







Sex as a Biological Variable in preclinical assays

Katia ANCELIN & Virginie DANGLES-MARIE

WG CEEA-IC #118





Why this presentation?

DAP / 3.4.12. Indicate the sex of the animals used and why

We will use only female mice because of less aggressiveness. We don't expect sex differences We will use males and females (without any information about distribution of the 2 sexes)

- Most applications for mouse work are applying for one sex
- When both sexes are included, results are more often analyzed together and not separately
- In general biology and immunology, less that half of publications specified sex (Beery & Zucker 2011)
- This impacts on results: reproducibility issues , increased variability
- And *latent sex effects are lost*

Meetings...



COLLOQUE

The Genetic and Epigenetic Basis of Sex Bias in Disease

21 avril 2023

Annbe

académigu

2022/2023

Thomas Rome COLLÈGE Administrateur du Collège de France DE FRANCE 11, place Marcelin-Berthelpt, 75005 Parts

www.college.de-france.fr 1530

21 avril 2023 de 9h à 18h Amphitheatre Maurice Halbwachs

The Genetic and Epigenetic Basis of Sex Bias in Disease

Edith Heard, Chaire Épigénétique & mémoire cellulaire Scientific co-organisers: James Cleland and Agnese Loda

Daniel Andergassen Technical University of Munich, Germany

Richard Festenstein Imperial College, London, UK

Cornelius Gross EMBL-Rome, Italy

Jean-Charles Guéry INSERM, University of Toulouse, France

Jamie Hackett EMBL-Rome, Italy

Irene Miguel-Aliaga Imperial College, London, UK

Jessica Tollkuhn Cold Spring Harbor Lab, New York, USA

Taru Tukiainen FIMM, Helsinki, Finland

Judith Zaugg EMBL Heidelberg, Germany

Colloquium in English, free entry, no registration require



November 17, 2023



Organisateurs Lionel LARUE Michel COHEN-TANNOUDJI Yann HÉRAULT Chantal THIBERT Martin HOLZENBERGER

Comité d'organisation loca Denise AUBERT, Delphine BAETZ, Sylvie DUCREUX, Bérengère FROMY Kiran PADMANABHAN, Bruno PILLOT, Fabienne TATOUT-RAJAS, Marlène WIART Christophe SOULAGE

Information et inscription gratuite mais obligatoire http://clubdesbellessouris.free.fr

Le Club des Belles Souris

Laurent LE CAM Intervenants

Magda MAGIERA

Daniel METZGER - IGBMC, Strasbourg Caractérisation des voies de signalisation impliquées dans la progression des cancers de la prostate : analyses *in viv*o et à l'aide d'organoïdes

Alexandra MONTAGNER - I2MC Inserm Toulouse Why sex matters in liver health and disease: a hormonal view

Philippe FAURE - ESPCI, PSL Université, Paris Étude en laboratoire de micro-sociétés de rongeur

Angèle VIOLA - CRMBM, CNRS, Aix-Marseille Université, Marseille Principe des 3R : réduire et raffiner grâce aux méthodes d'IRM in vivo

Maxime SCHLEEF - CarMeN, Université Claude Bernard, Lyon L'échographie de contraste et photo-acoustique rénale 3D permettent une évaluation non-invasive des lésions d'ischémie-reperfusion rénale

Zayna CHAKER - ENS Lyon, France & Biozentrum Bâle, Suisse Coordination spatiale et temporelle des cellules souches neurales adultes : étude longitudinale incluant analyse cellulaire, transcriptomique et comportement durant la grossesse et la gestation

Jérémy H. RAYMOND - Ludwig Institute for Cancer Research, Oxford, UK La perte de la E-cadhérine chez la femme rend les mélanomes sensibles aux estrogènes: usage de l'imagerie intra-vitale pour réduire le nombre d'animaux

Ludovic GOMEZ - CarMeN, Université Claude Bernard, Lyon Recherche translationnelle en cardioprotection : de la prédiction in silico à l'application in vivo

