Sex chromosome-modified mice and rats to identify factors causing sex differences in physiology and disease

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Complex Trait Community – RGD
October 3, 2024

UCLA

FCG

LNE

Major themes

1. 20th century models held that non-gonadal phenotypes are sexually differentiated exclusively by gonadal hormones, based on available experimental evidence.

2. The hormones-only theory has been replaced by a theory that includes large differences in XX and XY somatic tissues caused by cell-autonomous effects of sex chromosomes.

3. The Four Core Genotypes mouse model has emerged as the best first step for detecting sex chromosome effects that cause sex differences.

4. Using FCG and then XY* mouse models has major advantages.

5. We have manipulated *Sry* in rats to make XX and XXY and XYY rats with testes, XY and XXY rats with ovaries, expanding the investigation of sex chromosome effects to a new mammalian species.

Sex differences in diseases: One sex is protected.

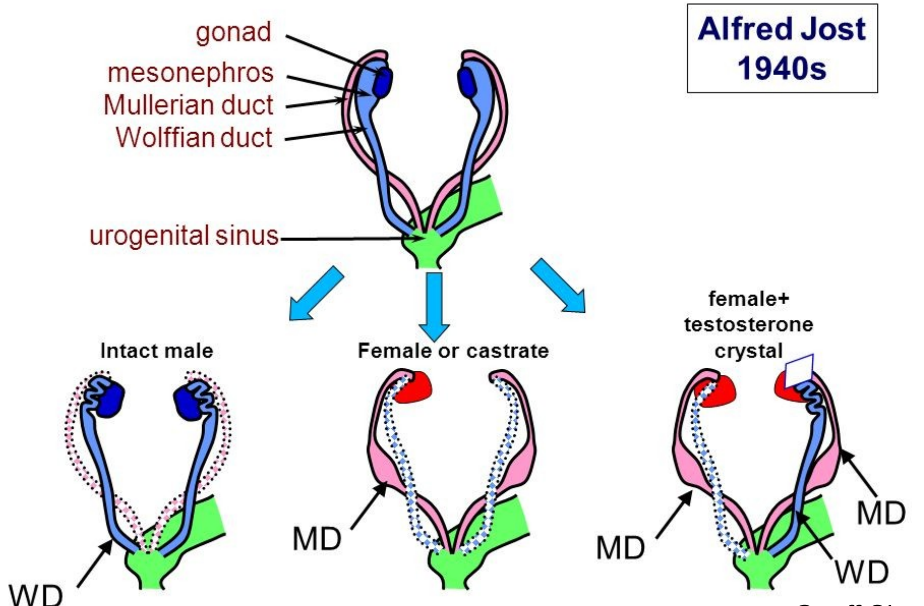
COVID19	M>F	1.25 : 1
Lupus (systemic lupus erythematosus)	F>M	9 : 1
Multiple sclerosis	F>M	3 : 1
Depression	F>M	1.7 : 1
Bulimia	F>M	10 : 1
ADHD	M>F	2:1 to 10:1
Tourette's syndrome	M>F	3 : 1
Heart disease	M>F	1.1 : 1
Schizophrenia	M>F	1.4 : 1

Can we develop therapies to enhance protective factors or inhibit harmful factors?

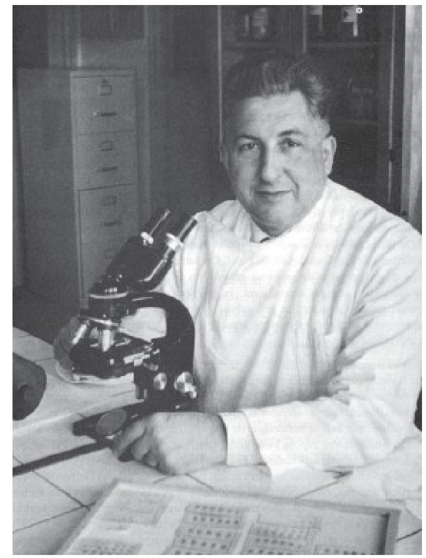
What sex-biased factors modify disease?

Alfred Jost (1940s)
The testes secrete two hormones (T and AMH) that cause male pattern of genital development.

Alfred Jost 1940s



Geoff Shaw



1916–1991

Control male	Control female or castrated male	Female + testes	Female + testosterone
sperm ducts ✓	sperm ducts 🚫	sperm ducts ✓	sperm ducts ✓
oviducts 🚫	oviducts ✓	oviducts 🚫	oviducts ✓
uterus 🚫	uterus ✓	uterus 🚫	uterus ✓

Testosterone causes persistence of sperm ducts.
Anti-Müllerian Hormone kills uterus and oviducts

Testosterone acts prenatally to masculinize behavior permanently.

ORGANIZING ACTION OF PRENATALLY ADMINISTERED TESTOSTERONE PROPIONATE ON THE TISSUES MEDIATING MATING BEHAVIOR IN THE FEMALE GUINEA PIG[1]

CHARLES H. PHOENIX, ROBERT W. GOY, ARNOLD A. GERALL AND WILLIAM C. YOUNG

Department of Anatomy, University of Kansas, Lawrence, Kansas

***Endocrinology* 1959 65:369-82.**

Testosterone treatment of female guinea pig fetuses ***permanently*** masculinizes / defeminizes their behavior.

Prenatal testosterone causes development of masculine traits that are permanent.

Permanent!

20^{th} Century Central Dogma (right, and wrong)

XY vs. XX

↓

Gonads: testes vs. ovaries

Sex Determination (first, genetic)

↓

Sex Differences in Non-Gonadal Phenotypes

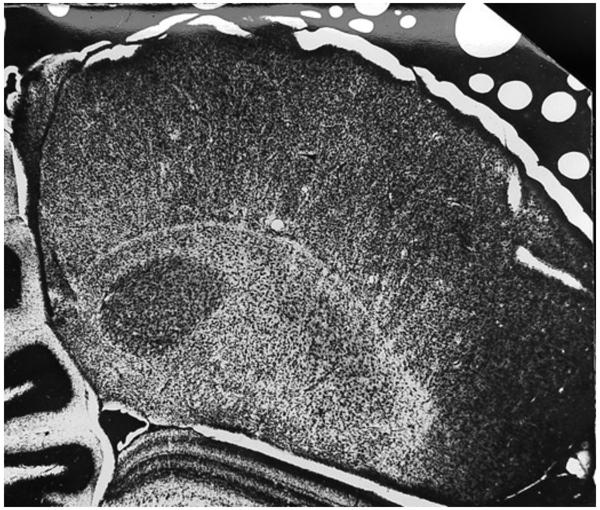
Sexual Differentiation (second, hormonal)

Zebra Finches

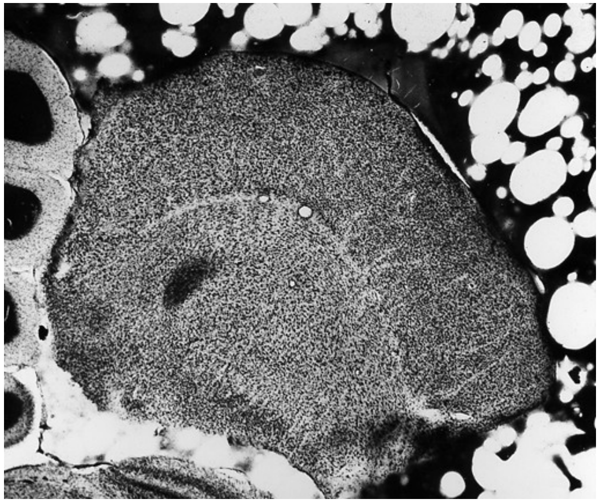
male
sings

female
does not

Neural Song Circuit



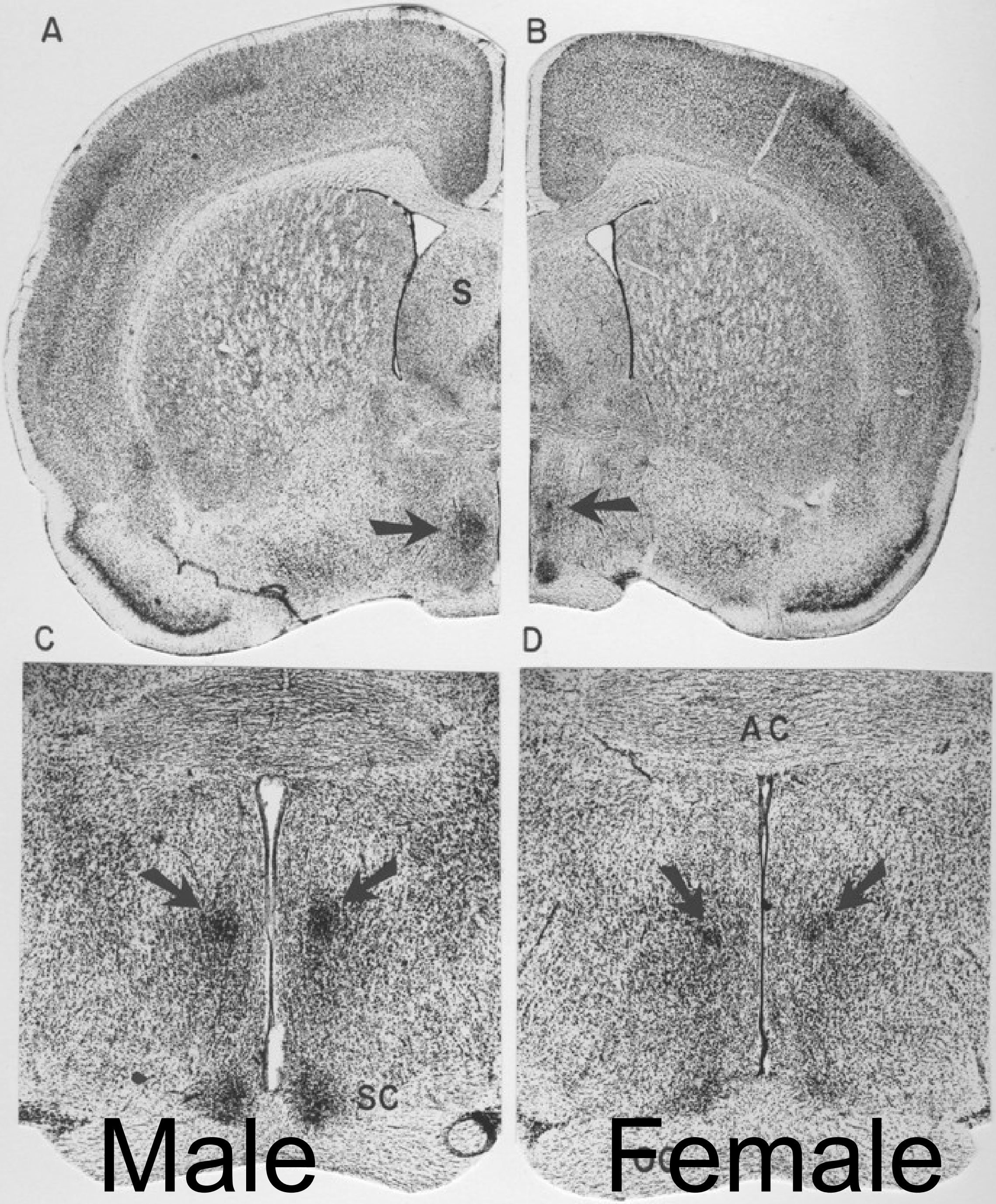
male



female

Nottebohm and Arnold
Science, 1976

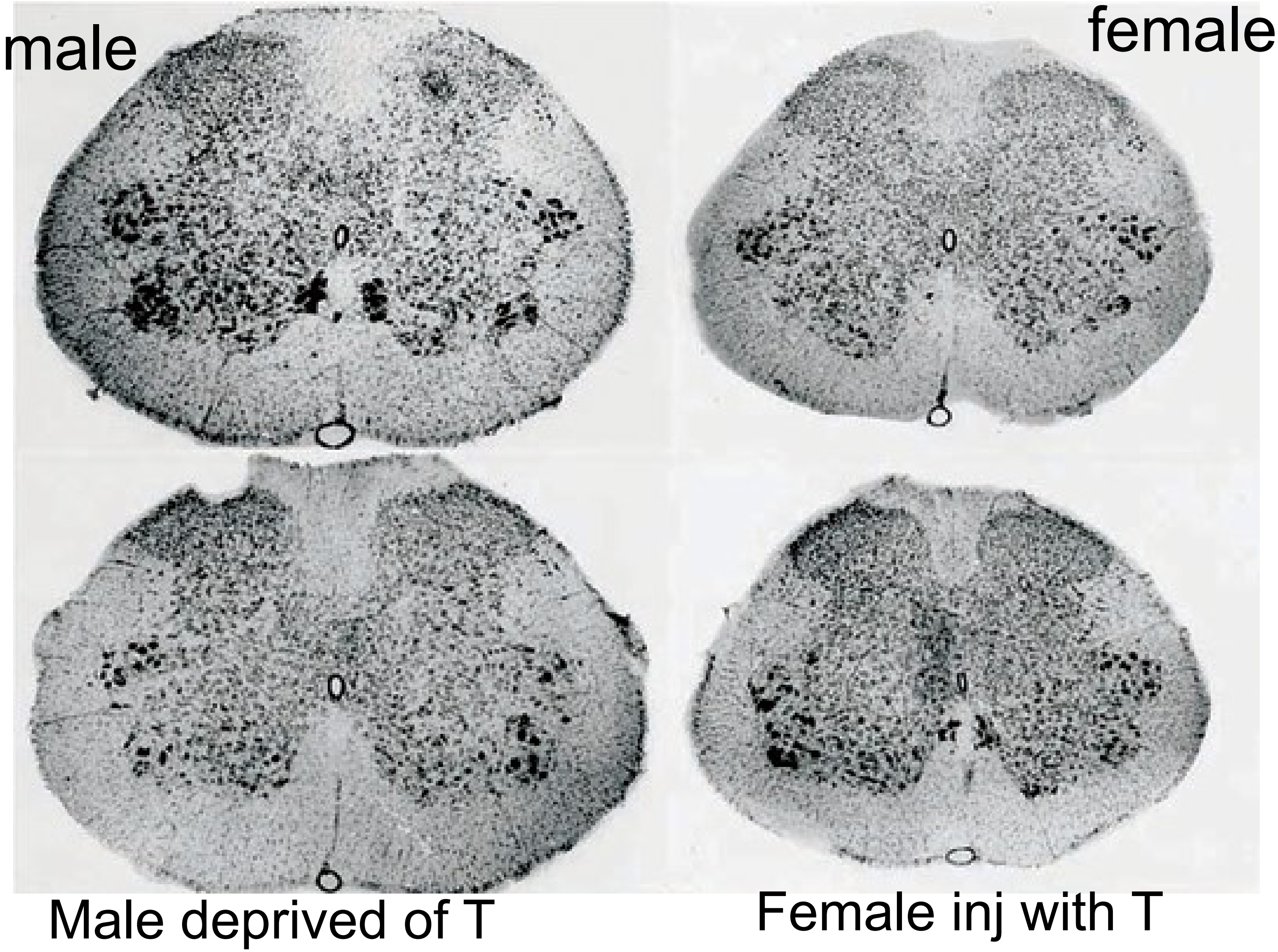
Sexually dimorphic nucleus of the preoptic area (rat POA)



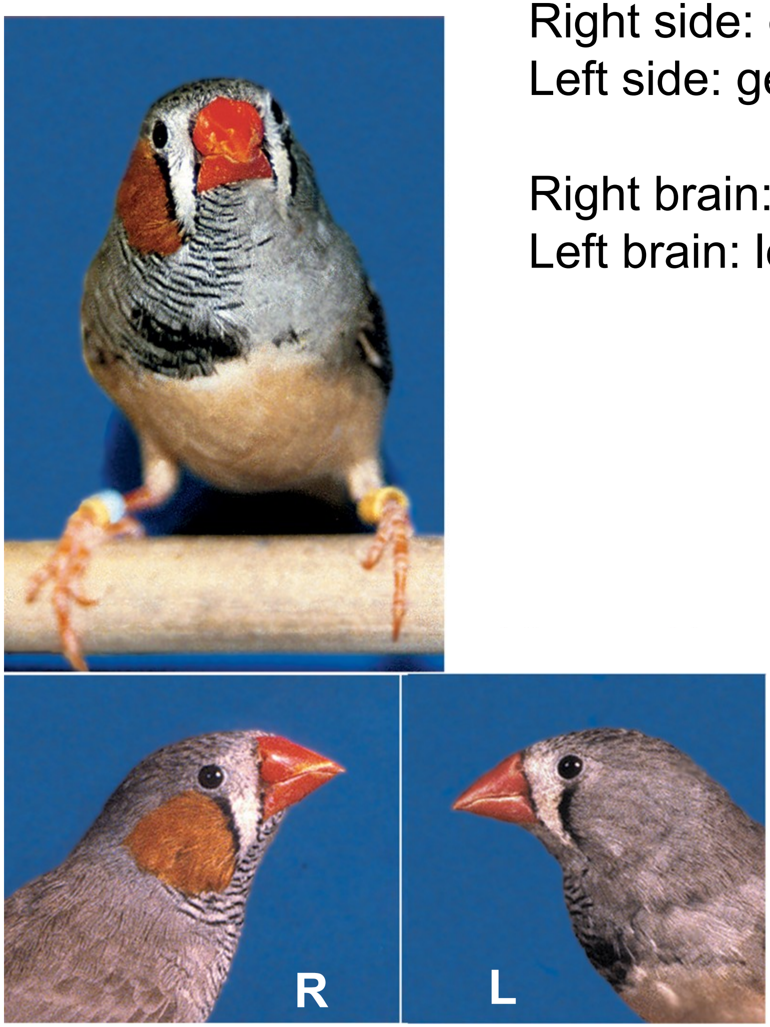
Roger Gorski, UCLA

Manipulations of hormones prenatally prove that the CNS is masculinized by prenatal testosterone.

Breedlove and Arnold, *Science* 1980

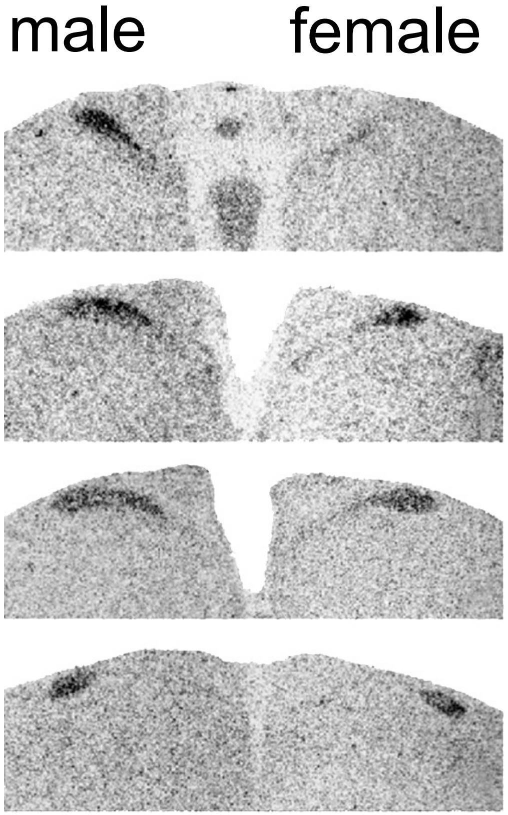


Gynandromorphic Zebra Finch suggests that brain sex chromosome genes cause sex differences by acting via cell-autonomous mechanisms.



