Y chromosome's roles in sex differences in disease

Arthur P. Arnold^{a,1}

In PNAS, Kremmentsov et al. (1) report that if you are a male mouse and catch the flu, the severity of your illness may depend on the type of Y chromosome (ChrY) that you have. In this study, influenza A virus was administered to consomic ChrY mouse strains in which numerous different versions of ChrY, derived from different mouse strains, were bred onto the same genetic background. This method varies the composition of ChrY while keeping the autosomes and X chromosome (ChrX) constant, so any difference in influenza pathogenesis reveals functional differences encoded by different ChrYs. The type of ChrY influences the frequency of $\gamma\delta$ T cells in the infected lung, and their production of IL-17. The Kremmentsov et al. report leads to the confident conclusion that some ChrY genetic elements regulate inflammatory immune responses and the pathogenesis of an infectious disease. Other work from the Teuscher laboratory, also using consomic ChrY strains, demonstrates ChrY effects on other viral infections and autoimmune diseases, including a mouse model of multiple sclerosis (2, 3). These reports are important in part because they bear on sex differences in immunity, but also have implications for a more general understanding of ChrY, and its partner, ChrX.

Further work will no doubt search for specific ChrY elements that affect immunity. Although ChrX contains many genes that regulate immune function, until recently one might have been pessimistic about finding ChrY elements controlling traits, including immune function. Methods that are useful for dissecting the roles of specific autosomal genes have been less informative for ChrY. For example, almost the entire ChrY is a single linkage unit, so linkage studies cannot localize specific ChrY regions that affect traits. Sequencing ChrY has been frustrated by its highly repetitive nature (4–6). Knocking out ChrY genes using homologous recombination has generally failed. Thankfully, the newest genome-editing and gene-knockdown methods are now able to manipulate ChrY gene expression (7, 8). Specific ChrY genes affecting traits are also discovered by adding back genes via transgenesis to offset effects of spontaneous

ChrY mutations (9). And, the triumphant sequencing of the entire ChrY in a few species has revolutionized our view of it and the study of its functions (4–6). We are going to find out a lot more about ChrY.

Sex Differences Versus Sexual Balance

A critical question is whether the Kremmentsov et al. study (1) shows that the large sex differences in immunity and autoimmune diseases (10, 11) are explained in part by a male-specific effect of ChrY. The results of this study are compatible with this idea, but also with a competing hypothesis that ChrY makes males more like females. Discovering the causes of sex differences is important because one sex is often more affected by infection or autoimmune disease, meaning that the other sex is protected by endogenous sex-biased mechanisms that might be useful targets for therapy. Most research on sex differences has been devoted to understanding the differentiating effects of gonadal hormones, because the hormones are potent, easy to manipulate, and are classically thought to be the only cause of sex differences outside of the gonads (12). Indeed, evidence supports the idea that levels of gonadal hormones alter autoimmunity and the course of infectious diseases (10, 11). Importantly, finding a role for gonadal hormones to cause sex differences in the immune system, and in the response to infection and inflammation, does not rule out direct sex-biasing effects of ChrX and ChrY ("sex chromosome effects"). Hormone and sex chromosome effects have been found to independently contribute to the same sex difference (11, 13). Moreover, finding a ChrY effect does not rule out a ChrX effect in causing sex differences related to the number and type of sex chromosomes. Indeed, for various other sex differences in mouse models of disease, the number of ChrXs appears to explain sex chromosome effects that are found (14). Below, I suggest an interpretation of Kremmentsov et al. study (1) that would increase the likelihood of finding sex-biasing effects of ChrX.

The current view of ChrY is conditioned by recent discoveries from the sequencing of several ChrYs and

^aDepartment of Integrative Biology & Physiology, Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, CA 90095

Author contributions: A.P.A. wrote the paper.

The author declares no conflict of interest.

See companion article on page 3491 in issue 13 of volume 114.

¹Email: arnold@ucla.edu.

comparison of ChrY across species (6, 15–17). We used to think of ChrY as a small bastion of pure maleness, the “masculinizer” of the genome. ChrY makes males different from females. ChrY contains the *Sry* gene, which causes testes to develop and secrete testicular hormones (18). The testicular hormones differ considerably in their effects relative to ovarian hormones, which develop in XX females in the absence of ChrY and *Sry*. Thus, ChrY masculinizes by setting up lifelong sex differences in the effects of gonadal hormones that we currently think are the root of most sex differences in physiology and disease (12). In addition, *Sry* has male-specific functions outside of the gonads (19). If ChrY is purely a masculinizer, then the Kremmentsov et al. study (1) shows that the masculinizing effect contributes to sex differences in response to influenza infection.