Sophie LAFFONT-PRADINES - Infinity, Université Paul Sabatier, Toulouse De l'importance de prendre en compte le sexe des animaux en immunologie

Amandine GAUTIER-STEIN - NUDICE, Université Claude Bernard, Lyon Les multiples fonctions de la production de glucose : apports des modèles murins tissu-spécifique

Lida KATSIMPARDI - Institut Necker Enfants Malades, Paris Étude du vieillissement cérébrovasculaire avec l'utilisation des organes-sur-puces pour réduire l'utilisation de souris

Minchul KIM - IGBMC, Strasbourg Deep dive into myonuclear heterogeneity and muscle domains with reduction and refinement of animal experiments







Keynotes Edith Heard - EMBL, Heidelberg, DE Muriel Darmon - CNRS. Paris, FR

Sex and Cancer Molly Ingersoll - Institut Cochin, Paris, FR Pierre Val - iGReD, Clermont Ferrand, FR

Sex and Immunology Hanna Lotter - Bernhard Nocht Institute for Tropical Medicine, Hamburg, DE Jean-Charles Guery - INFINITY,

Marcus Altfeld - Leibniz Institute of

Sex and Metabolism Sara Della Torre - University of Milan, IT Herve Guillou - Toxalim, Toulouse, FR Franck Mauvais Jarvis - University of