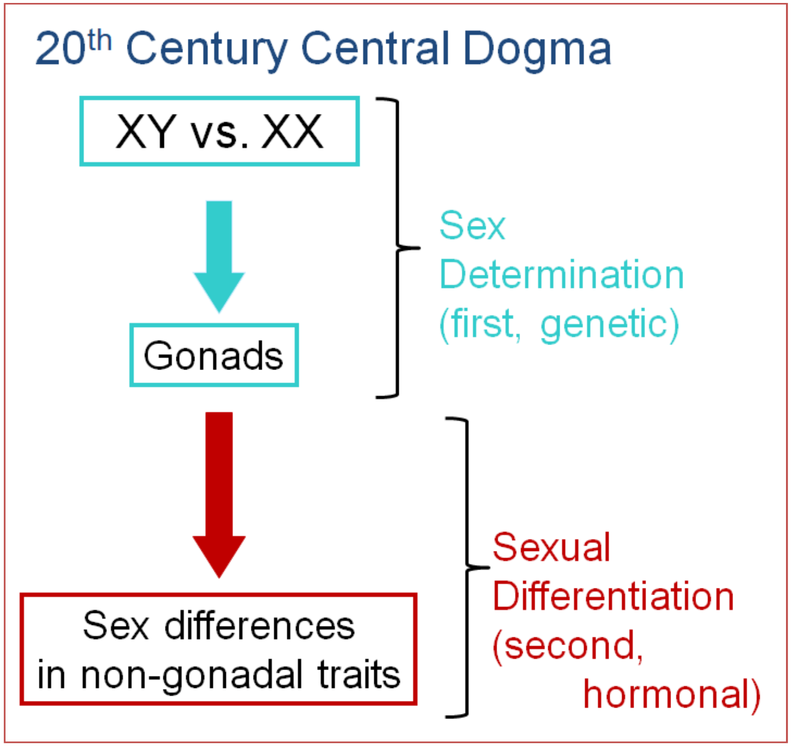
Right side: genetic male
Left side: genetic female

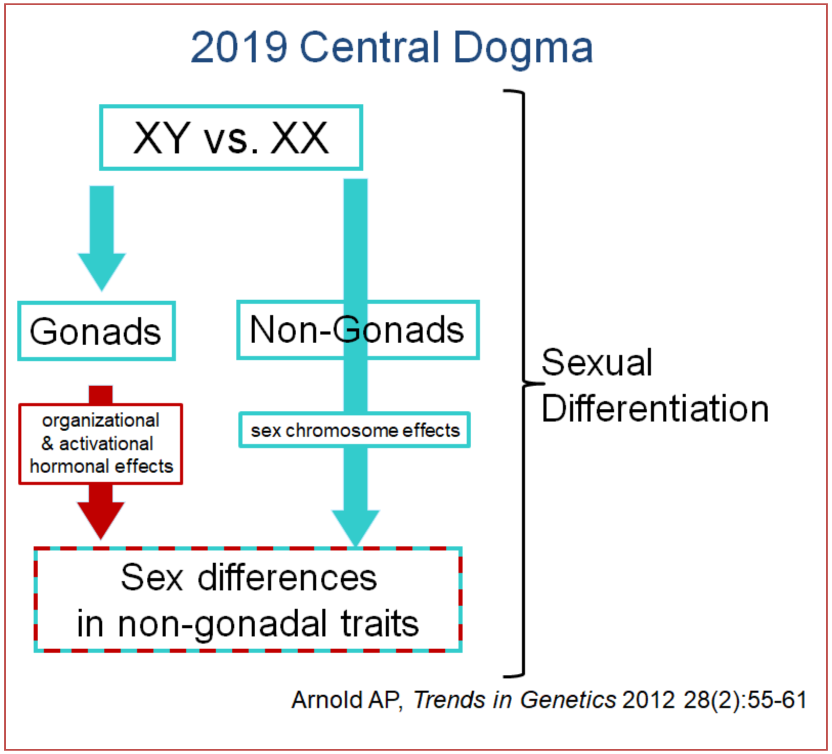
Right brain: more male-like
Left brain: less male-like



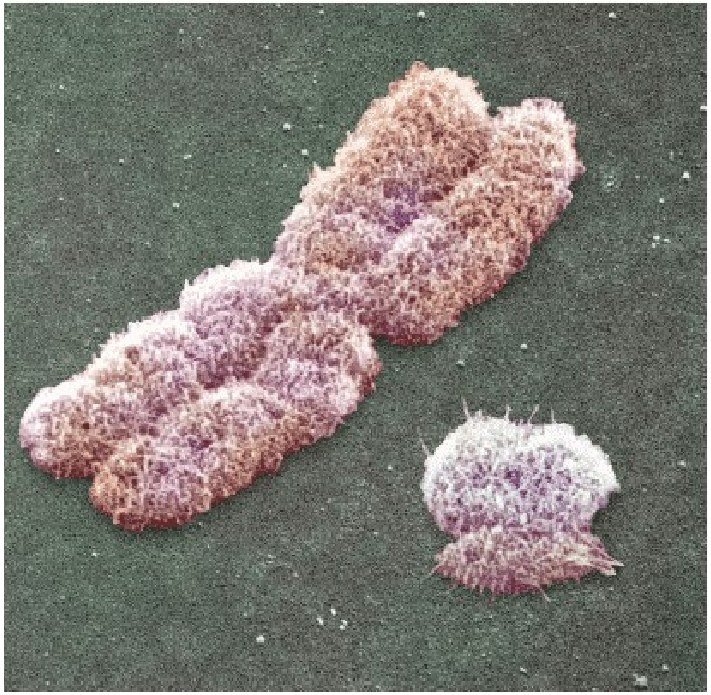
When only hormones could be manipulated, all non-gonadal sex differences were hormonal.

But when gonads and sex chromosomes are independently manipulated, both are found to cause sex differences.





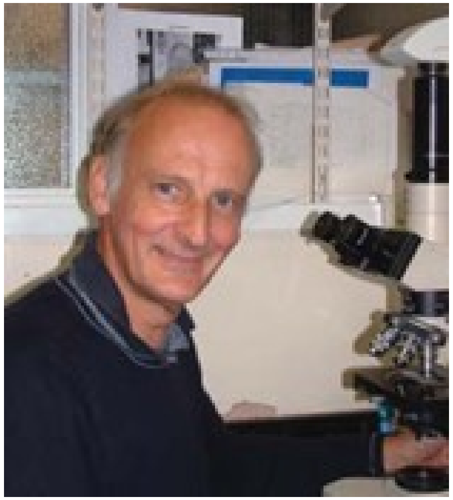
Sex chromosomes



The only factors in zygote
that are different
in males and females.

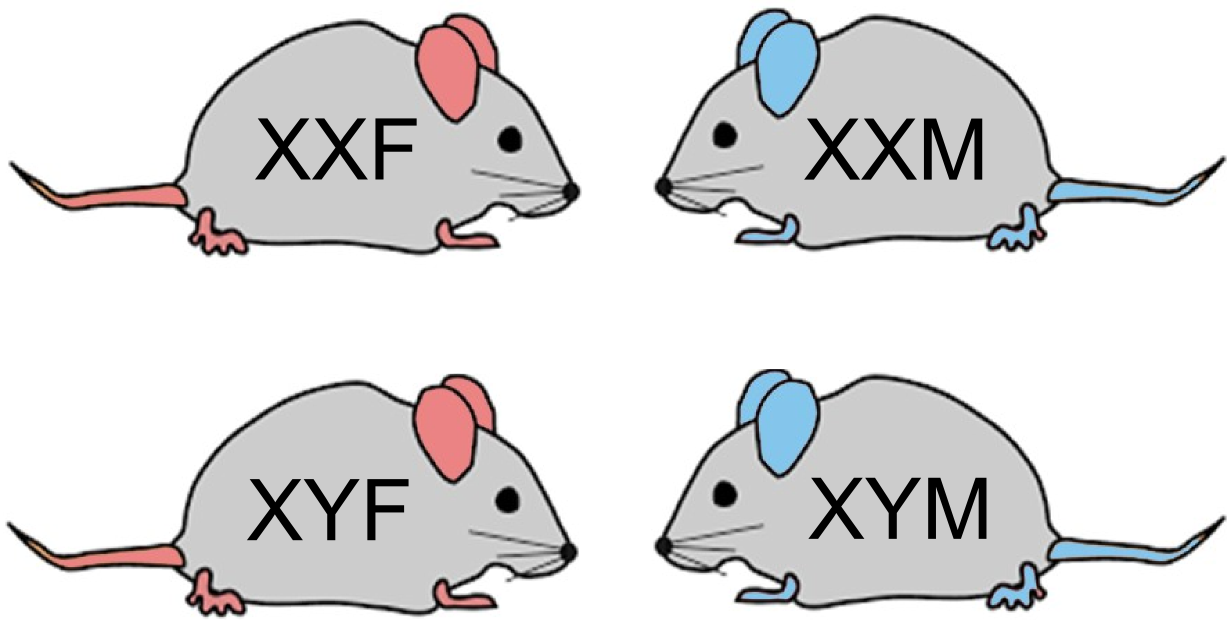
Thus, all sex differences
derive ultimately from sexual
inequality in X and Y
chromosomes.

“Four Core Genotypes” (FCG) Mice

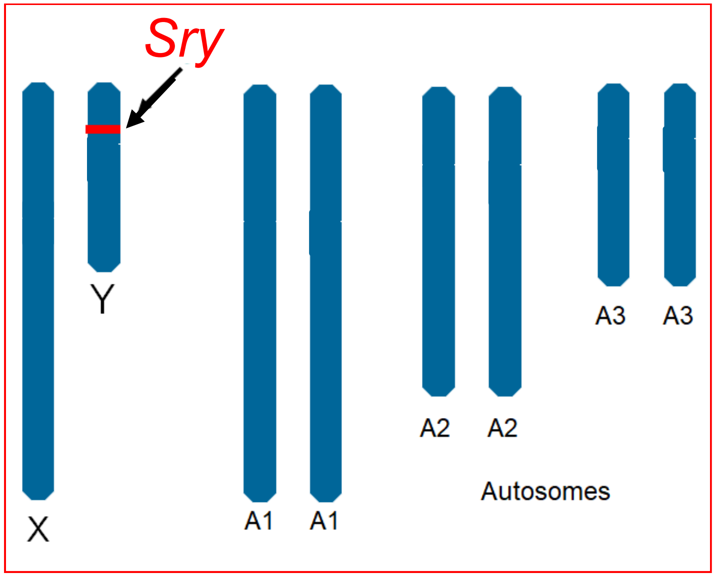

Paul S. Burgoyne
1946-2020


Robin Lovell-Badge

Make gonadal determination independent
of sex chromosome complement
to compare XX and XY mice
with the same type of gonads



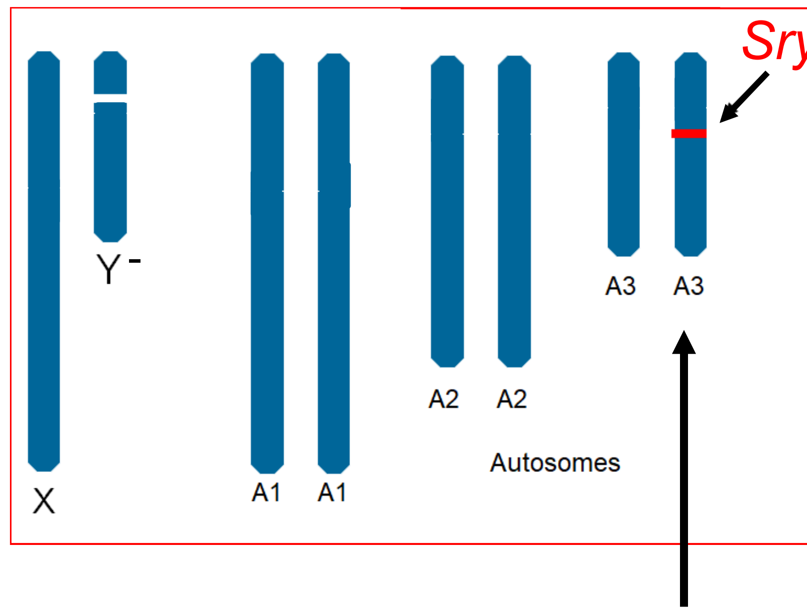
Typical XY male
Y-linked *Sry* is testis-determining.



2 kinds of sperm: X or Y

“Four Core Genotypes” (FCG) Mice

Sry “moved” to Chromosome 3.

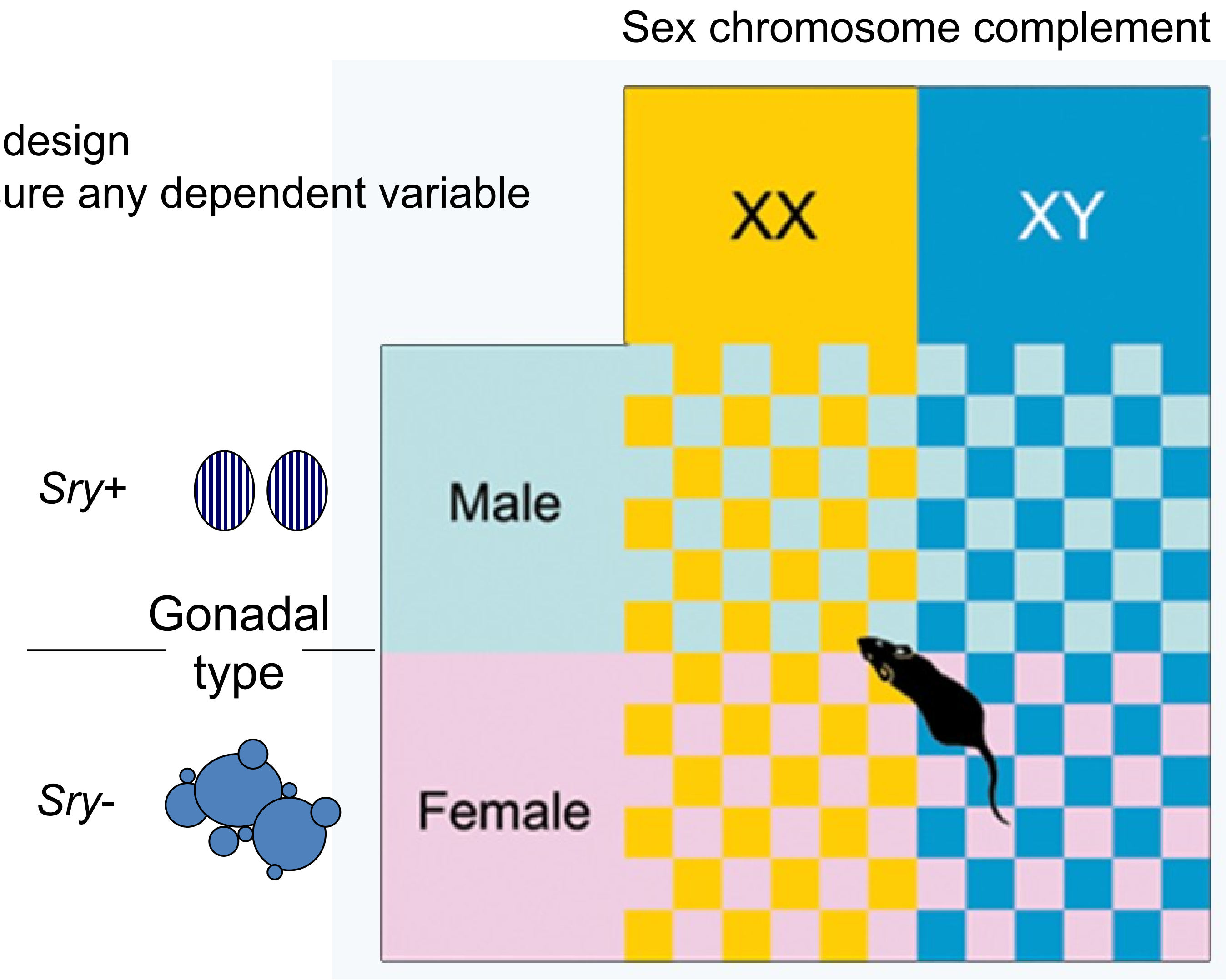


This version of Chrom3 becomes testis-determining.

