, testicular phenotype—with or without the Y chromosome.

The genetic manipulation of SRY generates XX and XY mice, each with either ovaries (O) or testes (T): XX(O), XX(T), XY(O), XY(T) (Figure 1a). Gonadal hormone levels in FCG mice with the same gonads are comparable, regardless of their sex chromosomes (Gatewood et al., 2006; McCullough et al., 2016). In FCG model mice, a sex difference with a main effect that statistically differs by genotype (XX vs. XY) is sex chromosome-mediated; one that differs by phenotype (ovaries vs. testes) is gonadal sex-mediated (Figure 1b).

Examples of age-relevant FCG mouse studies show that XX improves blood pressure regulation (Pessoa et al., 2015) and attenuates experimental brain injuries (Du et al., 2014; McCullough et al., 2016).

To explore sex-based differences in lifespan, we generated and aged over 200 mice from the FCG model on a congenic C57BL/6J background and investigated aging-dependent mortality from midlife to old age (12–30 months) (Figure 1c). We first examined whether mortality in “typical” females (XX,O) and males (XY,T) recapitulates the pattern of female longevity. Indeed, aging females (XX,O) lived longer than aging males (XY,T) (Figure 1d; Supporting Information Table S1).

We next measured main effects of sex chromosomes and gonads on survival in aging. XX mice with ovaries or testes lived longer than XY mice of either gonadal phenotype, indicating a main effect of sex chromosomes on lifespan (Figure 1e; Supporting Information Table S2). Mice with ovaries (XX & XY) tended to live longer than those with testes (XX & XY), suggesting a gonadal influence on lifespan (Figure 1f; Supporting Information Table S2). Collectively, these data indicate that the XX genotype increases survival in aging—and suggest a protective effect of ovaries.
To further understand benefits of femaleness on survival in aging, we directly compared the four groups of mice. In mice with ovaries, XX increased lifespan compared to XY (Figure 2a; Supporting Information Table S3). In mice with testes, mortality tended to be higher overall and did not differ between XX and XY genotypes (Figure 2b; Supporting Information Table S3). Ovaries increased lifespan in XX, but not XY mice (Figure 2c,d; Supporting Information Table S4). This suggests that female gonadal hormones, through organizational (long-term) or activational (short-term) effects, increase lifespan in the presence of a second X chromosome.

Since the XX genotype showed a main effect on overall survival, we next tested whether it increases resilience against death anytime during aging. We used the grid search method (Lerman, 1980) to determine the point in time when XX and XY lifespan curves change in relation to each other in mice with matching gonads. We then measured statistical differences between the two curves before and after that time point are shaded (significant differences = green grid pattern; no difference = shaded red). (e) In mice with ovaries, the relationship between XX and XY lifespan curves changed at 21 months with no difference before then (XX, \(HR = 0.52, SE = 0.64, p = 0.31\)) and significant difference afterward (XX, \(HR = 0.37, SE = 0.45, *p = 0.01\)). (f) In mice with testes, the relationship between XX and XY lifespan curves changed at 23 months with a significant difference before then (XX, \(HR = 0.36, SE = 0.60, *p = 0.05\)) and no difference afterward (HR = −0.78, SE = 0.33, \(p = 0.23\)).

HR = hazard ratio, CI = confidence interval, and SE = standard error; HR < 1 is decreased mortality risk (statistical details in Supporting Information Tables S3–S6).
after that point to assess whether XX increases survival at any time in aging. In mice with ovaries, XX increased survival after 21 months (Figure 2e; Supporting Information Table S5). In mice with testes, XX also increased survival, but the benefit was earlier, prior to 23 months, and did not alter maximal lifespan (Figure 2f; Supporting Information Table S6). Thus, independent of maximal lifespan, the XX genotype increased survival during aging in both male and female mice, albeit at different times.

It is important to note that lifespan and its interventions in mice are influenced by strain, substrain, environment, diet, and factors yet unidentified (Austad & Fischer, 2016). Thus, the presence, extent, and direction of sex bias in lifespan can vary across mouse colonies, even among C57BL6 substrains. Future studies examining mixed genetic backgrounds across geographic sites will be valuable. Nonetheless, our data are clear and indicate that female sex derived from the XX sex chromosome complement, combined with ovarian gonad exposure, extended lifespan; furthermore, the XX genotype itself increased survival in aging male and female mice.

Whether the presence of a second X chromosome or the lack of a Y dictates genetic causes of this intrinsic female advantage remains to be determined. Further, how hormone signaling induces ovarian-mediated survival in the presence of a second X chromosome deserves study. Major pathways underlying an XX-ovarian interaction could include IGF1 signaling (Brooks & Garratt, 2017), telomeres (Barrett & Richardson, 2011), or mitochondrial functions (Gaignard et al., 2015).

Evolutionary pressure may lie upon increased survival and longer lifespan in females to ensure additional care and better fitness for generations of genetic offspring. Alternatively, more male death could benefit the next generation by reducing competition for resources and mates. The identification and modulation of intrinsic XX-derived mechanisms of female advantage could open new pathways to modify and increase healthy aging in both sexes.

ACKNOWLEDGMENTS
We thank Dan Wang and Lauren Broestl for colony care. Supported by the NSF grant 1650113 (E.J.D.), NIH grants AG034531 and NS092918 (D.B.D.), Coulter-Weeks Foundation (D.B.D), Bakar Foundation (D.B.D), American Federation for Aging Research (E.J.D., D.B.D), and Glenn Foundation (D.B.D). D.B.D. has consulted for Unity Biotechnology.