Contact

Information and registration ww.institutcochin.fr/en/animation

Amphi Luton, Faculté de Médecine Cochin 24 rue du Fg St-Jacques, 75014 Paris

Sviesan

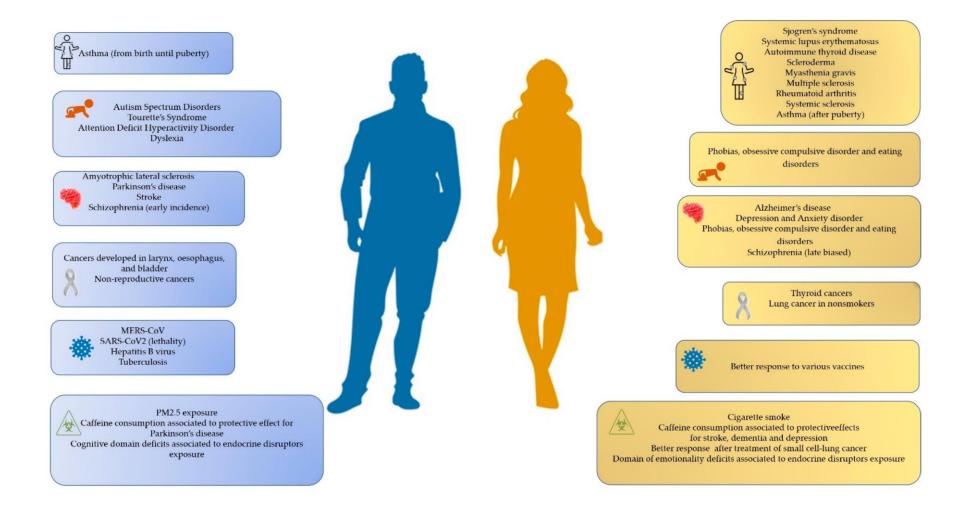
Toulouse, FR

Inserm

Virology, Hamburg, DE

Tulane, New-Orleans, US

Sex specific differences in disease susceptibility



From Migliore et al Biomedicines 2021

Sex As a Biological Variable in animal research

ARTICLE

COMMUNICATIONS

Received 27 Oct 2016 | Accepted 30 Mar 2017 | Published 26 Jun 2017

Prevalence of sexual dimorphism in mammalian phenotypic traits

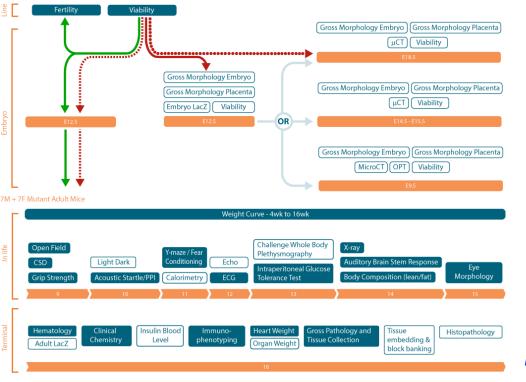
DOI: 10.1038/ncomms15475

OPEN

Data produced by the International Mouse Phenotyping Consortium

- 14,250 wildtype animals + 40,192 mutant mice
- From 2,186 single gene knockout lines
- 7 males and 7 females from each mutant line
- 10 phenotyping centers





Nat Commun. 2017 Jun 26;8:15475

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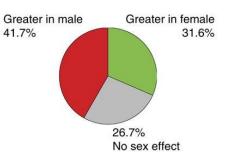
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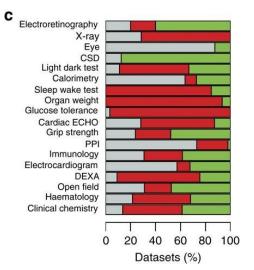
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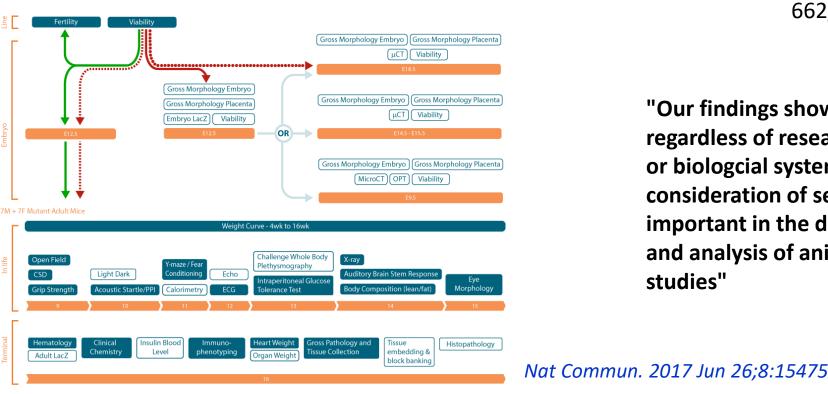


662/903 data sets

"Our findings show that regardless of research field or biologcial system, consideration of sex is important in the design and analysis of animal studies"

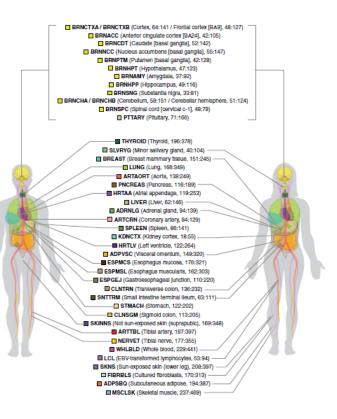


IMPReSS pipeline www.mousephenotype.org/impress/index



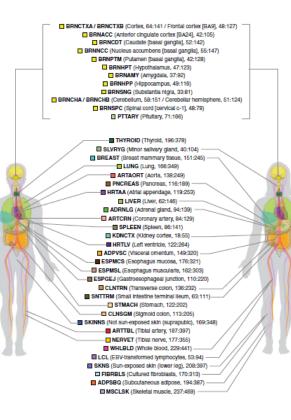
Oliva et al. *Science* 2020 The impact of sex on gene expression across human tissues