4 kinds of sperm: X or X*Sry* or Y or Y*Sry*

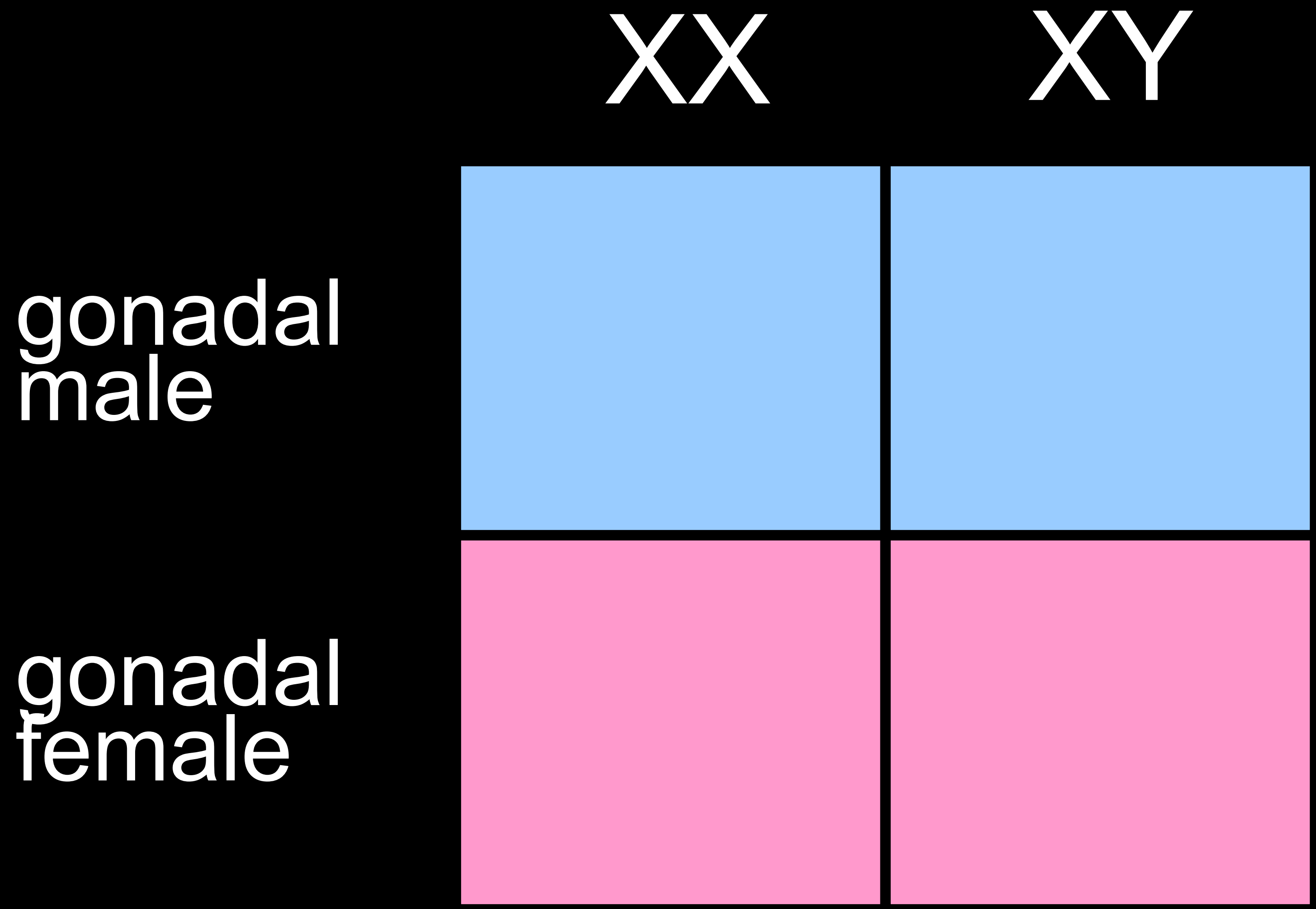
Four Core Genotypes

2 x 2 design
measure any dependent variable



Test trait in GDX mice (no activational effects)

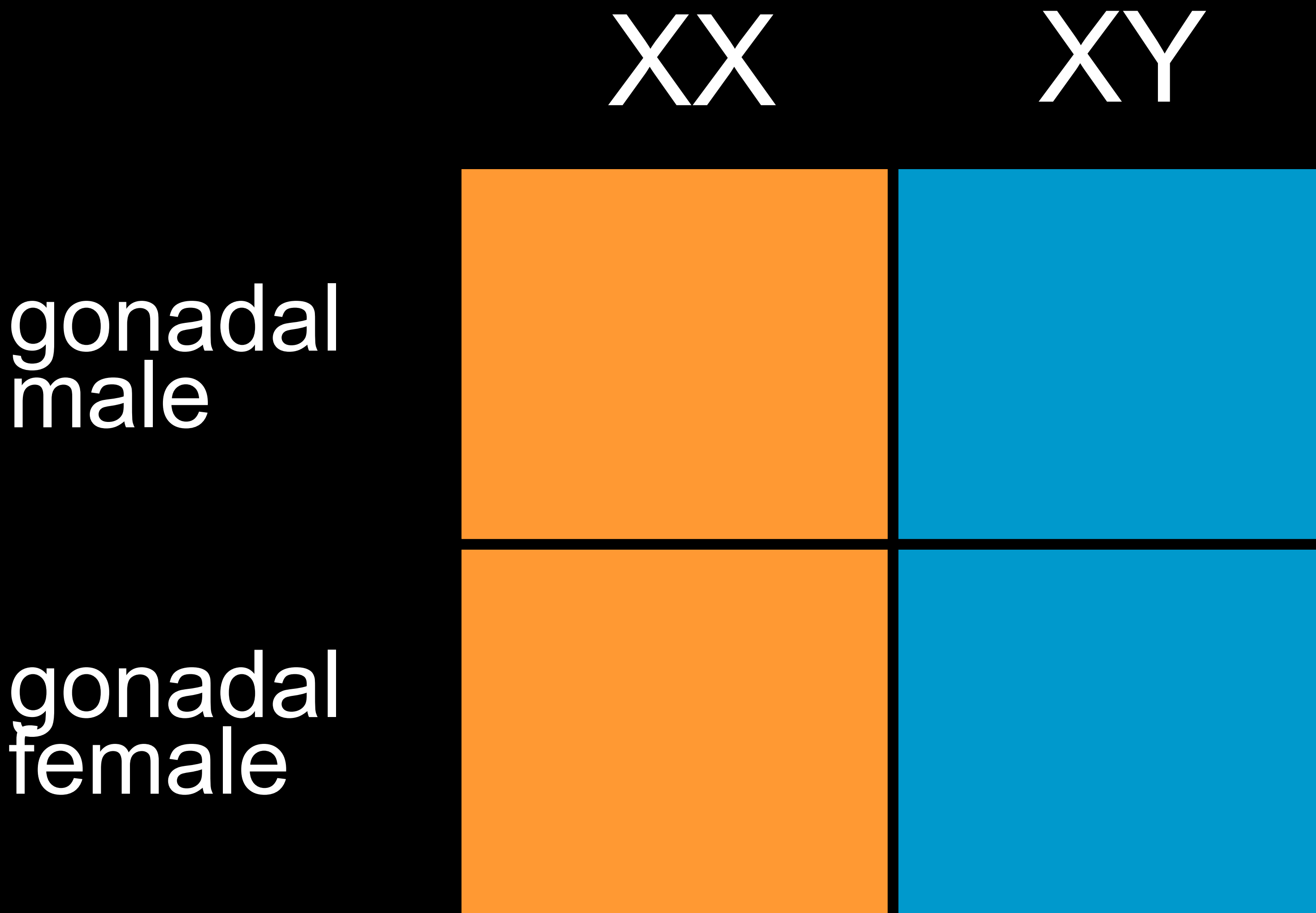
This is an organizational effect of gonadal hormones



Main effect of gonadal sex

Test trait in GDX mice (no activational effects)

This is an effect of sex chromosome complement



Main effect of XX vs. XY

OPEN ACCESS Freely available online

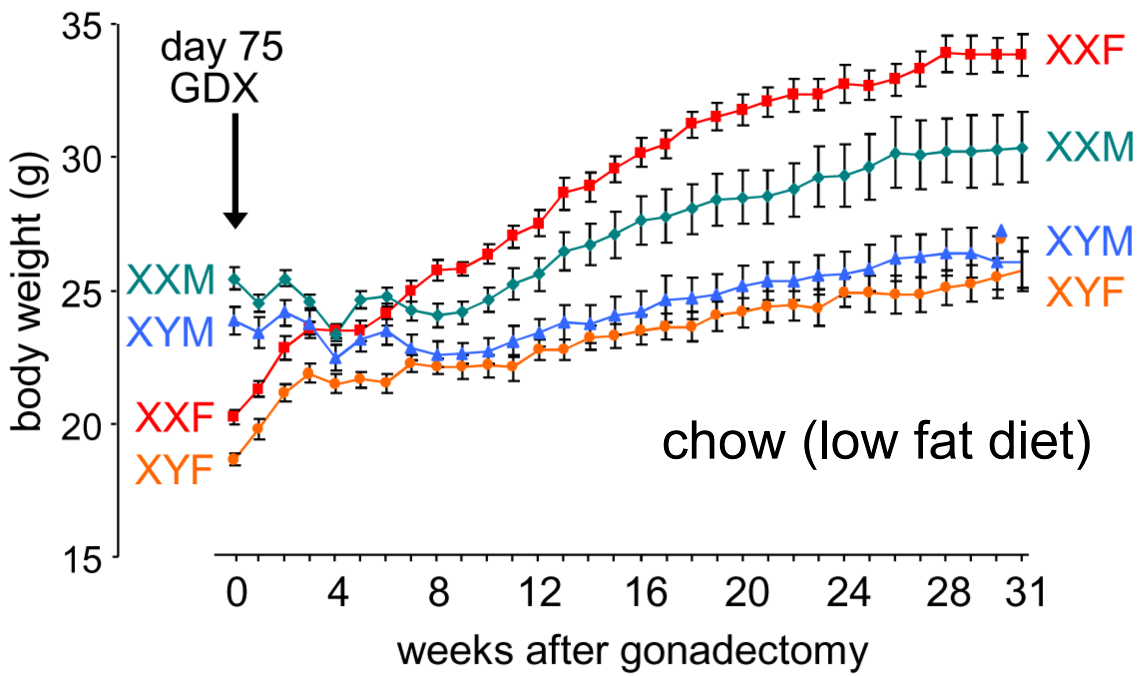
PLoS GENETICS

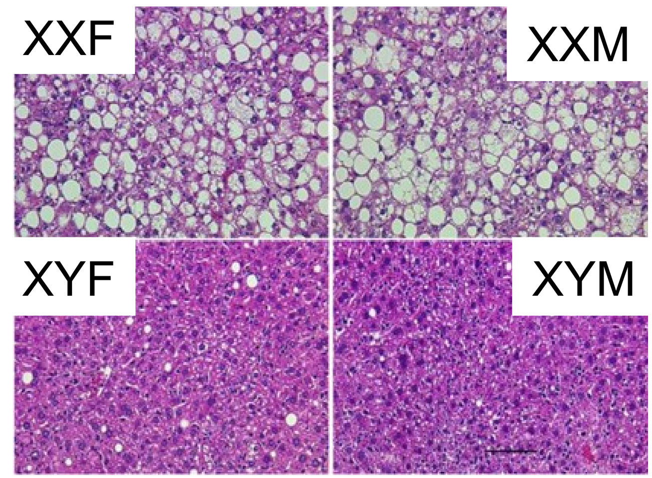
The Number of X Chromosomes Causes Sex Differences in Adiposity in Mice

2012

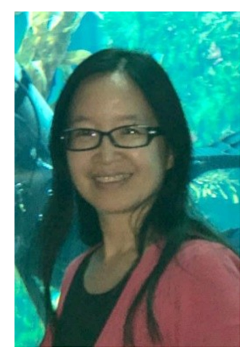
Xuqi Chen[1], Rebecca McClusky[1], Jenny Chen[2], Simon W. Beaven[3,4], Peter Tontonoz[4,5,6], Arthur P. Arnold[1], Karen Reue[2,3,6]*

Body weight dynamics following GDX

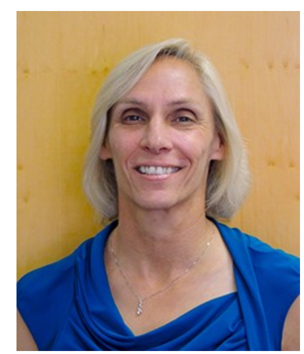




XX > XY fatty liver after high fat diet

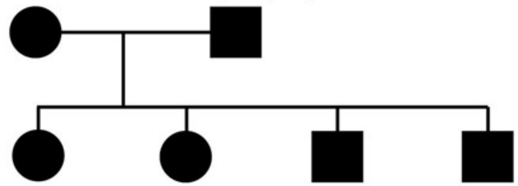


Xuqi Chen



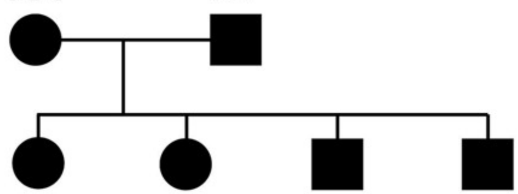
Karen Reue

Four Core Genotypes



genotype	XX	XY^{-}	XX(*Sry*+)	XY^{-}(*Sry*+)
abbreviation	XXF	XYF	XXM	XYM
Sry transgene	-	-	+	+
gonads	ovaries	ovaries	testes	testes
Y chrom	-	+	-	+
X chrom	two	one	two	one

XY* Model



genotype	XY^{*X}	XX	XY*	XX^{Y*}
shorthand	XO	XX	XY	XXY
gonads	ovaries	ovaries	testes	testes
Sry	-	-	+	+
Y chrom	-	-	+	+
X chrom	one	two	one	two

The Burgoynian Method

Questions answered:

FCG

1. Difference between XX and XY?
2. Difference between gonadal M and F?
3. Interaction of sex chrom and gonad?

XY*

4. Effect of X chrom number (1 vs. 2)?

Compare XO and XX, XY and XXY.

2. Effect of Y chrom (0 vs. 1)?

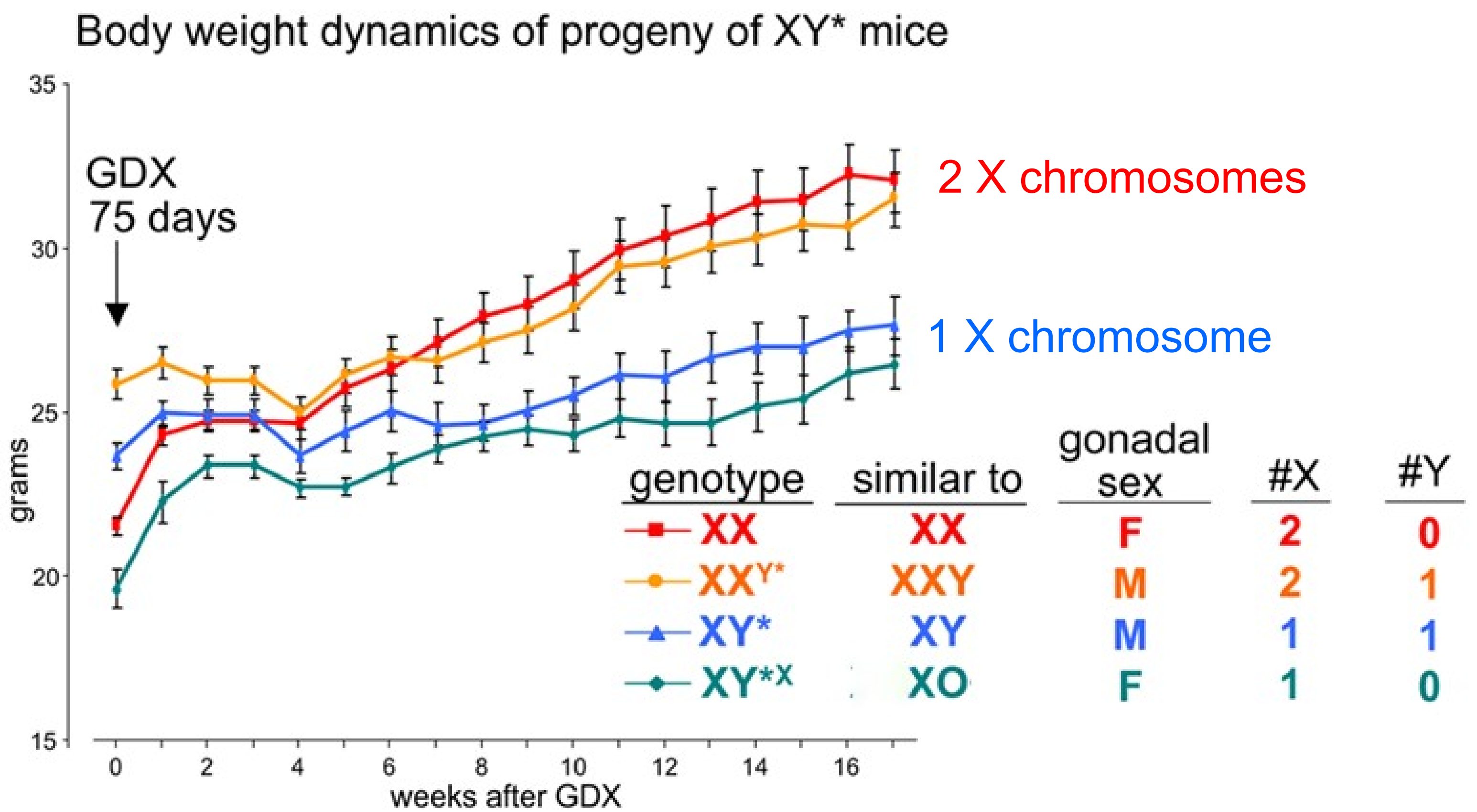
3. Interaction

Later

Effects of dose of specific X or Y genes.

XY* Model (Eicher)

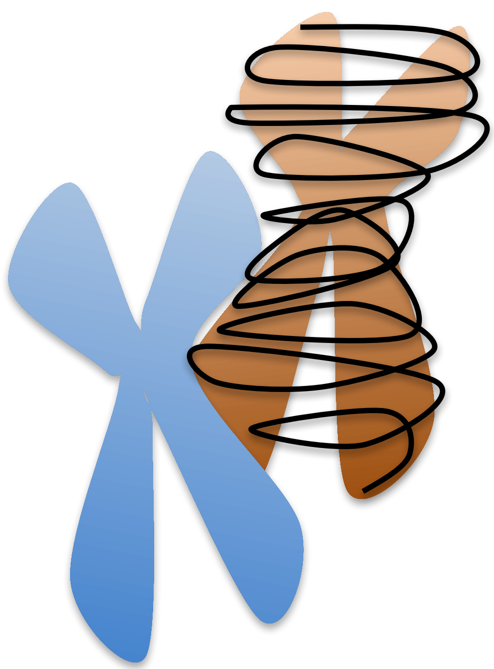
Mice with two X chromosomes are heavier and fatter than mice with one X chromosome