AUTHOR CONTRIBUTIONS
E.J.D., LL, and D.B.D. carried out experimental studies and analyses and wrote the manuscript. All authors discussed results and commented on the manuscript.

ORCID
Emily J. Davis http://orcid.org/0000-0001-9878-1252

REFERENCES
Arnold, A. P. (2004). Sex chromosomes and brain gender. Nature Reviews Neuroscience, 5(9), 701–708. https://doi.org/10.1038/nrn1494
Austad, S. N., & Fischer, K. E. (2016). Sex differences in lifespan. Cell Metabolism, 23(6), 1022–1033. https://doi.org/10.1016/j.cmet.2016.05.019
Barrett, E. L., & Richardson, D. S. (2011). Sex differences in telomeres and lifespan. Aging Cell, 10(6), 913–921. https://doi.org/10.1111/j.1474-9726.2011.00741.x
Berman, J. R., & Kenyon, C. (2006). Germ-cell loss extends C. elegans life span through regulation of DAF-16 by kri-1 and lipophilic-hormone signaling. Cell, 124(5), 1055–1068. https://doi.org/10.1016/j.cell.2006.01.039
Bronikowski, A. M., Altmann, J., Brockman, D. K., Cords, M., Fedigan, L. M., Pusey, A., … Alberts, S. C. (2011). Aging in the natural world: Comparative data reveal similar mortality patterns across primates. Science, 331(6022), 1325–1328. https://doi.org/10.1126/science.1201571
Brooks, R. C., & Garratt, M. G. (2017). Life history evolution, reproduction, and the origins of sex-dependent aging and longevity. Annals of the New York Academy of Sciences, 1389(1), 92–107. https://doi.org/10.1111/nyas.13302
Clutton-Brock, T. H., & Isvaran, K. (2007). Sex differences in ageing in natural populations of vertebrates. Proceedings of the Royal Society B: Biological Sciences, 274(1629), 3097–3104. https://doi.org/10.1098/rspb.2007.1138
Du, S., Itoh, N., Askarinam, S., Hill, H., Arnold, A. P., & Vasquez, R. R. (2014). XY sex chromosome complement, compared with XX, in the CNS confers greater neurodegeneration during experimental autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences of the United States of America, 111(7), 2806–2811. https://doi.org/10.1073/pnas.1307091111
Gaignard, P., Savouroux, S., Liere, P., Planos, A., Therond, P., Schumacher, M., … Guennoun, R. (2015). Effect of sex differences on brain mitochondrial function and its suppression by ovariectomy and in aged mice. Endocrinology, 156(8), 2893–2904. https://doi.org/10.1210/en.2014-1913
Gatewood, J. D., Wills, A., Shetty, S., Xu, J., Arnold, A. P., Burgoyne, P. S., & Rissman, E. F. (2006). Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. Journal of Neuroscience, 26(8), 2335–2342. https://doi.org/10.1523/JNEUROSCI.3743-05.2006
Gendron, C. M., Kuo, T. H., Harvanek, Z. M., Chung, B. Y., Yew, J. Y., Dierick, H. A., & Fletcher, S. D. (2014). Drosophila life span and physiology are modulated by sexual perception and reward. Science, 343(6170), 544–548. https://doi.org/10.1126/science.1243339
Hoffman, J. M., Creevy, K. E., & Promislow, D. E. (2013). Reproductive capability is associated with lifespan and cause of death in companion dogs. PLoS ONE, 8(4), e61082. https://doi.org/10.1371/journal.pone.0061082
Lerman, P. M. (1980). Fitting segmented regression models by grid search. Journal of the Royal Statistical Society. Series C (Applied Statistics), 29(1), 77–84. https://doi.org/10.2307/2346413
Maures, T. J., Booth, L. N., Benayoun, B. A., Izraylivit, Y., Schroeder, F. C., & Brunet, A. (2014). Males shorten the life span of C. elegans hermaphrodites via secreted compounds. Science, 343(6170), 541–544. https://doi.org/10.1126/science.1244160
McCullough, L. D., Mirza, M. A., Xu, Y., Bentivegna, K., Steffens, E. B., Ritzel, R., & Liu, F. (2016). Stroke sensitivity in the aged: Sex chromosome complement vs. gonadal hormones. Metabolism, 1033. https://doi.org/10.1001/ent.2016.1068. https://doi.org/10.1016/j.cell.2016.10.019
Min, K. J., Lee, C. K., & Park, H. N. (2012). The lifespan of Korean eunuchs. Current Biology, 22(18), R792–R793. https://doi.org/10.1016/j.cub.2012.06.036
Pessoa, B. S., Slump, D. E., Ibrahimi, K., Greffhorst, A., van Veghel, R., Garelds, I. M., ... van Esch, J. H. (2015). Angiotensin II type 2 receptor- and acetylcholine-mediated relaxation: Essential contribution of female sex hormones and chromosomes. *Hypertension, 66*(2), 396–402. https://doi.org/10.1161/HYPERTENSIONAHA.115.05303

United Nations (2015). World Population Ageing 2015. United Nations, Department of Economic and Social Affairs, Population Division (ST/ESA/SER.A/390), 1–164.

Zarulli, V., Barthold Jones, J. A., Oksuzyan, A., Lindahl-Jacobsen, R., Christensen, K., & Vaupel, J. W. (2018). Women live longer than men even during severe famines and epidemics. *Proceedings of the National Academy of Sciences of the United States of America, 115*(4), E832–E840. https://doi.org/10.1073/pnas.1701535115

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Davis EJ, Lobach I, Dubal DB. Female XX sex chromosomes increase survival and extend lifespan in aging mice. *Aging Cell*. 2019;18:e12871. https://doi.org/10.1111/acel.12871