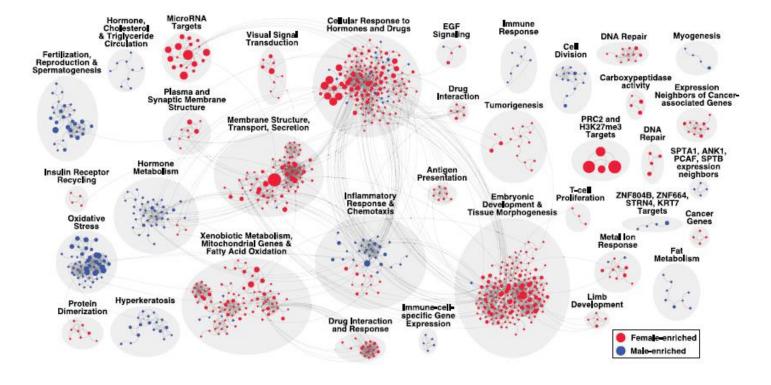
44 tissues GTEX project v8 release ; 838 individuals (557 males, 281 females)



Oliva et al. *Science* 2020 The impact of sex on gene expression across human tissues

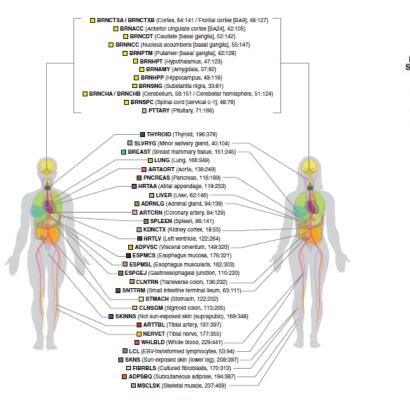
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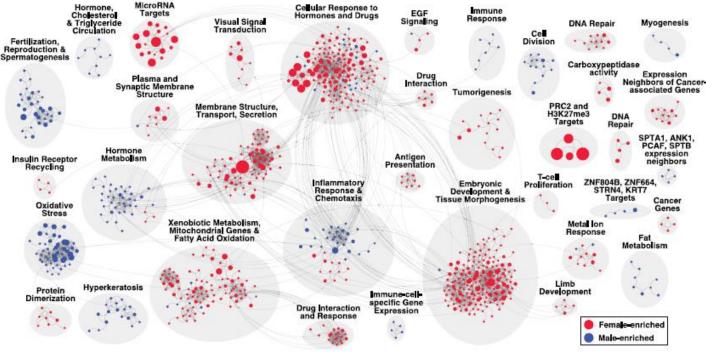




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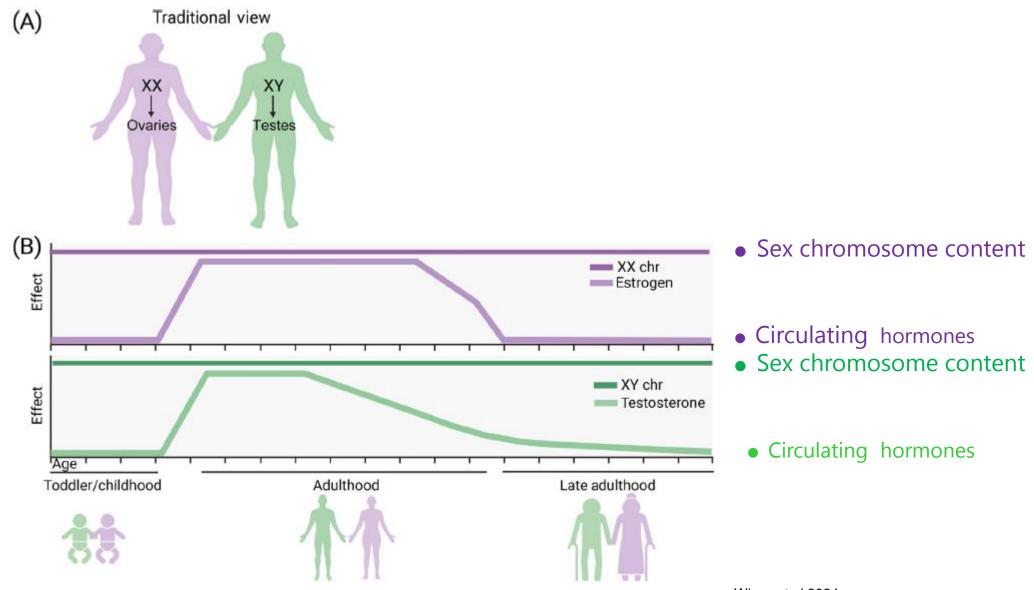




37,5% (13 294/35 431 genes; protein coding, IncRNA, & transcribed but less characterized genes) of the human transcriptome was differently expressed in at least one tissue.