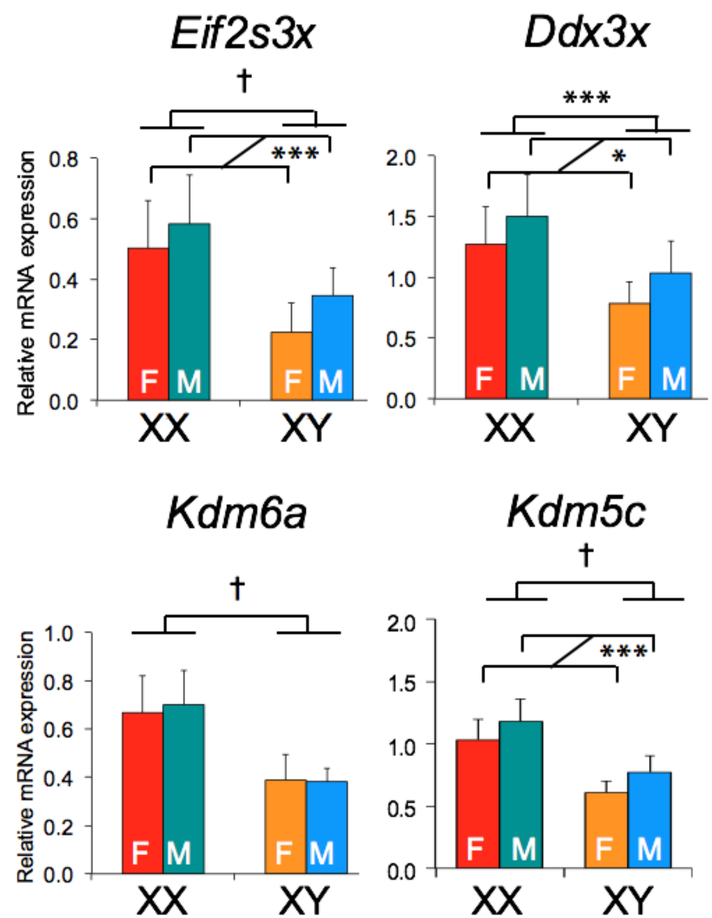


PLoS Genet. 2012;8(5):e1002709

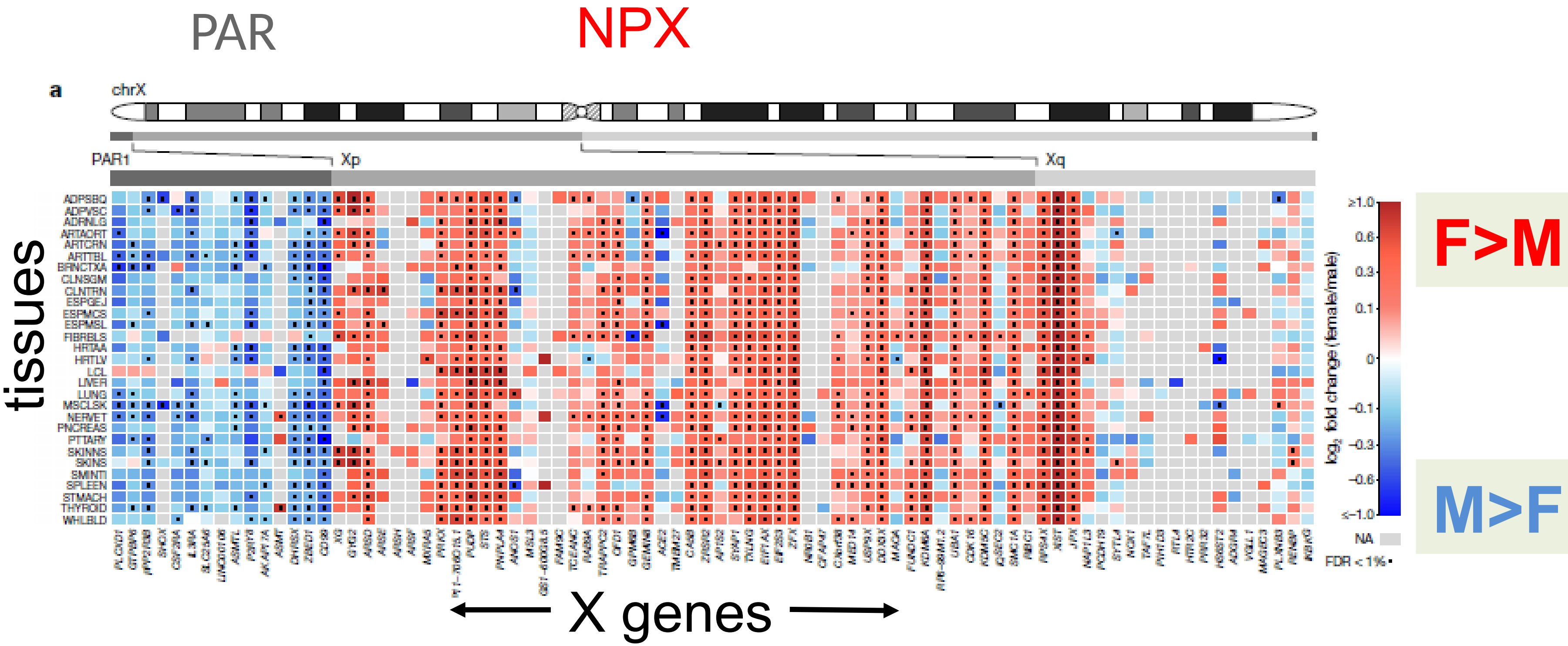
Escape from X inactivation
One genetic basis of XX-XY differences

Some X genes escape inactivation: XX > XY expression levels
"X escapees"





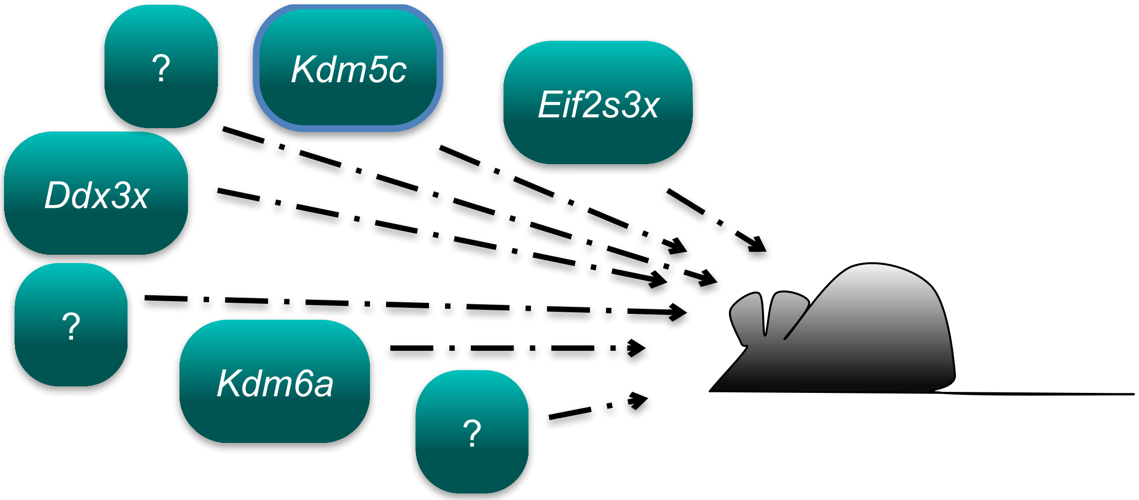
Human X chromosome: Escape from X inactivation 23% of X genes are expressed higher in F than M.



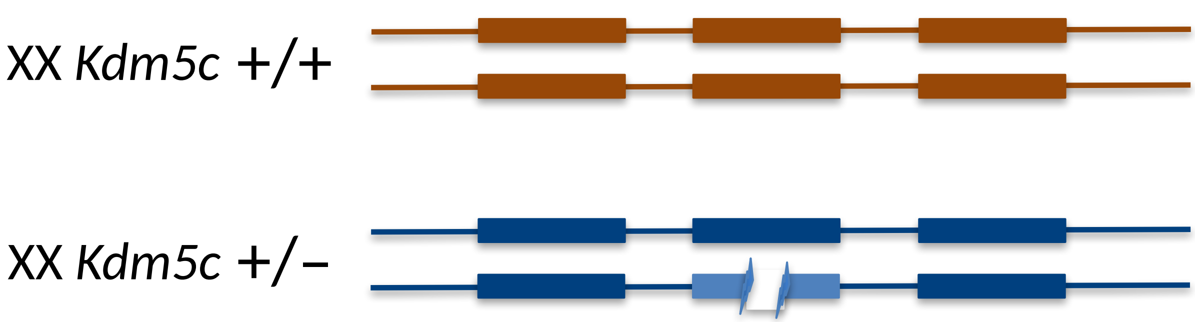
Red X escapees are potential drivers of sex differences in cell phenotypes.

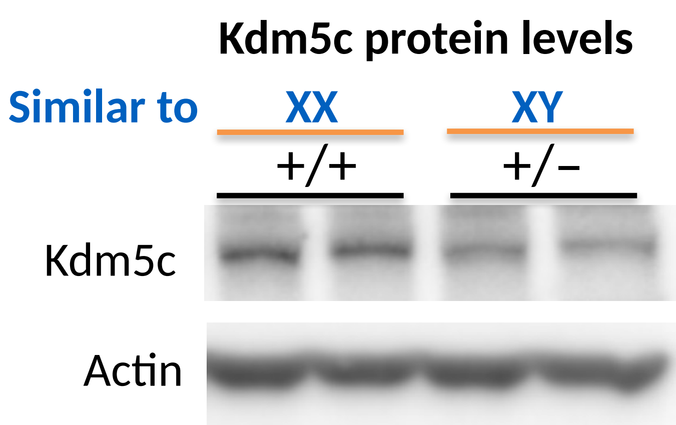
Tukiainen T et al.
Landscape of X chromosome inactivation across human tissues.
Nature 2017 550:244-248.

Test the contribution of X escapee gene to adiposity

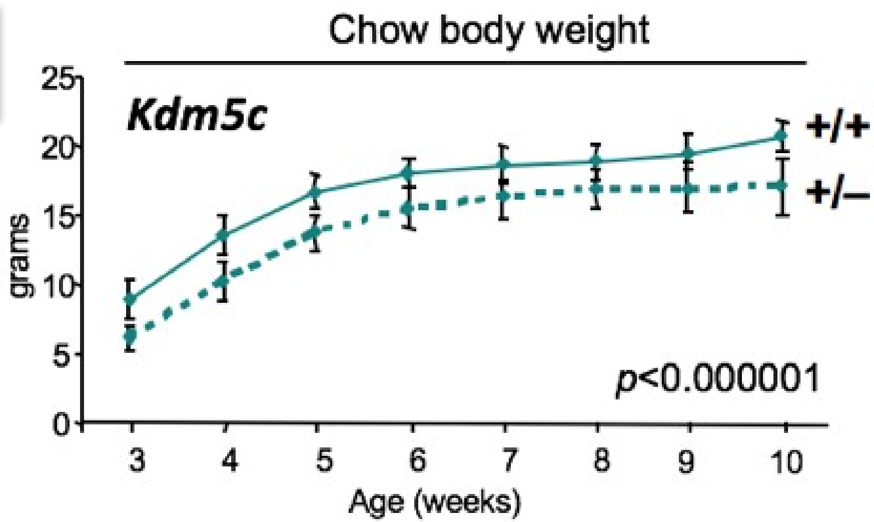


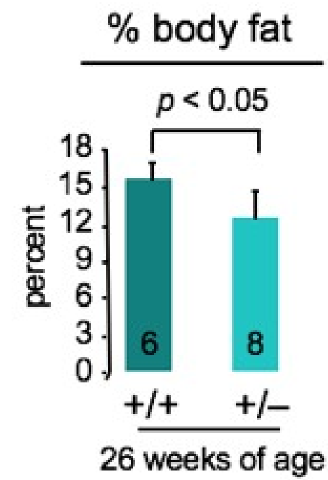
Does 1 vs. 2 copies of *Kdm5c* mimic effects of 1 vs. 2 copies of X chromosome?

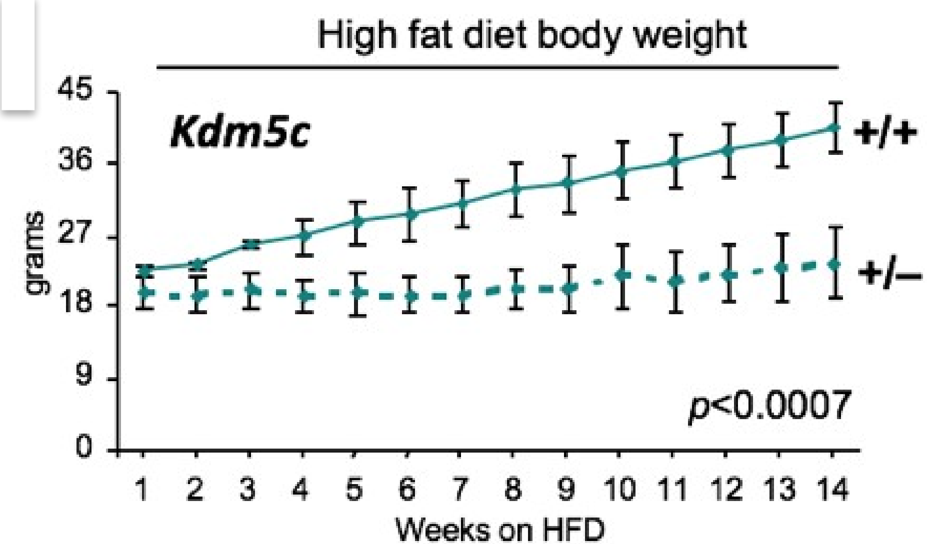


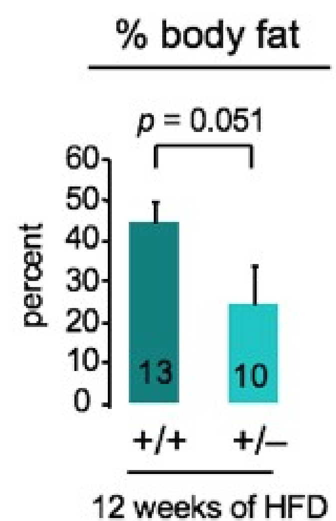


Like X chromosome dose, *Kdm5c* gene dose affects body weight and fat









Sex Chromosome Effects in Mouse Models

Autoimmune diseases (Voskuhl)
Metabolism and obesity (Reue)
Ischemic cardiovascular disease (Eghbali)
Stroke (McCullough)
Hypertension (Sandberg, Caeiro, etc.)
Abdominal Aortic Aneurysms (Cassis)
Atherosclerosis (Cassis and Reue)
Neural tube closure defects (Arnold)
Alzheimer's and aging (Dubal)
Pain and analgesia (A. Taylor and C. Evans)
Brain sex steroid synthesis (Cambiasso and Garcia Segura)
Chronic Stress, mood disorders (Seney & Sibille)
Behavior (Rissman, J. Taylor, Jentsch, etc.)
Brain Morphology (Lerch & Palmert)
Bladder Cancer (Li)
Pulmonary Hypertension (Eghbali)
Neonatal lung injury (Lingappan)
Cardiac proteome (Conlon)

Effects of X chromosome number in mice
XO ≠ XX, and/or XY ≠ XXY
not confounded by hormonal differences

1. Body weight, adiposity and lipid metabolism. (Chen et al. Plos Genet 2012)
2. Susceptibility to cardiac ischemia / reperfusion injury, infarct size (Li et al., Cardiovasc Res. 2014)
3. Neural tube closure defect (Chen et al., Dev Neurobiol 2008)
4. Gene expression in brain (Chen et al., Eur J Neurosci. 2009)
5. Fear reactivity (Isles et al., Hum Mol Genet. 2004)
6. Male sexual behavior (Bonthuis et al. Horm Behav 2012)
7. Social behavior of juveniles (Cox et al., Psychoneuroendo 2015)
8. Sexual partner preference (Ngun et al. Arch Sex Behav 2014)
9. Alzheimer's disease and longevity (Davis et al., *Sci Transl Med*, 2020)
10. K-opioid analgesia (Taylor et al., *J Neurosci Res*, 2020)
11. Cardiac proteome (Shi et al., *Developmental Cell*, 2021)

Nearly 100 papers have used FCG and/or XY* models

Table 1
The table lists primary literature articles using Four Core Genotypes (FCG) and/or XY* mouse models for detecting sex chromosome effects on mouse phenotypes. ChrX, effect of chromosome X; ChrY, effect of chromosome Y; CVD, cardiovascular disease, Xm, X chromosome with maternal imprint; Xp, X chromosome with paternal imprint; SCE, sex chromosome effect.

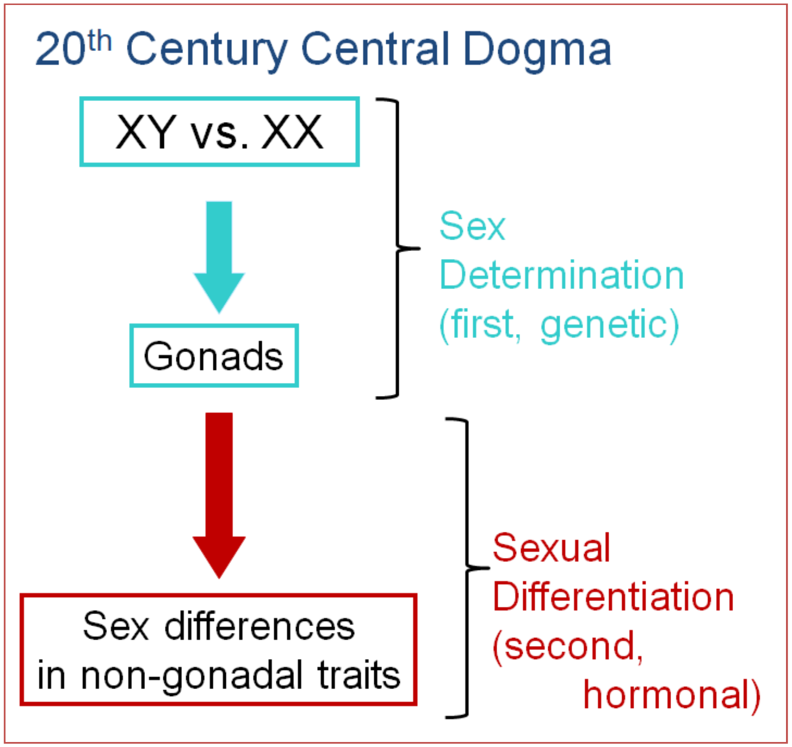
Reference	Model	Effect	Phenotype
(Davis et al., 2019)	FCG	SCE	Aging, longevity
(Golden et al., 2019)	FCG	ChrX, Xm vs. Xp	Autoimmunity, EAE
(Itoh et al., 2019)	FCG	SCE, ChrX	Autoimmunity, EAE
(Palaszynski et al., 2005)	FCG	SCE	Autoimmunity, EAE
(Sasidhar et al., 2012)	FCG	SCE	Autoimmunity, EAE
(Smith-Bouvier et al., 2008)	FCG	SCE	Autoimmunity, EAE and lupus
(Du et al., 2014)	FCG	SCE	Autoimmunity, EAE, brain
(Barker et al., 2010)	FCG	SCE	Behavior, addiction
(Quinn et al., 2007)	FCG	SCE	Behavior, addiction
(Martini et al., 2020)	FCG	SCE	Behavior, addiction
(Gatewood et al., 2006)	FCG	SCE	Behavior, aggressive, parental
(Kuljis et al., 2013)	FCG	SCE	Behavior, circadian
(Aguayo et al., 2018)	FCG	no SCE	Behavior, circadian feeding
(Chen et al., 2015)	FCG	SCE	Behavior, feeding
(Kopsida et al., 2013)	FCG	SCE	Behavior, feeding, anxiety
(Seu et al., 2014)	FCG	SCE	Behavior, learning and motivation
(Aarde et al., 2020)	FCG	SCE	Behavior, learning
(Gioiosa et al., 2008a)	FCG	SCE	Behavior, pain
(Gioiosa et al., 2008b)	FCG	SCE	Behavior, pain
(Ehlen et al., 2013)	FCG	SCE	Behavior, sleep
(Cox and Rissman, 2011)	FCG	SCE	Behavior, social
(McPhie-Lalmansingh et al., 2008)	FCG	SCE	Behavior, social
(Tejada and Rissman, 2012)	FCG	no SCE	Behavior, social
(Cisternas et al., 2015)	FCG	SCE	Brain, aromatase expression
(Cisternas et al., 2017)	FCG	SCE	Brain, aromatase expression
(Moore et al., 2013)	FCG	SCE	Brain, callosal remyelination
(Markham et al., 2003)	FCG	no SCE	Brain, cortical thickness
(Abel et al., 2011)	FCG	SCE	Brain, gene expression
(Barko et al., 2019)	FCG	SCE	Brain, gene expression, stress
(Puralewski et al., 2016)	FCG	SCE	Brain, gene expression, stress
(Seney et al., 2013a)	FCG	SCE	Brain, gene expression, stress
(Seney et al., 2013b)	FCG	SCE	Brain, gene expression, stress
(Quinnies et al., 2015)	FCG	SCE	Brain, hypothalamus gene expression
(van Veen et al., 2020)	FCG	no SCE	Brain, hypothalamus gene expression
(Kuo et al., 2010)	FCG	no SCE	Brain, hypothalamus in vitro
(Scerbo et al., 2014)	FCG	SCE	Brain, hypothalamus in vitro
(Carruth et al., 2002)	FCG	SCE	Brain, midbrain expression in vitro
(Corre et al., 2016)	FCG	SCE	Brain, MRI morphology
(Vousden et al., 2018)	FCG	SCE	Brain, MRI morphology
(Wagner et al., 2004)	FCG	no SCE	Brain, neonatal hypothalamus
(De Vries et al., 2002)	FCG	SCE	Brain, septal anatomy
(Alsiraj et al., 2017)	FCG	SCE	CVD, abdominal aortic aneurysms
(Alsiraj et al., 2018)	FCG	SCE	CVD, aortic aneurysms
(AlSiraj et al., 2019)	FCG	SCE	CVD, atherosclerosis
(Manwani et al., 2015)	FCG	no SCE	CVD, brain, stroke
(McCullough et al., 2016)	FCG	SCE	CVD, brain, stroke
(Caeiro et al., 2011)	FCG	SCE	CVD, heart rate
(Ji et al., 2010)	FCG	SCE	CVD, Hypertension
(Pessoa et al., 2015)	FCG	SCE	CVD, hypertension
(Dadam et al., 2017)	FCG	SCE	CVD, hypertension, renal