However, ChrY also has “balancer” functions that offset the effects of ChrX and make the two sexes more similar (6, 20). ChrX and ChrY evolved from a pair of ancient autosomes that existed 180 Myr ago (16). The emergence of *Sry* on one of those autosomes initiated a series of degenerative ChrY events and major compensatory changes in ChrX (21, 22). ChrY lost most of its original genes, as well as genes added more recently to both ChrX and ChrY. This left the remaining ChrX genes out of balance in males (1X) vs. females (2X), relative to the rest of the genome. To compensate, females shut off one of the two ChrXs in their cells to bring ChrX expression into the male range, and both sexes up-regulate ChrX expression to the level of autosomes (20). However, some X genes escape X inactivation, and are expressed in two doses in XX cells and one dose in XY cells. For a few X escapee genes, at least, ChrY plays the role of balancer by retaining dosage-compensating genes that are very similar to some X genes that escape inactivation (16, 17). These similar X-Y gene pairs are orthologs of the ancient autosomal genes from which they both evolved, and they overlap in their functions (23). Females express two copies of the X genes, whereas males express one X and one Y copy, achieving some balance. ChrY therefore participates in dosage compensation, a job that is normally thought to be performed exclusively by the female genome. Dosage compensation on ChrY makes males more like females, the opposite of the masculinizer role. The balancer ChrY genes likely compensate for a lack of a second ChrX in males (23).

Another example of balancer functions is illustrated in the tight coevolution of ChrX and ChrY (21, 22). Over 95% of the present-day mouse ChrY is composed of ampliconic genes, comprising repetitions of only three gene families that were not present on the ancestral autosomal precursors of ChrY and ChrX (5). The evolutionary duplication of these genes on ChrY is matched by their amplification on ChrX. The massive expansion of ampliconic genes is explained by meiotic drive, in which a driver emerges on one sex chromosome, favoring inheritance of that sex chromosome relative to the other (5, 6, 8, 24). A driver on one sex chromosome sets up countervailing selection pressures that favor the emergence of a suppressor on the other sex chromosome. If the driver and suppressor are dosage-sensitive, each becomes dominant and then is counteracted by the other in successive reciprocal waves of amplification of each. Thus, 95% of ChrY acts to offset and counteract the effects of ChrX, at least in the male germ line, making the two chromosomes more similar.

In consomic ChrY strains, as used by Kremmentsov et al. (1), are the variable effects of different ChrY's evidence for varying degrees of masculinizing functions of ChrY? Or are they evidence that the balance achieved between ChrY and ChrX in separately

evolving mouse populations (causing greater sexual equality) has been disrupted when the strain of ChrY does not match that of ChrX and the autosomes? In my view, both are likely, and the jury is out for the specific effects in consomic ChrY strains. As mouse populations diverged over the last 0.5–1.0 Myr (5), separate mutations of ChrY in each population might have given rise to selection pressures favoring adaptive changes in ChrX and rest of the genome, to keep the X-Y balance. In other words, selection pressure to keep males and females similar, in immune or other physiological functions

In PNAS, Kremmentsov et al. report that if you are a male mouse and catch the flu, the severity of your illness may depend on the type of Y chromosome (ChrY) that you have.

that are important in both sexes, may have operated to match ChrY gene function to ChrX gene function differently in different mouse populations. The breeding of inbred strains may have favored or undermined this match.

ChrY as Masculinizer Versus Balancer

A long-known example of mismatch of ChrY to background stain uncovers differences in ChrY as masculinizer. When a poschiavinus strain ChrY (ChrY^{POS}), derived from wild-caught mice from the Val Poschiavo in Switzerland, is crossed to a C57BL/6 background, testis differentiation fails in some XY progeny (25). The ChrY^{POS} *Sry*, which reliably causes formation of testes within a ChrY^{POS} genetic background, is mismatched to the C57BL/6 background and, therefore, has biological consequences that impact the health of consomic ChrY males.

An illustrative example of offsetting X-Y effects comes from the study of sex chromosome effects on body weight and adiposity. Adding a ChrY to an XO mouse increases body weight and adiposity in one strain of mice (26), and the effect of ChrY is mimicked by that of ChrX; XX and XY mice are similar, but each differs from XO. In this case, a second sex chromosome, either ChrX or ChrY, affects the phenotype in the same manner, suggesting that ChrY prevents deleterious effects of the lack of a second ChrX. In another strain, however, adding a second ChrX to XO increases body weight/adiposity, but adding a ChrY does not (13). Thus, the balancing or out-of-balance effects of ChrX and ChrY, which may make male and female mice more or less similar, are strain-dependent. The balancing effects of ChrX and ChrY are likely to be labile. If sexual equality is favored, then selection pressures will make the two chromosomes more similar. When sex differences are favored, then the two chromosomes move out of balance. The X-Y balance may come and go, producing or reducing sex differences. From this viewpoint, a variable effect of ChrY (1) leads to the prediction that variation in ChrX sequence would also produce or reduce sex differences.