531 are X linked & 12763 are autosomal (47% and 37% of all tested genes respectively)

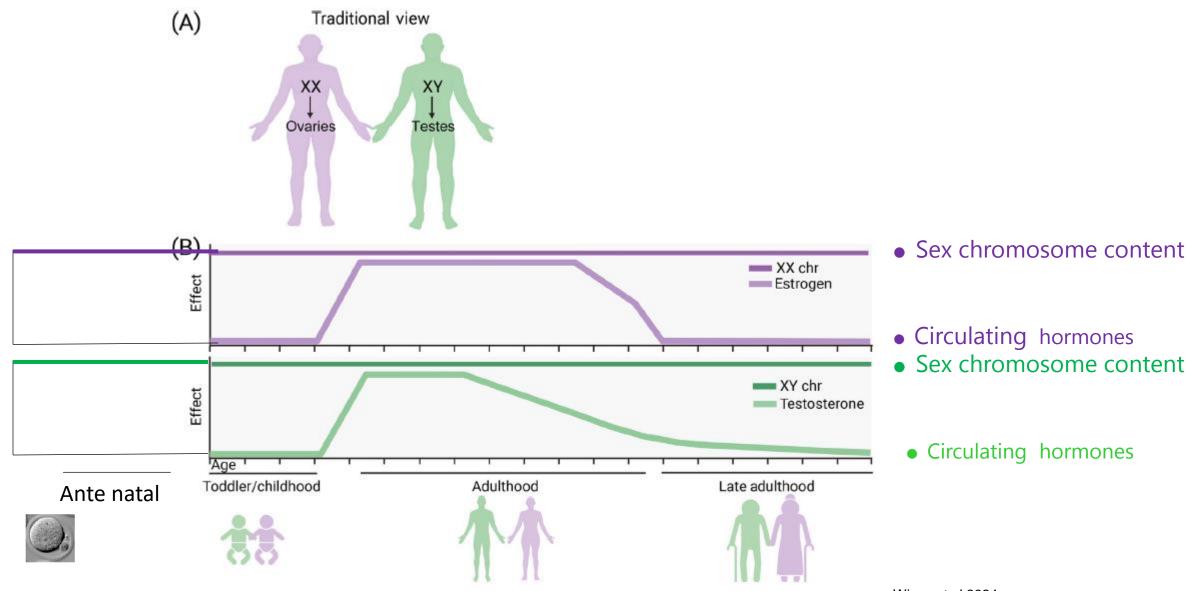
Sex differences in phenotype



Trends in Endocrinology & Metabolism

Wiese et al 2024

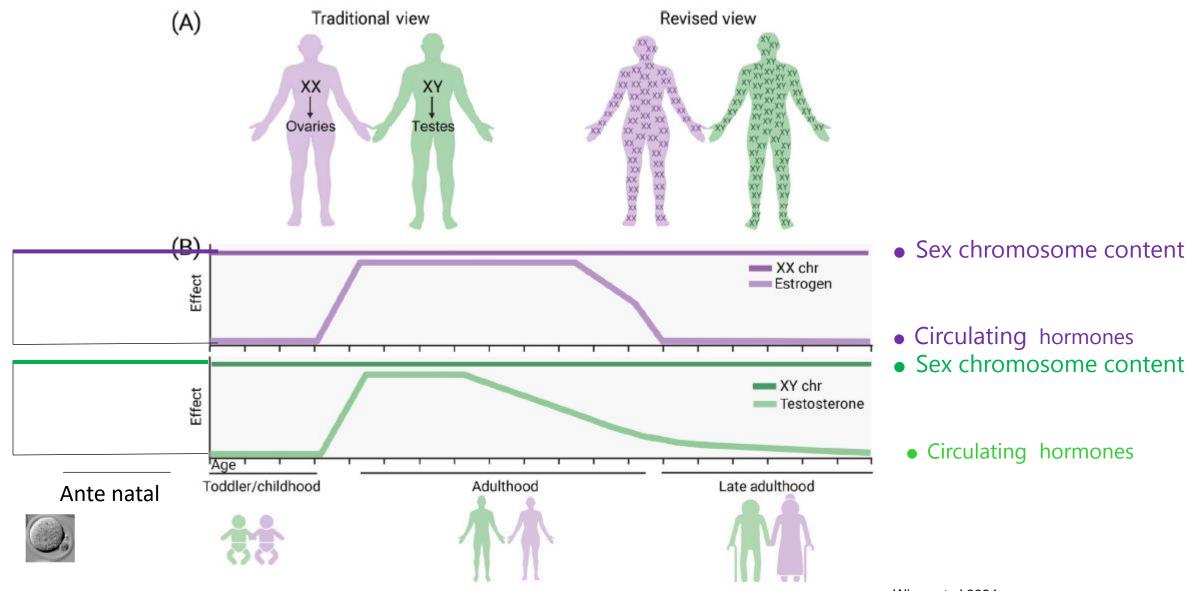
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Trends in Endocrinology & Metabolism