Table 1 (continued)

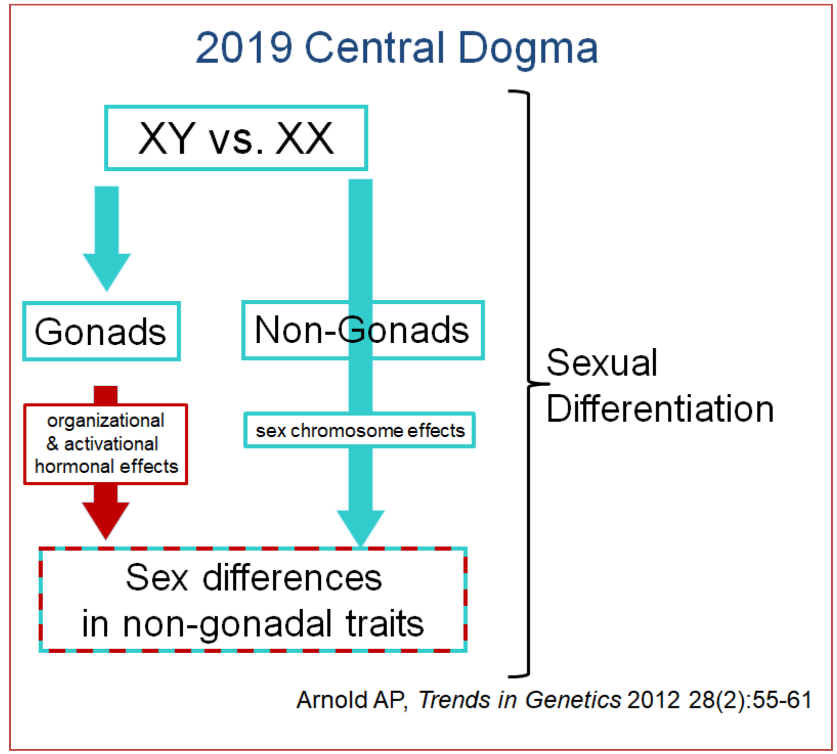
Reference	Model	Effect	Phenotype
(Liu et al., 2010)	FCG	no SCE	CVD, renal gene expression
(Dadam et al., 2014)	FCG	SCE	CVD, salt regulation
(Van Nas et al., 2009)	FCG	SCE	Gene expression
(Xu et al., 2002)	FCG	SCE	Gene expression, brain
(Xu et al., 2005a)	FCG	SCE	Gene expression, brain
(Xu et al., 2005b)	FCG	SCE	Gene expression, brain
(Xu et al., 2006)	FCG	SCE	Gene expression, brain
(Xu et al., 2008a)	FCG	SCE	Gene expression, brain
(Xu et al., 2008b)	FCG	SCE	Gene expression, brain
(Xu and Arnold, 2005)	FCG	no SCE	Gene expression, kidney
(Durcova-Hills et al., 2004)	FCG	SCE	Germline
(Sangrithi et al., 2017)	FCG	SCE	Germline
(Itoh et al., 2015)	FCG	no SCE	Growth, anogenital distance
(Holaskova et al., 2015)	FCG	SCE	Immunity, drug effect
(Dill-Garlow et al., 2019)	FCG	no SCE	Immunity, lymph node
(Robinson et al., 2011)	FCG	SCE	Immunity, viral infection
(Link et al., 2017)	FCG	SCE	Metabolism, adipose miRNA expression
(Link et al., 2020)	FCG	ChrX	Metabolism, adipocyte differentiation
(Wijchers et al., 2010)	FCG, XO	ChrX	Gene expression, autosomal
(Bonthuis et al., 2012)	FCG, XY*	ChrX	Behavior, reproductive
(Chen et al., 2009)	FCG, XY*	ChrX	Brain, striatum gene expression
(Davis et al., 2020)	FCG, XY*	Chr X	Alzheimer's Disease, longevity
(Li et al., 2014)	FCG, XY*	ChrX	CVD, cardiac ischemia / reperfusion injury
(Umar et al., 2018)	FCG, XY*	ChrY	CVD, pulmonary hypertension
(Chen et al., 2008)	FCG, XY*	ChrX	Developmental defect, neural tube closure
(Ishikawa et al., 2003)	FCG, XY*	SCE	Growth, placental
(Burgoyne et al., 2002)	FCG, XY*	SCE	Growth, postnatal
(Link et al., 2015)	FCG, XY*	SCE	Metabolism, adipose
(Chen et al., 2012)	FCG, XY*	ChrX	Metabolism, adiposity, body weight
(Chen et al., 2013)	FCG, XY*	ChrX, ChrY	Metabolism, adiposity, body weight
(Taylor et al., 2020)	FCG, XY*	Chr X	Behavior, pain
(Davies et al., 2007)	XY*	ChrX	Behavior, attention
(Davies et al., 2009)	XY*	ChrX	Behavior, attention
(Davies et al., 2005)	XY*	ChrX, Xm vs. Xp	Behavior, cognitive
(Isles et al., 2004)	XY*	ChrX	Behavior, fear
(Aarde et al., 2019)	XY*	SCE	Behavior, learning
(Lewejohann et al., 2009)	XY*	ChrX	Behavior, memory
(Wolstenholme et al., 2012)	XY*	ChrX	Brain, gene expression
(Bonthuis and Rissman, 2013)	XY*	ChrX	Brain, gene expression, body weight
(Cox et al., 2015)	XY*	ChrX	Brain; Behavior, social and anxiety
(Hinton et al., 2015)	XY*	ChrX, Xm vs. Xp	CVD, aortic morphology
(Werler et al., 2011)	XY*	ChrX	Gene expression, multiple tissues
(Werler et al., 2014)	XY*	ChrX	Testis function, germline
(Wistuba et al., 2010)	XY*	ChrX	Testis function, plasma hormones

Use of FCG and XY* mice catalyzed a major shift in sexual differentiation theory.

It’s all hormonal…



It’s both hormonal and sex chromosomal.



Sex differences evolved because of different adaptations to male and female modes of reproduction.

Sex differences also evolved as side effect of genomic forces causing inherent XX-XY inequality.

Summary so far

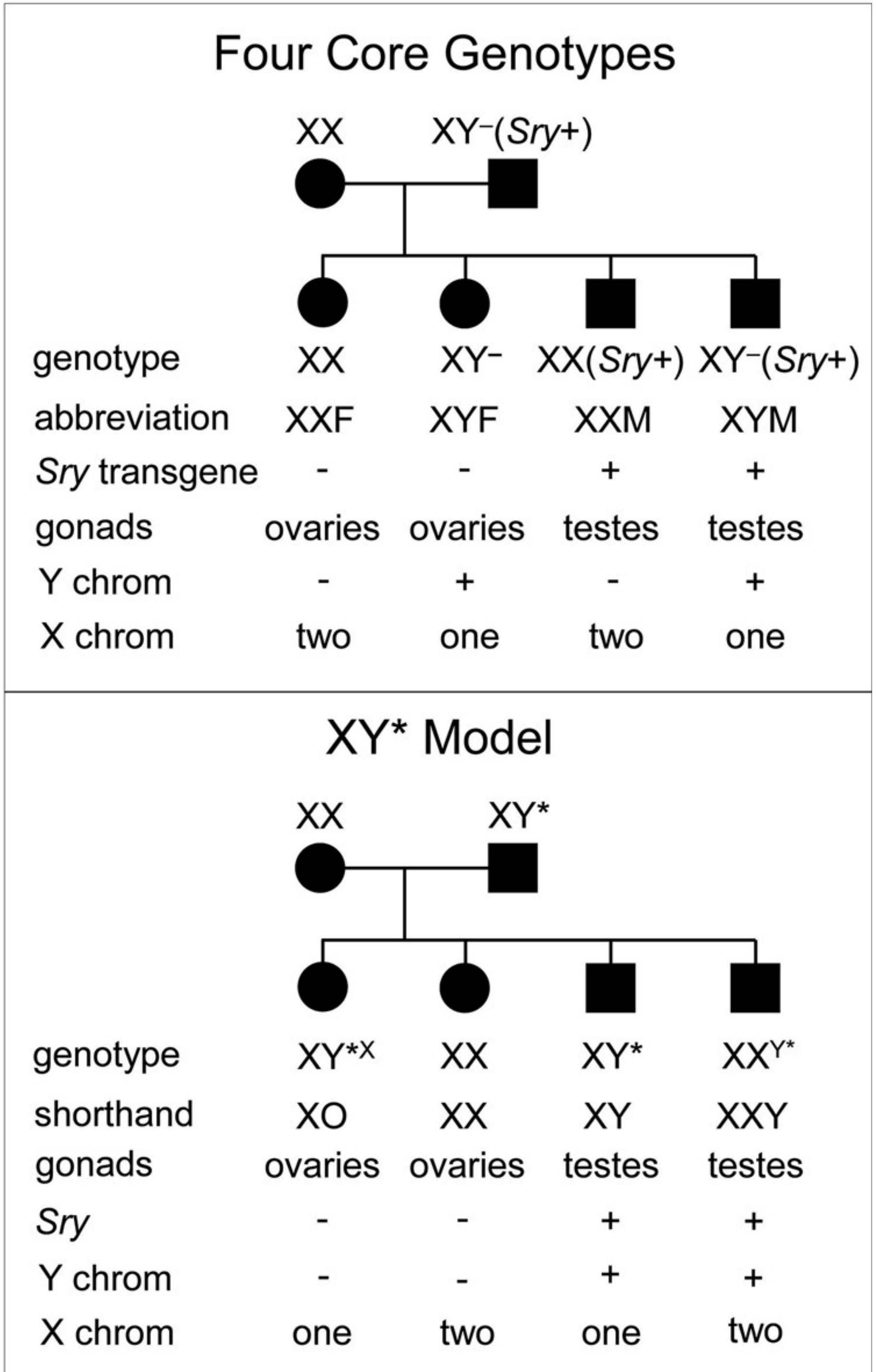
X and Y genes act within cells in every organ. They cause sex differences in physiology, and disease susceptibility and progression.

X genes that escape X inactivation are one type of gene that can cause sex differences in tissue function. Expression XX > XY

If you want to study sex chromosome effects on any mouse phenotype, the Four Core Genotypes model is the best first step.

The FCG model powerfully tests for sex chromosome effects, gonadal hormone effects, and their interaction, to cause sex differences in any phenotype.

One-two punch: Use FCG mice then XY* mice to discover X chromosome or Y chromosome effects causing sex differences in any phenotype.



The Burgoynian Method

Questions answered:

FCG
1. Difference between XX and XY?
2. Difference between gonadal M and F?
3. Interaction of sex chrom and gonad?

XY*
4. Effect of X chrom number (1 vs. 2)?

Compare XO and XX, XY and XXY.

2. Effect of Y chrom (0 vs. 1)?

3. Interaction

Later

Effects of dose of specific X or Y genes.

Why make an FCG rat model?

The study of any important biological question must be performed in multiple species to test the generality of answers.

Ask a question of a mouse, and you get a mousified answer.

How are the effects of sex chromosomes similar or different in rats and mice?

Two manipulations

(*Sry* causes testis development and blocks ovary development.)

1. Knock out *Sry* from Y chromosome (producing "Y^{-}")

   produces XY^{-} gonadal female
   compare XX and XY^{-} gonadal females (both with ovarian hormones)

2. Insert *Sry* transgene into autosome

   produces XXTG gonadal male
   compare XX(*Sry*TG+) and XY^{-}(*Sry*TG+) gonadal males
   (both with testicular hormones)

NIH R21 OD026560 to make FCG rats
2018-2020

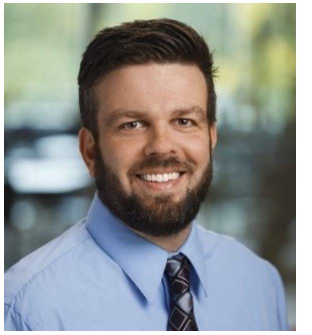
Two aims

1. KO *Sry* from Y chromosome using CRISPR to make XYF.

2. Insert *Sry* transgene onto autosome to make XXM.

3 mPIs
Aron Geurts, Melinda Dwinell, Art Arnold
Medical College of Wisconsin & UCLA

Then
NIH R01 OD030496, 5 years 2021-2026 to develop model
“Transformative rat models to study sex differences in disease”



Aron Geurts

Mindy Dwinell

Mike Grzybowski

Xuqi Chen

Vince Harley

Challenge

The rat Y chromosome has 10+ different *Sry* genes, interspersed with genes important for spermatogenesis.

Prokop *et al. BMC Genomics* 2013, **14**:792
http://www.biomedcentral.com/1471-2164/14/792

2013 BMC Genomics

RESEARCH ARTICLE **Open Access**

Analysis of Sry duplications on the Rattus norvegicus Y-chromosome

Jeremy W Prokop[1,2,3], Adam C Underwood[4], Monte E Turner[1,2], Nic Miller[1], Dawn Pietrzak[1], Sarah Scott[1], Chris Smith[1] and Amy Milsted[1,2*]

Which Sry genes are testis-determining?

Prokop *et al. Biology of Sex Differences* (2020) 11:28
https://doi.org/10.1186/s13293-020-00305-8

Biology of Sex Differences
2020

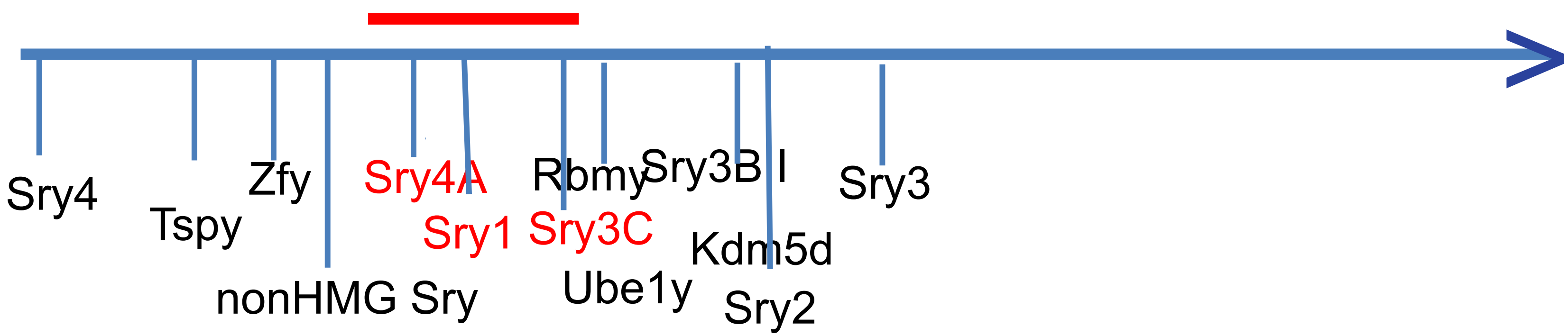
RESEARCH Open Access

Transcriptional analysis of the multiple *Sry* genes and developmental program at the onset of testis differentiation in the rat

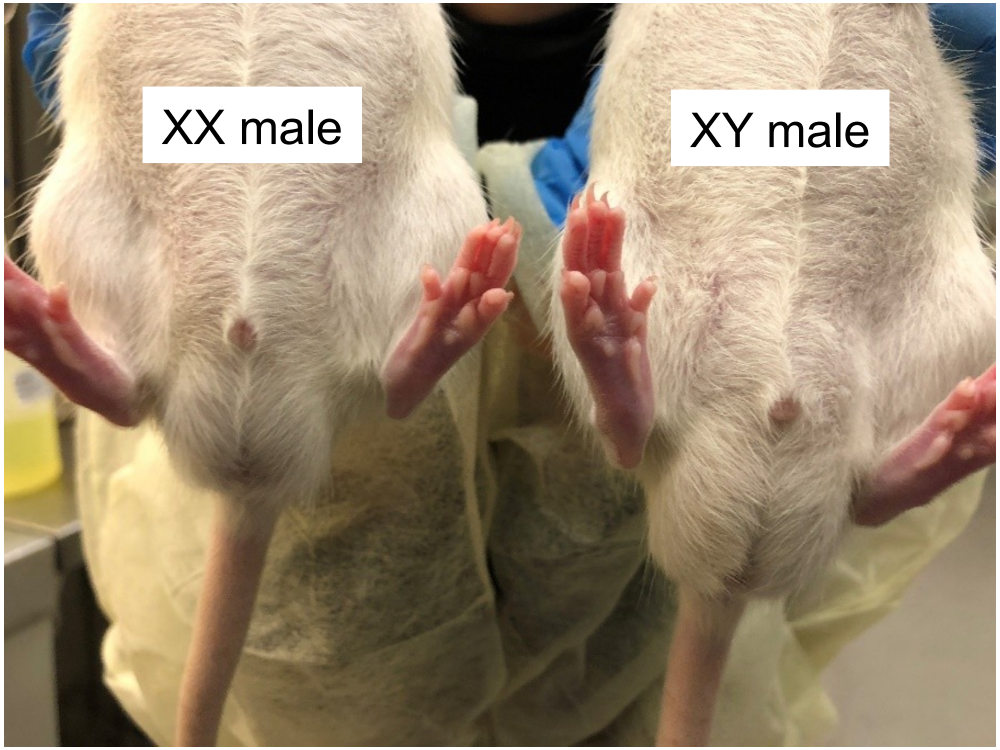
Jeremy W. Prokop[1,2,3], Surya B. Chhetri[3,4], J. Edward van Veen[5], Xuqi Chen[5], Adam C. Underwood[1,6], Katie Uhl[1], Melinda R. Dwinell[7], Aron M. Geurts[7], Stephanie M. Correa[5] and Arthur P. Arnold[5*]

Sry1, *Sry4A*, and *Sry3C* are upregulated in gonad at the onset of testis differentiation.

