"Four Core Genotypes" Rats: Comparing XX and XY Rats with the Same Type of Gonads to Detect Sex Chromosome Effects that Cause Sex Differences in Physiology and Disease

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Rationale

The mouse Four Core Genotypes (FCG) model has successfully allowed comparison of XX and XY mice with the same type of gonads, to determine if sex differences in mouse traits are caused by cell-autonomous effects of X and Y genes ("sex chromosome effects", SCEs). For example, among 30 brain regions shown by MRI to have different volumes in FCG mice, almost 45% showed differences caused SCEs (XX vs. XY), whereas others showed differences caused by gonadal hormones (PMID 25445841). We sought to produce an FCG-like model in rats, because many sex differences are studied in rat brain and behavior (and other traits), but measuring SCEs has not been possible in rats.

Aims

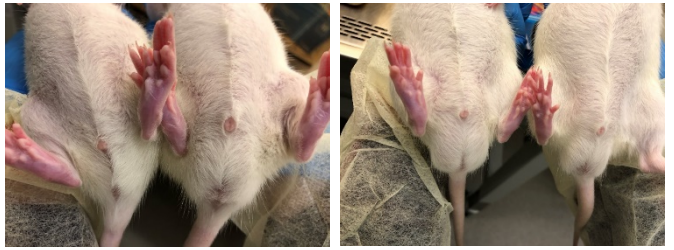
1. Insert a transgene of *Sry* (testis-determining gene) onto an autosome to make XX rats with testes. Compare phenotypes of XX and XY rats with testes.
2. Knock out testis-determining function from the Y chromosome to make XY rats with ovaries. Compare phenotypes of XX and XY rats with ovaries.

Complication

The rat has up to 11 *Sry* genes. We first determined which *Sry* transcripts are expressed in the XY gonad as it begins to differentiate into a testes: *Sry4A, Sry1, and Sry3C*. Prokop et al., *Biology of Sex Differences* 2020, PMID 32398044

Autosomal Sry transgene

A BAC clone encoding *Sry4A, Sry1, and Sry3C*, and no other genes, was inserted onto an autosome to make founder XY(*Sry*-tg+) males. These males bred with WT XX females to produce 3 kinds of gonadal males: WT XY, XY(*Sry*-tg+), and XX(*Sry*-tg+), allowing comparison of XX and XY gonadal males in the same litters.



WT XX female XX(*Sry*-tg+) male WT XY male XX (*Sry*-tg+)male

Fig. 1

CRISPR KO of testis-determining factor (TDF) on Y chromosome

Guide RNAs aimed at *Sry*4A caused elimination of TDF, producing XY$^{\Delta}$ rats with ovaries that produced litters containing XX and XY$^{\Delta}$ females.

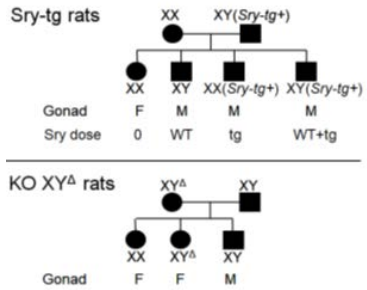


Fig 2. Breeding scheme to produce XX and XY rats with the same type of gonads, either testes, or ovaries. Comparison of phenotypes of XX and XY rats shows effects of sex chromosome complement.

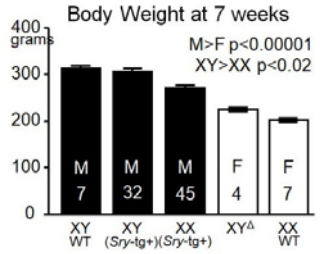


Fig. 3. Body weight was larger in rats with testes than ovaries (hormone effect), but also larger in XY than XX rats with the same type of gonad (sex chromosome effect).

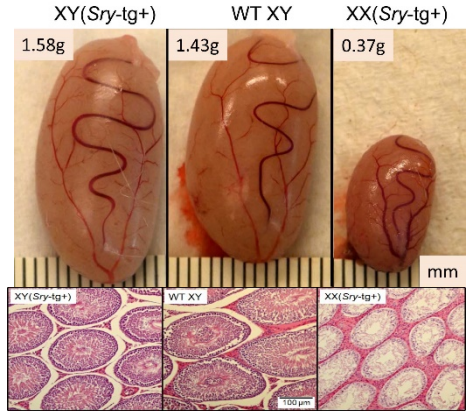


Fig. 4. As expected, XX male rats have smaller testes than XY male rats, because of the lack of sperm.

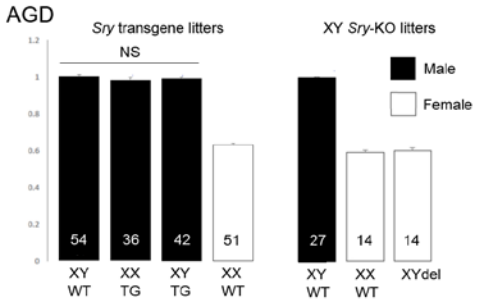
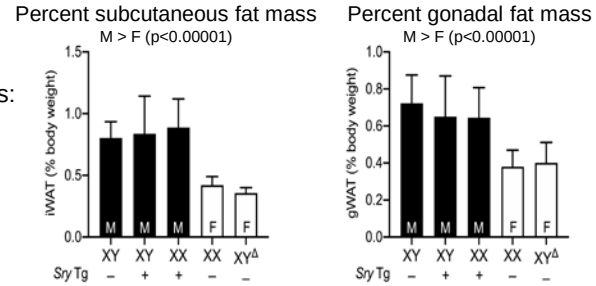


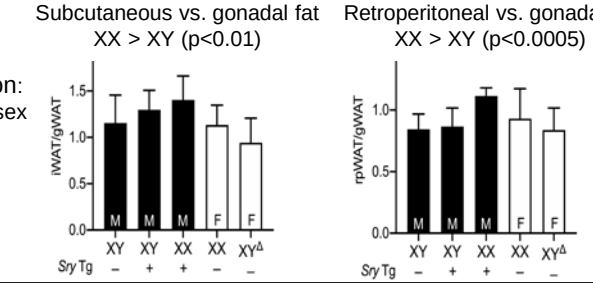
Fig. 5. Anogenital distance, a measure of prenatal androgen levels, was similar among all types of gonadal males, and smaller and similar in XX and XY females, implying no difference in prenatal androgen levels in XX and XY rats with the same type of gonad.

Fig. 6. Body fat depots show sex differences in total mass (relative to body weight), caused by different gonadal hormones (M>F), or in ratios of mass of different depots caused by sex chromosome complement (XX>XY).

Total fat mass:
Gonadal sex
M > F



Fat distribution:
Chromosomal sex
XX > XY



Conclusion: These rat resources allow comparison of XX and XY rats that have the same type of gonad, and thus permit measurement of the effects of sex chromosome complement (XX vs. XY) on any rat phenotype.

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