Wiese et al 2024

Sex differences in phenotype



Trends in Endocrinology & Metabolism

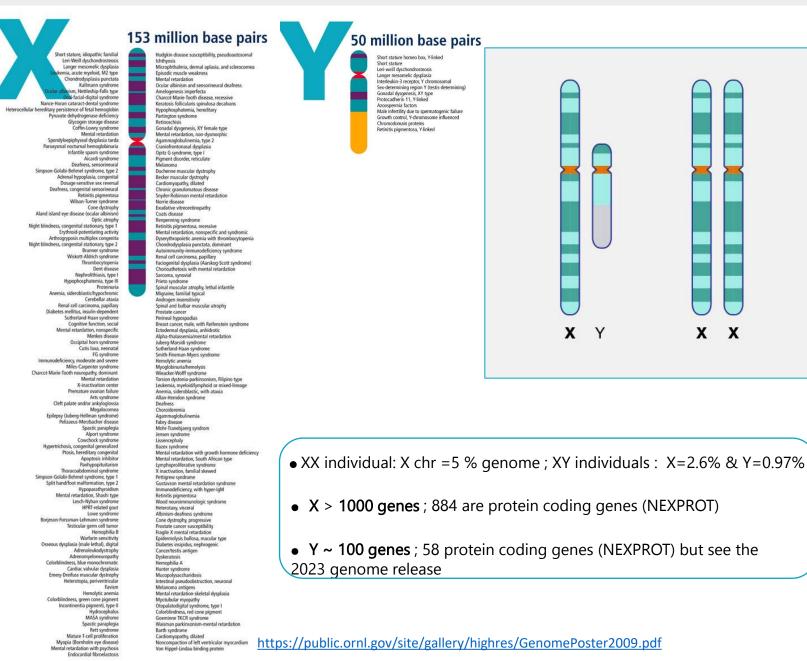
Wiese et al 2024

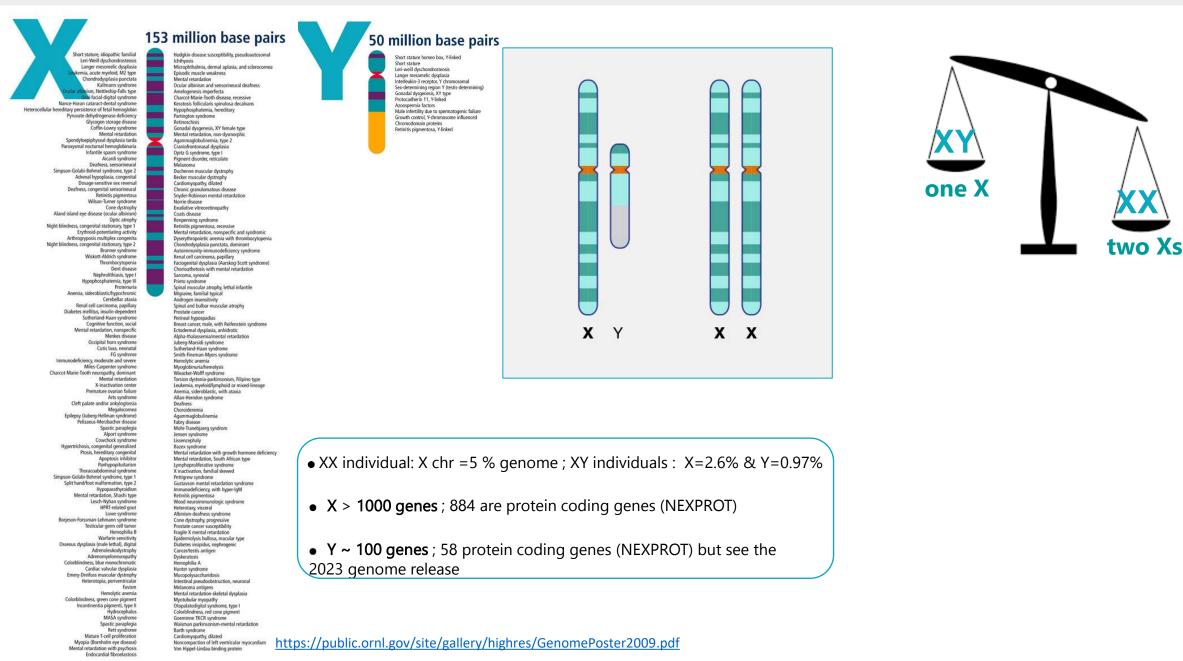
Sex As a Biological Variable in animal research