Proximal end of rat Y chromosome

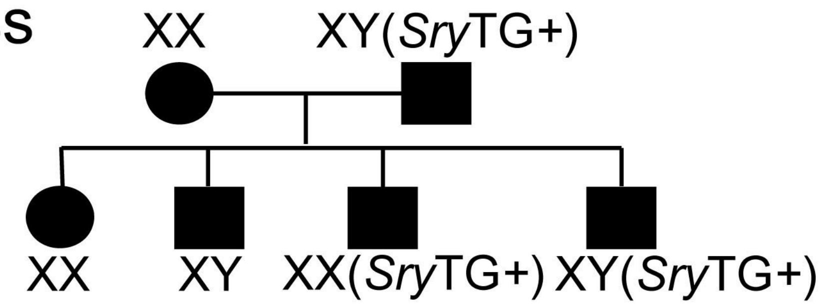


“Four core genotypes” rats



Arthur P. Arnold, Xuqi Chen, Michael N. Grzybowski, Janelle M. Ryan,
Dale R. Sengelaub, Tara Mohanroy, V. Andree Furlan, William Grisham,
Lynn Malloy, Akiko Takizawa, Carrie B. Wiese,
Laurent Vergnes, Monika Tutaj, Jeremy Prokop,
Helen Skaletsky, David C. Page, Karen Reue,
Vincent R. Harley, Melinda R. Dwinell, and Aron M. Geurts

SryTG+ cross

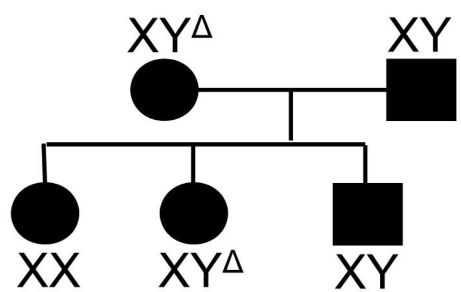


Gonad	F	M	M	M
Sry dose	0	WT	TG	WT+TG

Compare:

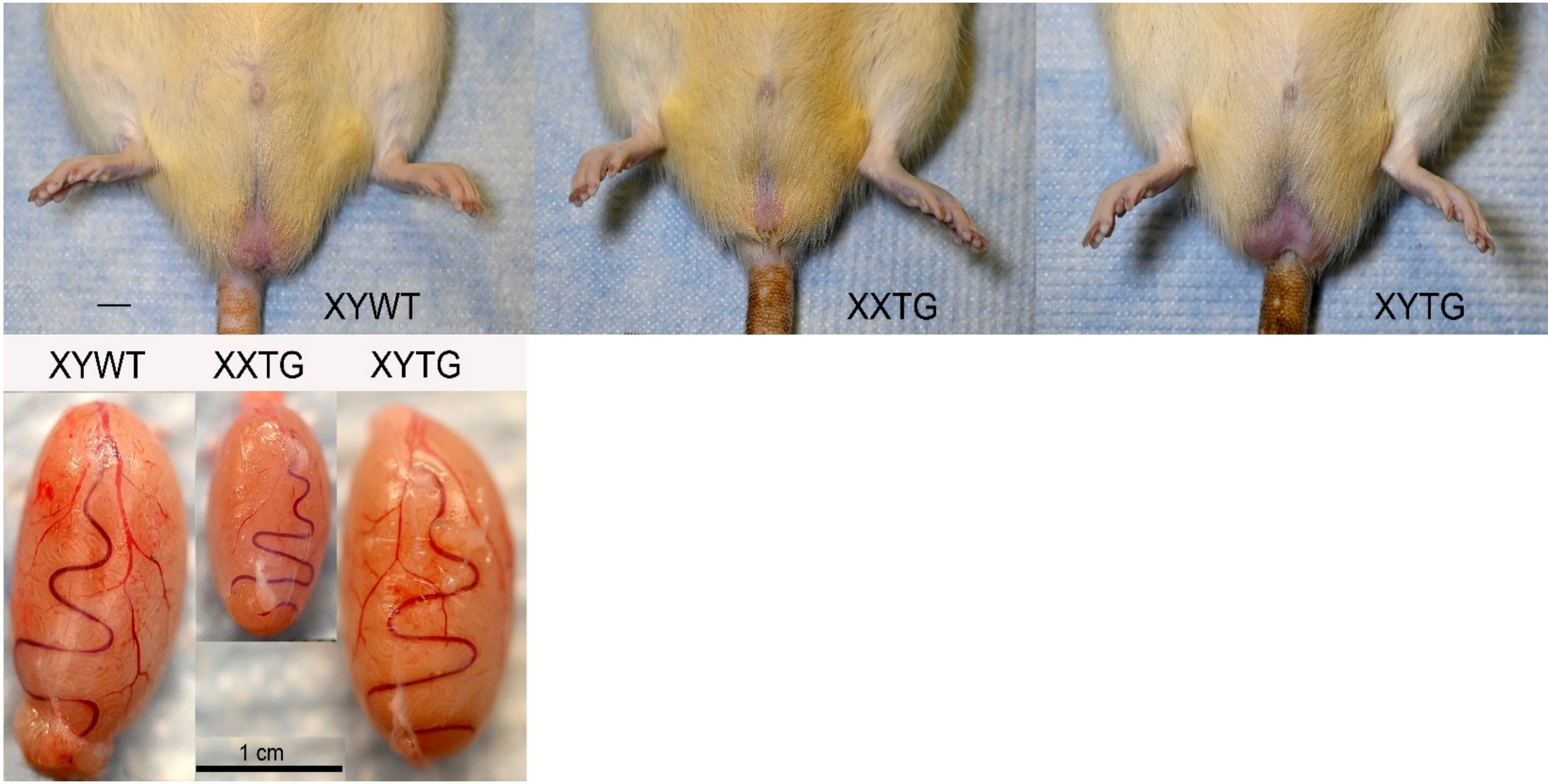
XX and XY rats with testes

XY$^{\Delta}$ cross



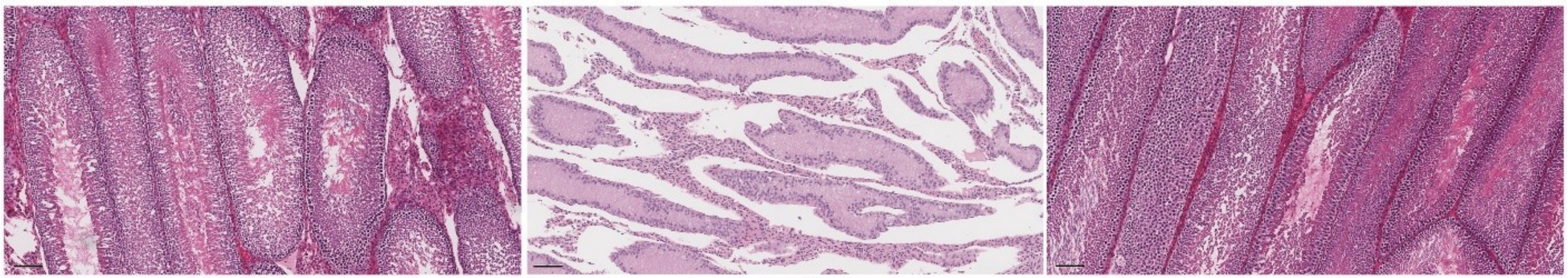
Gonad	F	F	M
Sry dose	0	Y$^{\Delta}$	WT

XX and XY rats with ovaries


XYWT
XXTG
XYTG
XYWT
XXTG
XYTG
1 cm

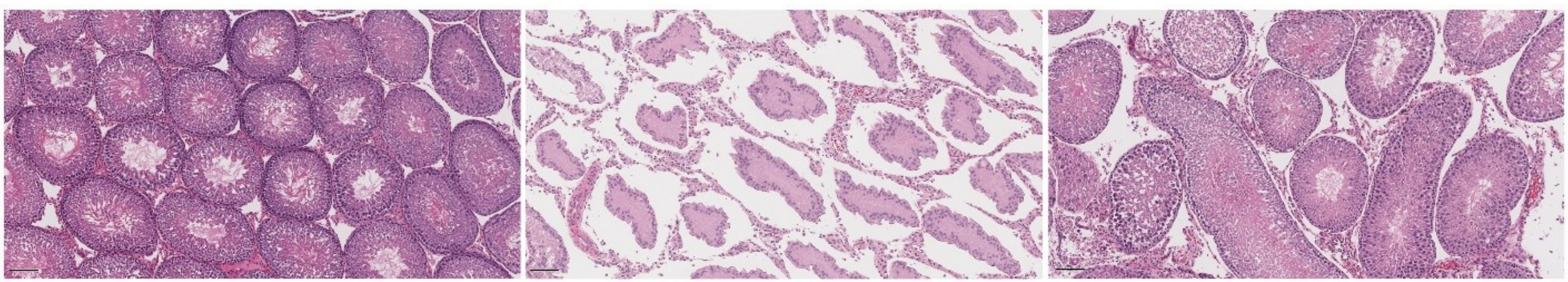
Testis histology from *Sry*-transgenic males

Line 208



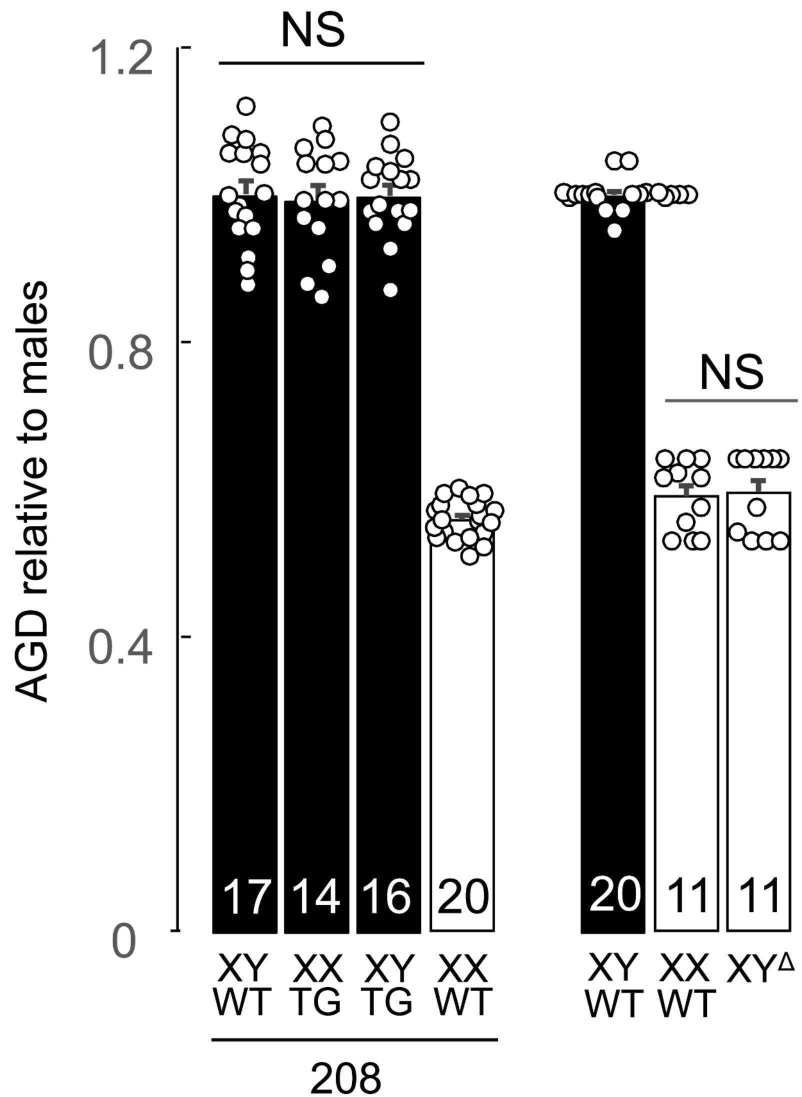
XYWT XXTG XYTG

Line 733

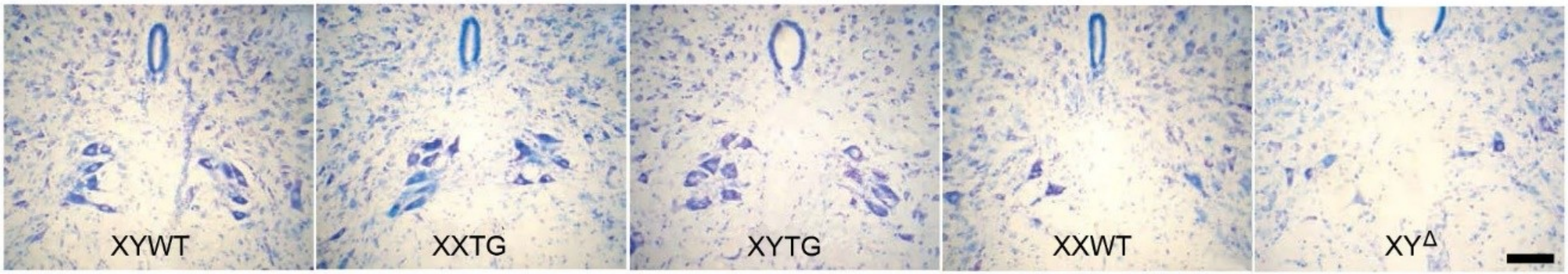


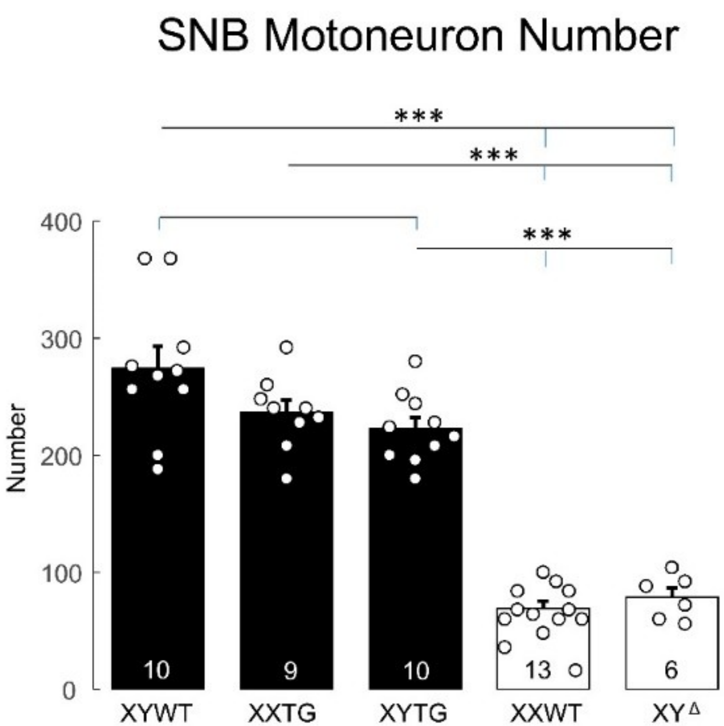
XYWT XXTG XYTG

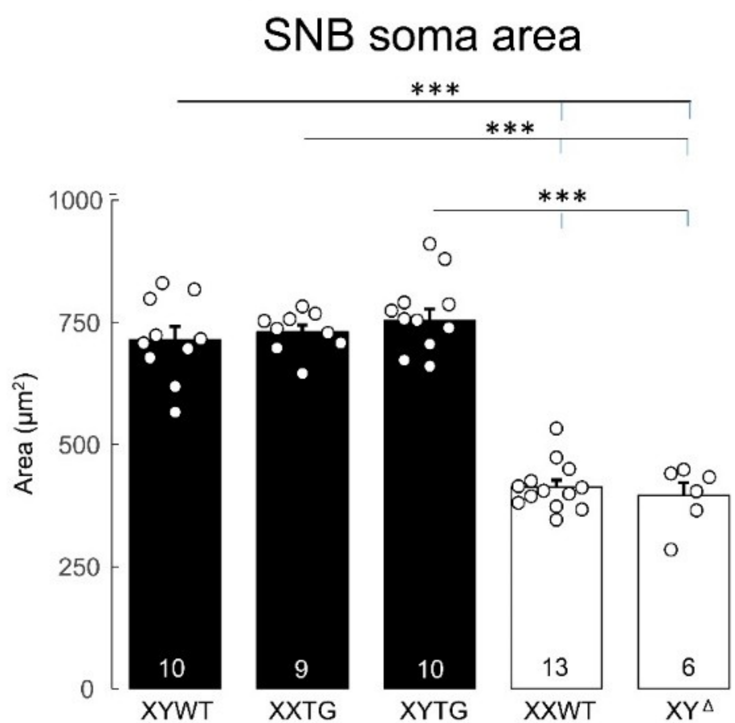
Anogenital Distance
a measure of androgen effects prenatally



Rat CNS Sexual Dimorphism: Spinal Nucleus of the Bulbocavernosus (SNB)

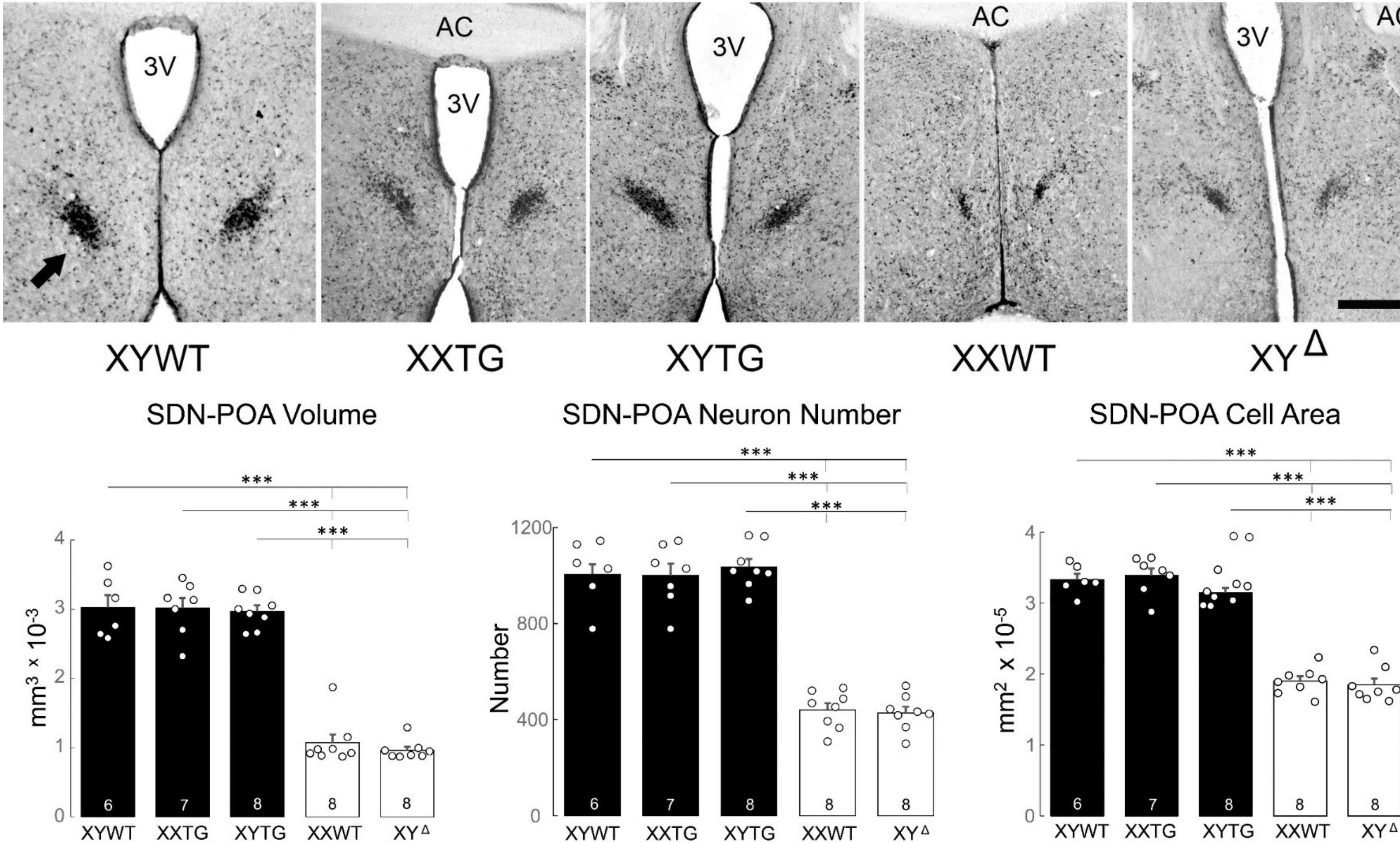






Dale Sengelaub
Indiana University

Rat CNS Sexual Dimorphism: Hypothalamic Nucleus SDN-POA



Tara Mohanroy

A "Four Core Genotypes" rat model to distinguish mechanisms underlying sex-biased phenotypes and diseases

Arthur P. Arnold[1], Xuqi Chen[1], Michael N. Grzybowski[2], Janelle M. Ryan[3], Dale R. Sengelaub[4], Tara Mohanroy[1], V. Andree Furlan[1], William Grisham[5], Lynn Malloy[2], Akiko Takizawa[2], Carrie B. Wiese[6], Laurent Vergnes[6], Helen Skaletsky[7], David C. Page[7], Karen Reue[6], Vincent R. Harley[3], Melinda R. Dwinell[2], and Aron M. Geurts[2]

[1] Department of Integrative Biology & Physiology, and Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, Los Angeles 90095 CA USA

[2] Department of Physiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA.