For studies of immunity and autoimmune disease, the fascinating use of consomic ChrY strains (1–3) puts ChrY on the map as a source of factors that can either produce sex differences or balance them out. These exciting studies provide much food for thought and rationalize future studies to find the ChrY and ChrX factors that influence immunity.

Acknowledgments

The author is supported by NIH Grants HD076125, HL131182, and DK083561.

- 1 Kremtsov DN, et al. (2017) Genetic variation in chromosome Y regulates susceptibility to influenza A virus infection. *Proc Natl Acad Sci USA* 114(13):3491–3496.
- 2 Case LK, et al. (2013) The Y chromosome as a regulatory element shaping immune cell transcriptomes and susceptibility to autoimmune disease. *Genome Res* 23:1474–1485.
- 3 Case LK, Teuscher C (2015) Y genetic variation and phenotypic diversity in health and disease. *Biol Sex Differ* 6:6.
- 4 Skaletsky H, et al. (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* 423:825–837.
- 5 Soh YQ, et al. (2014) Sequencing the mouse Y chromosome reveals convergent gene acquisition and amplification on both sex chromosomes. *Cell* 159:800–813.
- 6 Hughes JF, Page DC (2015) The biology and evolution of mammalian Y chromosomes. *Annu Rev Genet* 49:507–527.
- 7 Nakasuji T, et al. (2017) Complementary critical functions of Zfy1 and Zfy2 in mouse spermatogenesis and reproduction. *PLoS Genet* 13:e1006578.
- 8 Cocquet J, et al. (2009) The multicopy gene *Sly* represses the sex chromosomes in the male mouse germline after meiosis. *PLoS Biol* 7:e1000244.
- 9 Mazeyrat S, et al. (2001) A Y-encoded subunit of the translation initiation factor Eif2 is essential for mouse spermatogenesis. *Nat Genet* 29:49–53.
- 10 Klein SL, Flanagan KL (2016) Sex differences in immune responses. *Nat Rev Immunol* 16:626–638.
- 11 Voskuhl RR, Gold SM (2012) Sex-related factors in multiple sclerosis susceptibility and progression. *Nat Rev Neurol* 8:255–263.
- 12 Arnold AP (2012) The end of gonad-centric sex determination in mammals. *Trends Genet* 28:55–61.
- 13 Chen X, et al. (2012) The number of X chromosomes causes sex differences in adiposity in mice. *PLoS Genet* 8:e1002709.
- 14 Arnold AP, et al. (2016) The importance of having two X chromosomes. *Philos Trans R Soc Lond B Biol Sci* 371:20150113.
- 15 Livernois AM, Graves JA, Waters PD (2012) The origin and evolution of vertebrate sex chromosomes and dosage compensation. *Heredity (Edinb)* 108:50–58.
- 16 Cortez D, et al. (2014) Origins and functional evolution of Y chromosomes across mammals. *Nature* 508:488–493.
- 17 Bellott DW, et al. (2014) Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. *Nature* 508:494–499.
- 18 Eggers S, Ohnesorg T, Sinclair A (2014) Genetic regulation of mammalian gonad development. *Nat Rev Endocrinol* 10:673–683.
- 19 Loke H, Harley V, Lee J (2015) Biological factors underlying sex differences in neurological disorders. *Int J Biochem Cell Biol* 65:139–150.
- 20 Disteche CM (2012) Dosage compensation of the sex chromosomes. *Annu Rev Genet* 46:537–560.
- 21 Vallender EJ, Lahn BT (2004) How mammalian sex chromosomes acquired their peculiar gene content. *BioEssays* 26:159–169.
- 22 Graves JAM (2006) Sex chromosome specialization and degeneration in mammals. *Cell* 124:901–914.
- 23 Shpargel KB, Sengoku T, Yokoyama S, Magnuson T (2012) UTX and UTY demonstrate histone demethylase-independent function in mouse embryonic development. *PLoS Genet* 8:e1002964.
- 24 Cocquet J, et al. (2012) A genetic basis for a postmeiotic X versus Y chromosome intragenomic conflict in the mouse. *PLoS Genet* 8:e1002900.
- 25 Eicher EM, Washburn LL, Whitney JB, 3rd, Morrow KE (1982) *Mus poschiavinus* Y chromosome in the C57BL/6J murine genome causes sex reversal. *Science* 217:535–537.
- 26 Chen X, McCluskey R, Itoh Y, Reue K, Arnold AP (2013) X and Y chromosome complement influence adiposity and metabolism in mice. *Endocrinology* 154:1092–1104.