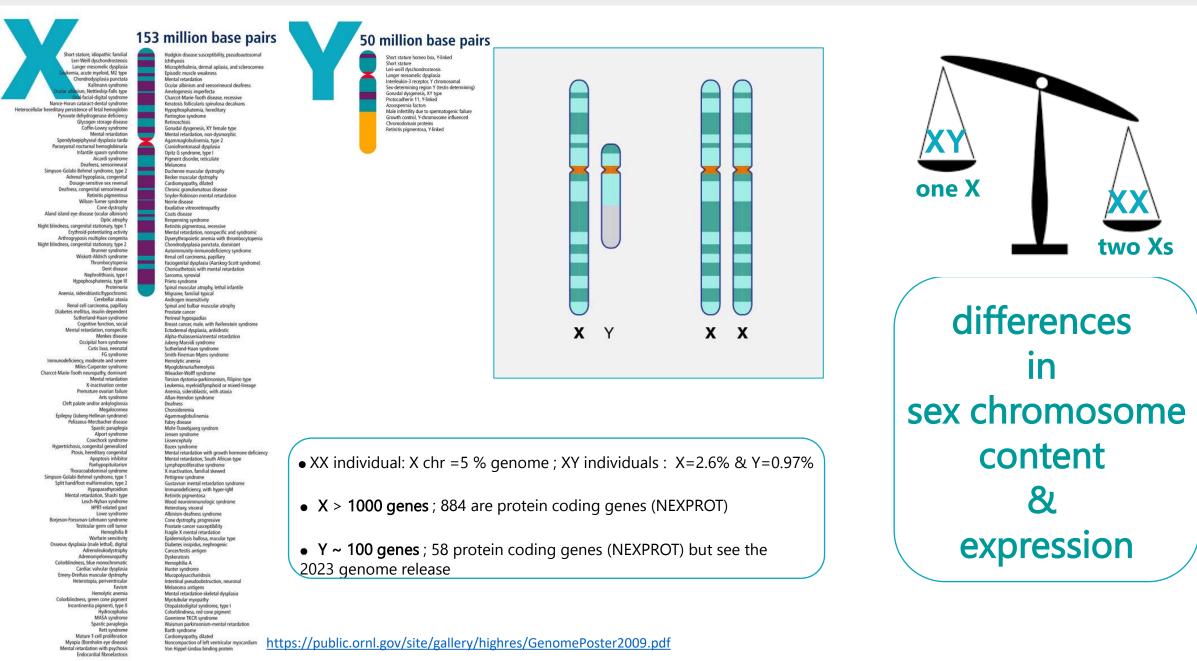
- Evidences of sex impacting biology: an overview about sex chromosomes
- Specific illustrations in cancer and immunology
- Tools: How to apply Sex As a Biological Variable?