[3] Hudson Institute of Medical Research, Monash Medical Centre, Melbourne, Australia.

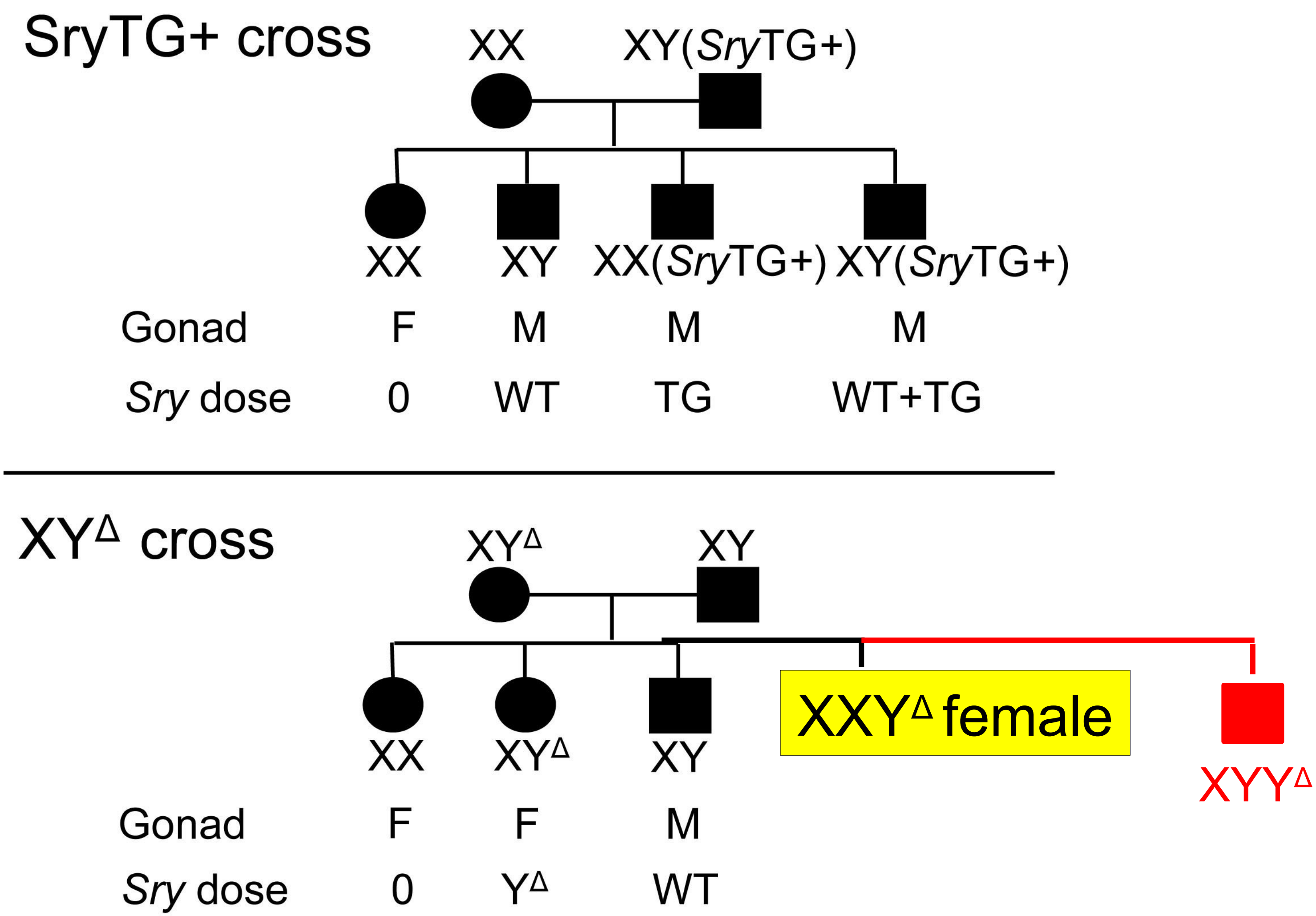
[4] Department of Psychological and Brain Sciences, Indiana University, Bloomington, IN, USA

[5] Department of Psychology, University of California, Los Angeles, Los Angeles, CA 90095, USA

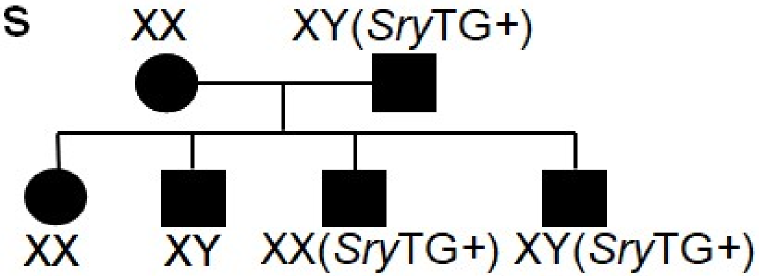
[6] Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, 90095 CA, USA

[7] Howard Hughes Medical Institute, Whitehead Institute, Cambridge MA, USA

Error in genotyping

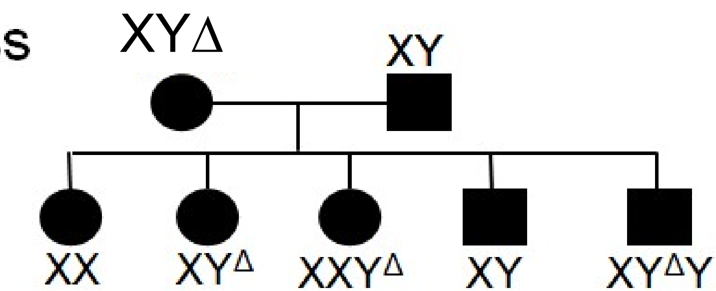


SryTG+ cross



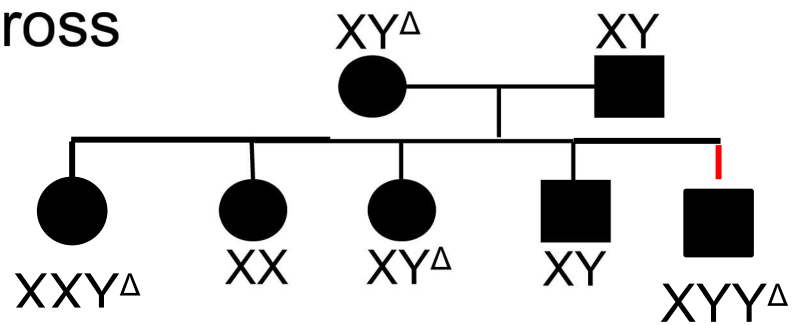
	XX	XY	XX(*Sry*TG+)	XY(*Sry*TG+)
Gonad	F	M	M	M
Sry dose	0	WT	TG	WT+TG
X number	2	1	2	1
Y number	0	1	0	1

XY^{Δ} cross



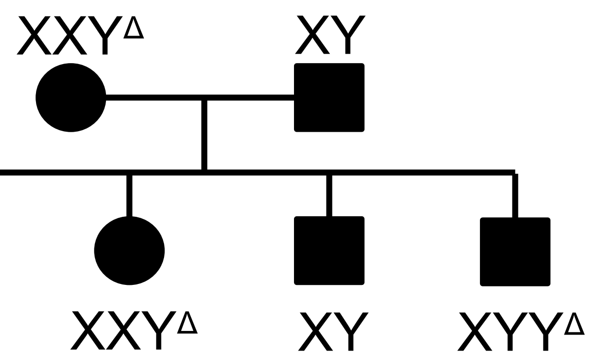
	XX	XY^{Δ}	XXY^{Δ}	XY	$XY^{\Delta}Y$
Gonad	F	F	F	M	M
X number	2	1	2	1	1
Y number	0	Y^{Δ}	Y^{Δ}	Y	Y^{Δ} + Y
Sry dose	0	Y^{Δ}	Y^{Δ}	WT	Y^{Δ} + WT

XY^{Δ} cross



XY^{Δ} make:
X eggs,
Y^{Δ} eggs,
XY^{Δ} eggs.

$XXY\Delta$ Cross



XXY^{Δ} make:
X eggs,
XY^{Δ} eggs.

Catch-22

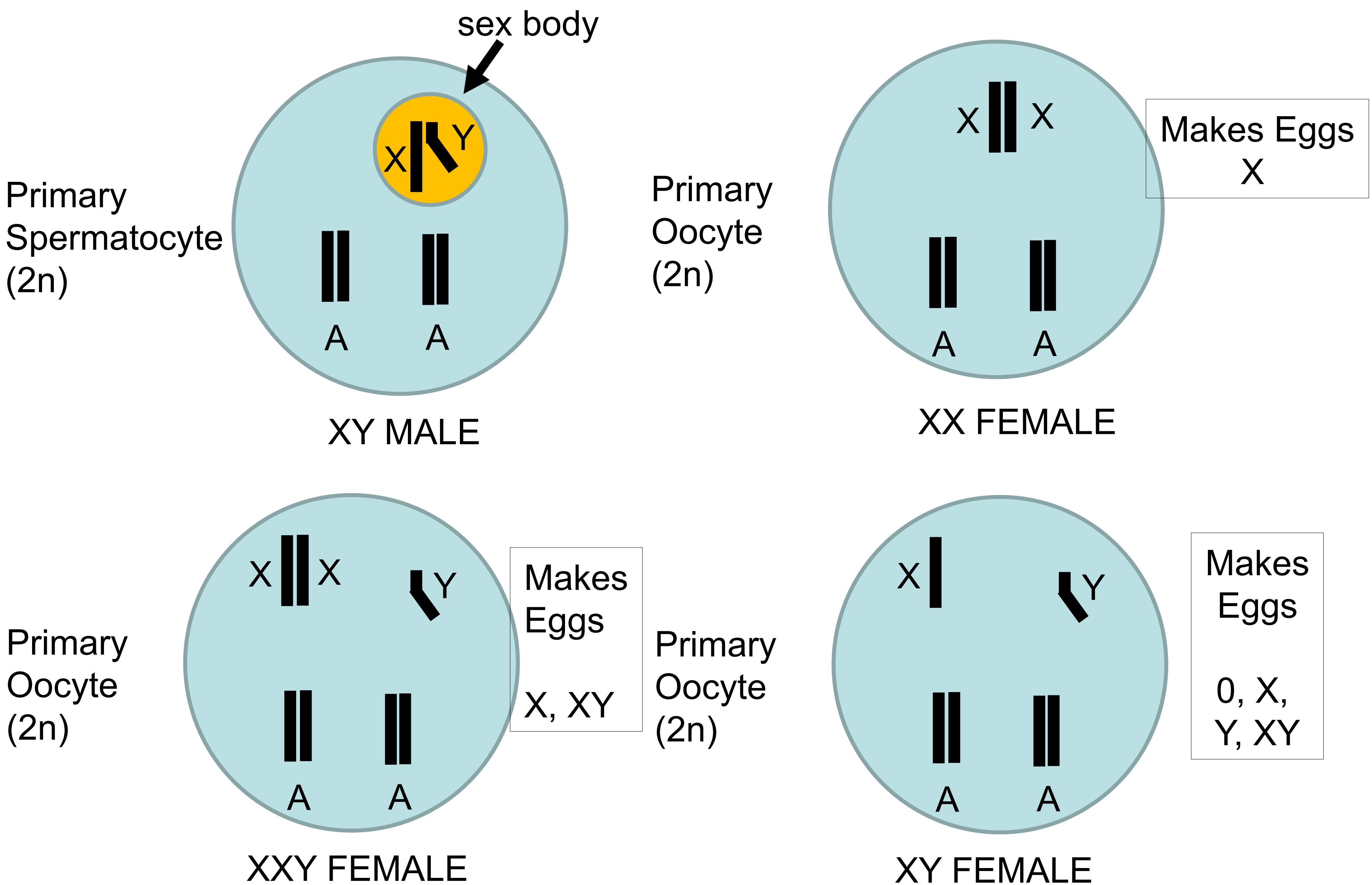
1. XY^{Δ} females make XX and XXY^{Δ} daughters, but few XY^{Δ} and XO daughters

2. XXY^{Δ} females make XX and XXY^{Δ} daughters, not XY^{Δ} daughters.

3. Inevitably and inadvertently, we had lost all XY^{Δ} females in our colonies.

Upside: We can make XXY females and XYY males.

Non-disjunction of X and Y chromosomes in ooctyes



Strategies to recover XY^{Δ}

- Geurts lab made a second line of XY^{Δ}, which do not make many XY^{Δ} daughters. Not attractive as easy-to-produce model.

- Instead of KO of Sry, make KO of *Sry*'s genomic binding site where it induces testis differentiation.

Knockout of Enhancer 13 makes XY female mice.

SEX DETERMINATION

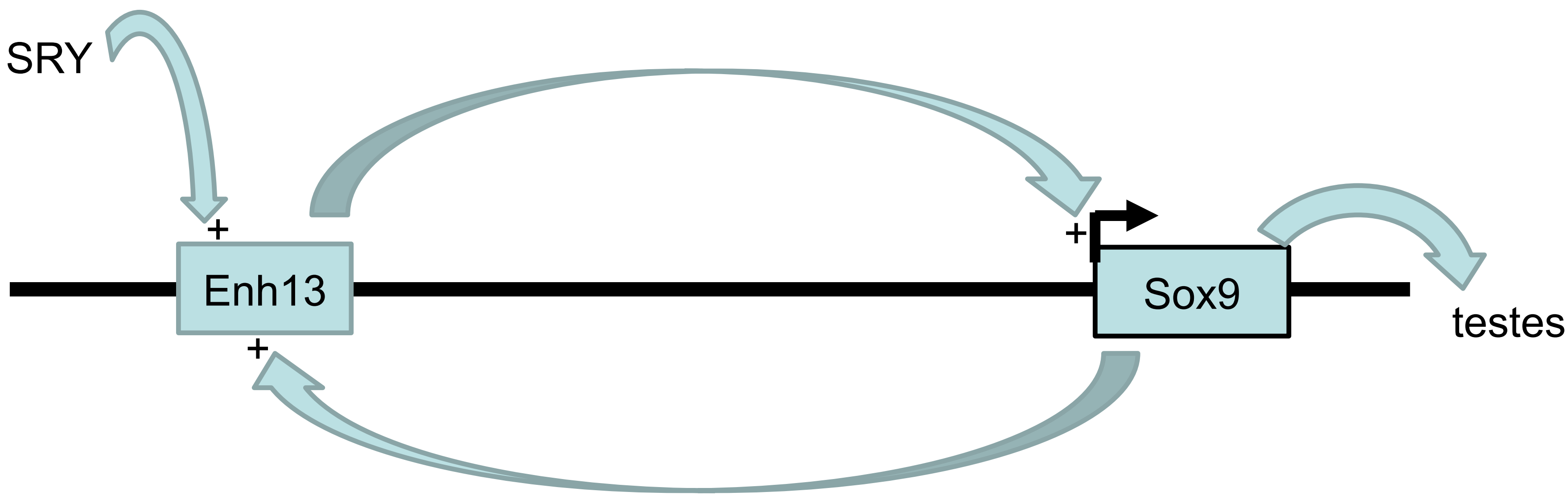
Sex reversal following deletion of a single distal enhancer of *Sox9*

Nitzan Gonen[1], Chris R. Futtner[2], Sophie Wood[1], S. Alexandra Garcia-Moreno[2], Isabella M. Salamone[2]*, Shiela C. Samson[1]†, Ryohei Sekido[3]‡, Francis Poulat[4], Danielle M. Maatouk[2]§||, Robin Lovell-Badge[1]§¶

Science, 2018

Enhancer 13 (Enh13) is site of SRY and SOX9 activation of *Sox9*, a critical causal link in testis determination.

SRY binds upstream of *Sox9* (ancient testis gene), increases *Sox9*.
SOX9 binds upstream of *Sox9*, self-activates (positive feedback).
SOX9 activates multiple testis genes which also reduce ovary genes.



KO of Enh13 in mice blocks testis development (XY gonadal female).

We make 5 male groups and 2 female groups.

Males

XYWT
XXTG
XYTG

Females

XXY^{Δ}
XYY^{Δ}

Current genotypes and strategy

1. Compare XYM and XXF and XXM to detect sex chromosome and hormonal effects.
2. If XXM and XYM differ, then
   (a) Search for X chromosome effect, compare XYM and XXYM.
   (b) Search for Y chromosome effect, compare XXF and XXYF, XYM and XYYM.

UCLA

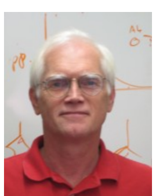
Metabolism
Karen Reue
Jenny Link

Autoimmune Disease
Rhonda Voskuhl
Yuichiro Itoh, Lisa Golden

Cardiovascular & Pulmonary
Mansoureh Eghbali
Jingyuan Li, Soban Umar,
Christine Cunningham

Sex chromosome trisomies, etc.
Eric Vilain
Negar Ghahramani, Tuck Ngun

Behavior & Cognition
David Jentsch



Systems Biology
Jake Lusis

Systems Bio
Xia Yang

Crick Institute, London

Paul Burgoyne
Robin Lovell-Badge

Arnold Lab

Xuqi Chen
Yuichiro Itoh
Haley Hrncir
Tara Mohanroy
Andree Furlan

Jason Lerch, Oxford
Kamila Szulc-Lerch, Oxford
Armin Raznahan, NIMH
Allan Mackenzie-Graham, UCLA
Aron Geurts, Med College Wisc.
Mindy Dwinell, MCW
Mike Grzybowski, MCW
Vince Harley, Hudson Inst.
Janelle Ryan, Hudson Inst.

Support: thank you!

NICHD, ORWH, NIDDK,
I, NINDS, NIMH, NIDCD,
OD

Society for Women's Health Research

UCLA Iris Cantor Center, CURE,
Center for Neurobiology of Stress,

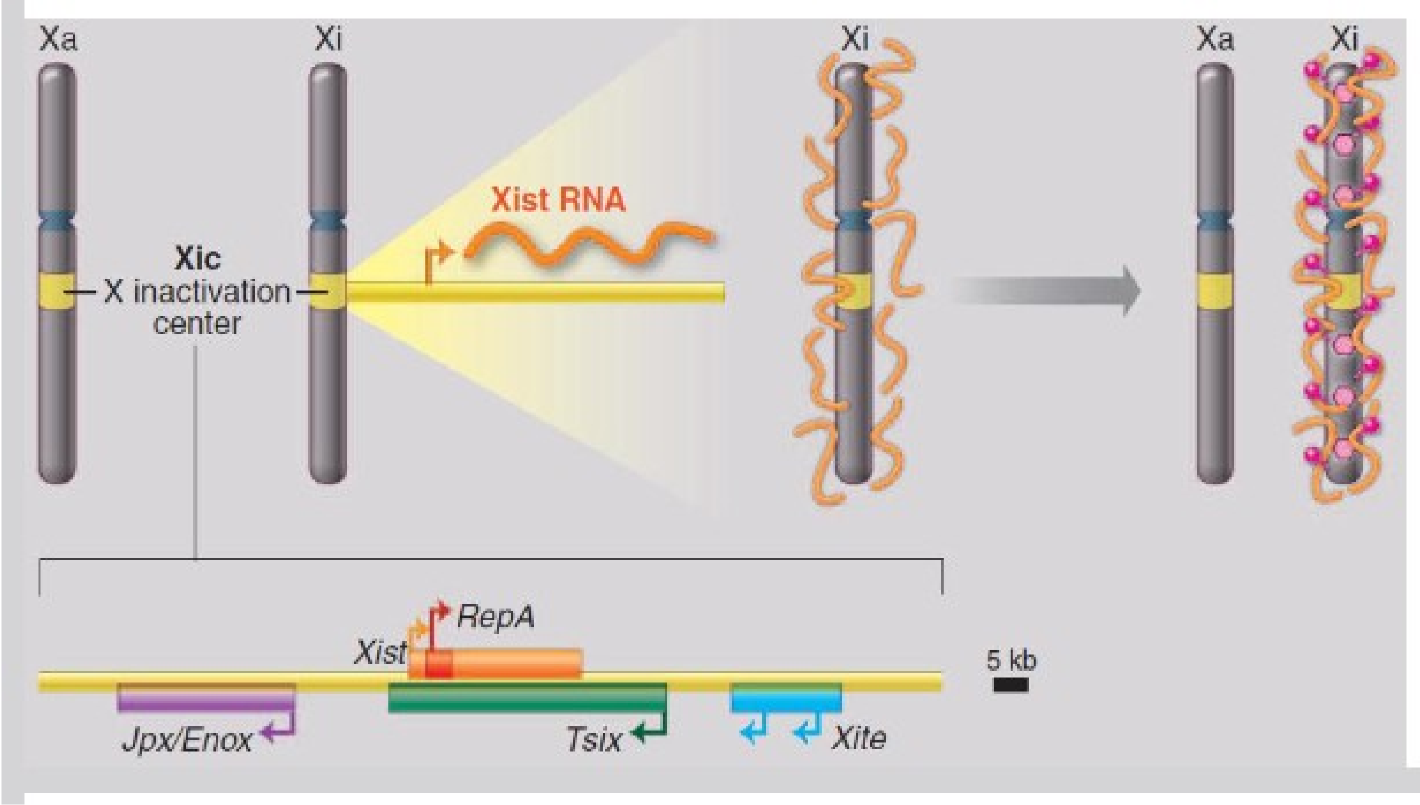
Sex-specific balancers also cause sex differences.

1. The evolution of *Xist* made XX cells more like XY cells,
   but also made XX cells unlike XY cells
   ➡ XX cells depend on *Xist* and XY cells do not.
   ➡ Inherent sex differences in *underlying molecular regulation*.

2. Survival of Y genes dosage compensators, similar effects of X and Y chromosome. But Y genes were free to evolve male-specific effects.

Disruptions in X inactivation or X-Y balance lead to sex differences, side effects of these 2 types of dosage compensation. In other words, selection for sexual equality paradoxically resulted in mechanisms of sexual inequality.

Xist non-coding RNA expressed from future Xi and coats Xi in cis, recruiting DNA methylation and histone modifications to repress transcription of most X genes.



Small problem, we thought

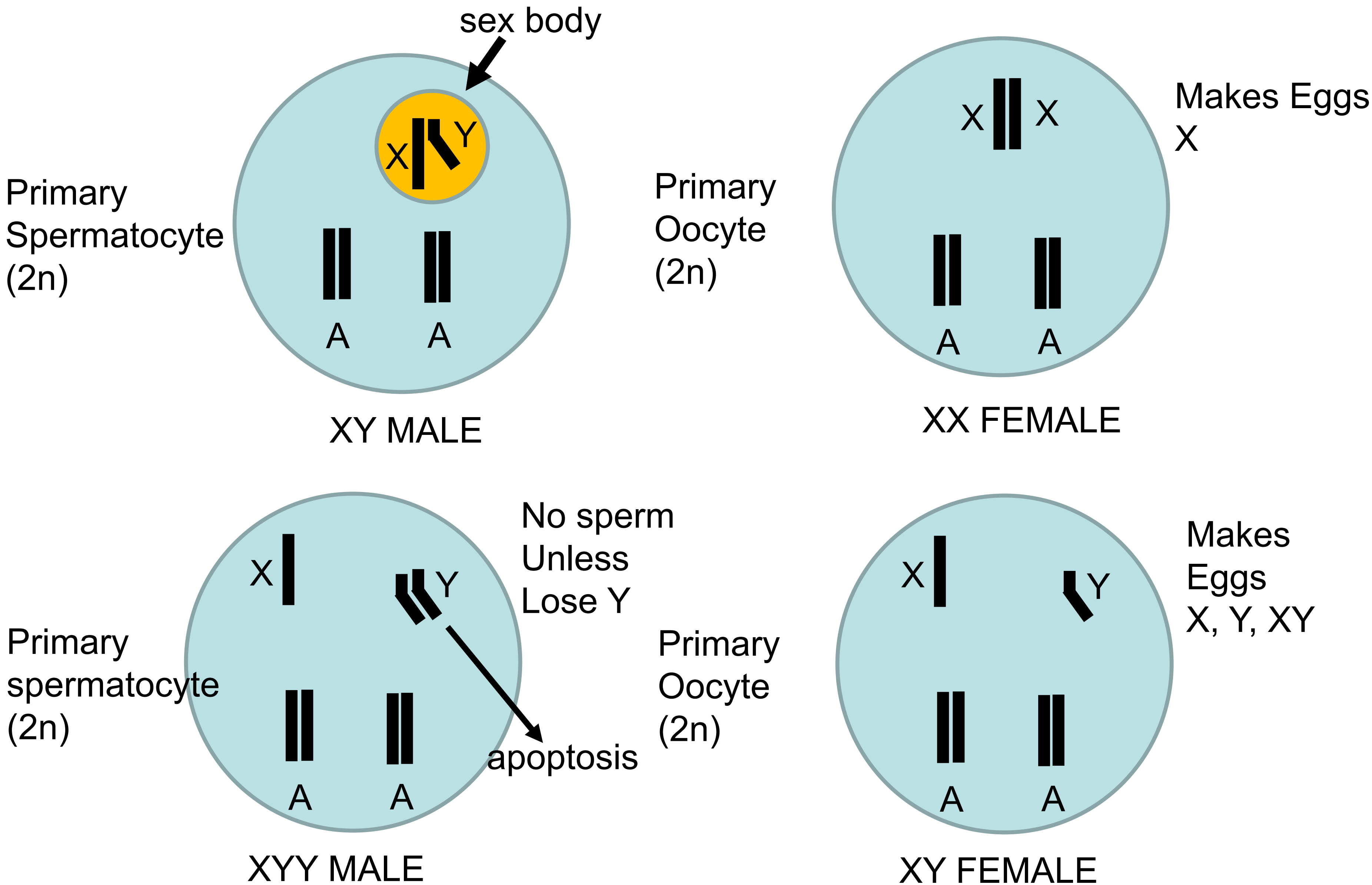
- Breeding XY(*Sry*TG+) males with XY^{Δ} females did not produce “true FCG father” XY^{Δ}(*Sry*TG+) who should be able to make all 4 genotypes as in the mouse model.

- Therefore, we had to have two breeding schemes to make XX and XY rats with either testes or ovaries.

- The inability to pass the Y^{Δ} chromosome from the father loomed larger as the project developed.

- XY^{Δ} mothers do not produce many XY^{Δ} daughters.

Alignment of chromosomes before first meiotic division



REVIEWS

Check for updates

X chromosome agents of sexual differentiation

Arthur P. Arnold

NATURE REVIEWS | ENDOCRINOLOGY

2022

Is there evidence for effects of *Xist* that make XX different from XY?

1. *Xist* expression is regulated differently according to tissue type, immune activation, life stage, and across cells within a single tissue type.
2. *Xist* is female-specific tumor suppressor, successful survival of females is anchored by a female-specific mechanism. Deleting *Xist* in adult hematopoietic cells causes hematological cancer in 100% of females but 0% males (Yildirim et at. *Cell* 2013).
3. Deletion of *Xist* in gastrointestinal tract makes XX females susceptible to tumorigenic effects of a carcinogen (Yang et al. *PNAS* 2020).
4. Mutations of p53 cause female-specific neural tube defects, due to number of X chromosomes (Chen et al. *J Neurobiol* 2008) because p53 binds to X inactivation center and reduces *Xist* expression and binding to Xi, reducing H3K27me3 associated with Xi (Delbridge et al., *Cell Rep* 2019).

Cell

CellPress
OPEN ACCESS

Article

Xist ribonucleoproteins promote female sex-biased autoimmunity

Diana R. Dou,[1] Yanding Zhao,[1] Julia A. Belk,[1] Yang Zhao,[1] Kerriann M. Casey,[2] Derek C. Chen,[1] Rui Li,[1] Bingfei Yu,[1] Suhas Srinivasan,[1] Brian T. Abe,[1] Katerina Kraft,[1] Ceke Hellström,[3] Ronald Sjöberg,[3] Sarah Chang,[4] Allan Feng,[4] Daniel W. Goldman,[5] Ami A. Shah,[5] Michelle Petri,[5] Lorinda S. Chung,[4] David F. Fiorentino,[6] Emma K. Lundberg,[7,8] Anton Wutz,[9] Paul J. Utz,[4,10] and Howard Y. Chang[1,11,12,*]

Dou et al., 2024, Cell *187*, 733–749
February 1, 2024
https://doi.org/10.1016/j.cell.2023.12.037

Upregulation of *Xist* in XX cells causes down-regulation of X escapees

Escape from X inactivation is directly modulated by levels of Xist non-coding RNA

Antonia Hauth[1,15*], Jasper Panten[6,7,8*], Emma Kneuss[1], Christel Picard1[1,13], Nicolas Servant[10], Isabell Rall[1,14], Yuvia A. Pérez-Rico[1], Lena Clerquin[1], Nila Servaas[5], Laura Villacorta[4], Ferris Jung[4], Christy Luong[11], Howard Y. Chang[11,12], Oliver Stegle[3,7], Duncan T. Odom[6,8,9], Agnese Loda[1#] & Edith Heard[1,2#]

Variations in *Xist* expression in differentiated cells can regulate expression from Xi, thus changing the XX/XY ratio of expression of X escapees and changing the level of XX vs XY inequality.

CellPress
OPEN ACCESS

Cell

Article

XIST directly regulates X-linked and autosomal genes in naive human pluripotent cells

Iris Dror,[1,7] Tsotne Chitiashvili,[1,7] Shawn Y.X. Tan,[1,8] Clara T. Cano,[1,8] Anna Sahakyan,[1,8] Yolanda Markaki,[1,2] Constantinos Chronis,[1,3] Amanda J. Collier,[1] Weixian Deng,[1] Guohao Liang,[4] Yu Sun,[1] Anna Afasizheva,[1] Jarrett Miller,[1] Wen Xiao,[5] Douglas L. Black,[5] Fangyuan Ding,[4,6] and Kathrin Plath[1,9,*]
[1]Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

Y Chromosome Survivors

1. Genes that are very important to males: *Sry*, spermatogenesis, testis genes.

2. Genes that are critical for basic cellular functions (X-Y genes). When mutation of a Y gene (homolog of an X gene) was lethal because of haploinsuffiency of the X partner gene, that Y chromosome dropped out of the population, leaving the Y gene intact in other Y chromosomes.

The whole explanation for survival of Y gene partners of X genes is that they perform the same function as the X partners, thus the Y chromosome compensates for the lack of second X.

(But, the Y survivors may evolve male-specific functions too….)

Survivors: X-Y gene pairs that participate in dosage compensation

1. The Y genes compensate for lack of second copy of X gene.

2. These are “dosage sensitive” genes, in which the X partner gene is haplo-insufficient.

3. Every cell needs two doses:
Two X doses in females, or one X and one Y dose in males.
The X gene escapes inactivation.   X + X = X +Y

Big conclusion

2. Survival of Y genes dosage compensators, similar effects of X and Y chromosome. But Y genes were free to evolve male-specific effects.

Disruptions in X inactivation or X-Y balance lead to sex differences, side effects of these 2 types of dosage compensation. In other words, selection for sexual equality paradoxically resulted in mechanisms of sexual inequality.

Utx (Kdm6a) and Uty are X-Y gene pair
but Uty has lost histone demethylase activity

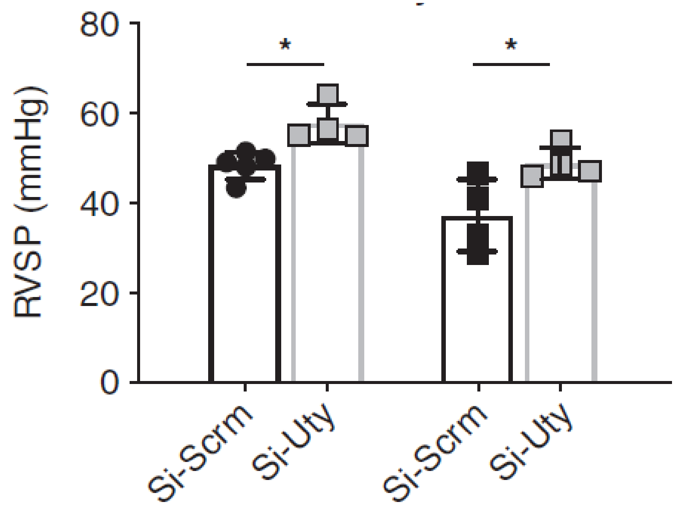
Y-Chromosome Gene, *Uty*, Protects Against Pulmonary Hypertension by Reducing Proinflammatory Chemokines

Christine M. Cunningham[1,2,3], Min Li[1], Gregoire Ruffenach[1], Mitali Doshi[1,4], Laila Aryan[1], Jason Hong[1,5], John Park[1], Haley Hrncir[6], Lejla Medzikovic[1], Soban Umar[1], Arthur P. Arnold[6], and Mansoureh Eghbali[1]

[1]Division of Molecular Medicine, Department of Anesthesiology, [5]Division of Pulmonary and Critical Care Medicine, Department of Medicine, and [6]Department of Integrative Biology & Physiology, University of California, Los Angeles, Los Angeles, California; [2]School of Medicine, Stanford University, Stanford, California; [3]VA Palo Alto Health Care System, Palo Alto, California; and [4]University of Massachusetts Medical School, Worcester, Massachusetts

Right Ventricular Hypertension

Uty knockdown



Cell Genomics

Article

The human Y and inactive X chromosomes similarly modulate autosomal gene expression

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San Roman et al., 2024, Cell Genomics *4*, 100462
January 10, 2024
https://doi.org/10.1016/j.xgen.2023.100462

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Metabolism
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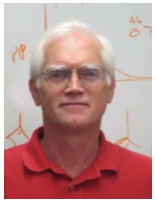
Autoimmune Disease
Rhonda Voskuhl
Yuichiro Itoh, Lisa Golden

Cardiovascular & Pulmonary
Mansoureh Eghbali
Jingyuan Li, Soban Umar,
Christine Cunningham

Sex chromosome trisomies, etc.
Eric Vilain
Negar Ghahramani, Tuck Ngun

Behavior & Cognition
David Jentsch



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Armin Raznahan, NIMH
Allan Mackenzie-Graham, UCLA
Aron Geurts, Med College Wisc.
Mindy Dwinell, MCW
Mike Grzybowski, MCW
Vince Harley, Hudson Inst.
Janelle Ryan, Hudson Inst.

Support: thank you!

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I, NINDS, NIMH, NIDCD,
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Society for Women's Health Research

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Important X-Y gene pairs

Kdm5c (X) / *Kdm5d* (Y)

Dose of *Kdm5c* causes sex difference in adiposity and body weight (Link et al., 2020)

The Journal of Clinical Investigation RESEARCH ARTICLE

X chromosome dosage of histone demethylase KDM5C determines sex differences in adiposity

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Important X-Y gene pairs

Kdm6a aka Utx (X) / *Uty* (Y)

Kdm6a proposed to cause sex difference in adiposity and body weight (Itoh et al., 2019)

RESEARCH ARTICLE

The Journal of Clinical Investigation

The X-linked histone demethylase *Kdm6a* in $CD4^+$ T lymphocytes modulates autoimmunity

Yuichiro Itoh,[1] Lisa C. Golden,[1,2] Noriko Itoh,[1] Macy Akiyo Matsukawa,[1] Emily Ren,[1] Vincent Tse,[1] Arthur P. Arnold,[3] and Rhonda R. Voskuhl[1]

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X and Y chromosome genes that are agents of sexual differentiation

1. *Kdm6a*: Autoimmune disease (Voskuhl), Bladder Cancer (S. Li), Alzheimer's Disease and aging (Dubal)
2. *Kdm5c*: Adiposity, metabolism (Reue), Autoimmune EAE (Rangachari)
3. *Uty*: Pulmonary hypertension (Eghbali)
4. *Ogt*: Prenatal stress (Bale)
5. *Xist*: hematologic cancer (J. Lee)

Common properties: Pleiotropic, epigenetic, dosage sensitive, linkage to sex chromosomes

Not common property: relation to reproduction