• **Perspectives:** further complement on other topics and species

	153 million base pair	rs 50 million base pairs	
Short stature, idiopatric familia la veir Weil dysknortostosis la nejer mesomelic dysplasis la veirenia, acute myskoli A2 type Dondodysplasia punctata in veirenia, acute myskoli A2 type Dondodysplasia punctata in facial digital syndrome weither facial digital syndrome Provent delystogenesis deliciency provest del deliciency provest deliciency p	Hodgin disease susceptibility, pseudoutosonal ityryosis Microphtalmia, demal aplasia, and sciencomea Episofic muscle weakness Metal retardiation Costa alteriation Costa alter	Abs data later l	xx
K kiactivation cetter Premature ovarian failure Arts syndrome Cleft palte and/or antysfogiosia Megalacomes Epilepy (Juberg Helinaus yndrome) Retizaes Merzbacher disease Solowie (Solowie Solowie Solowie Preize Solowie Solowie Solowie Retizaes Solowie Solowie Solowie Prosis, hereditary congenital Apoptosis inhibitor Prosis, hereditary congenital Apoptosis inhibitor Prosis, hereditary congenital Apoptosis inhibitor Prosis, hereditary congenital Apoptosis inhibitor Prosis, hereditary congenital Apoptosis Joanni yne Spith Aafofor malformati Menal retraditoris, Salah injee Leiter (Solowie Solowie Solowie Kerne Borjeson-Forsman-Lehman syndrome Borjeson-Forsman-Lehman syndrome Borjeson-Forsman-Lehman syndrome Borjeson-Gossman-Lehman syndrome Borjeson-Gossman-Lehman syndrome Borjeson-Gossman-Lehman syndrome Borjeson-Gossman-Lehman syndrome Borjeson-Gossman-Lehman syndrome Borjeson-Costania Costania Colobilindenes, Sule menochonate Colobilindenes, Sule	Leukemia, myedioli/myboid or mixed-lineage Anemia, sideroblatti, with ataxia Allan-Hendon syndrome Dealhers Chorciciderenia Agammaglobulinemia Fabry disease Moto Tandykeng syndrom tissencephaly Bases syndrome Alland Fabry and Alland Statistics Metal retardiation with growth homone deficiency Metal retardiation with spen-typh Pattigrew syndrome Giustavon metal retardiation syndrome Immunodeficiency, with hyper-typh Retenting syndrome Giustavon metal retardiation syndrome Hettereasy, visceral Albinion-dankers, syndrome Epidemotypis buildos, muchar type Diabetes insplate, negative Prostate cancer suscephility Fragile X mental retardiation Dyskeratosis Hemophilia A Hanter syndrome Metal retardiation Schedid syndrome Hettereasy discone Cancerbists antigen Metal retardiation Hetteress, stringen Dyskeratosis Hemophilia A Hanter syndrome Metal retardiation functione Calcolinging Syndrome, type I Colchindhess: red cone pigment Goamine TKCR syndrome Bash String, Glated Hincorage.time al Het ventricolar myocardium Von figget Lindou binding protein	https://public.ornl.gov/site/gallery/highres/GenomePoster2009.pdf	



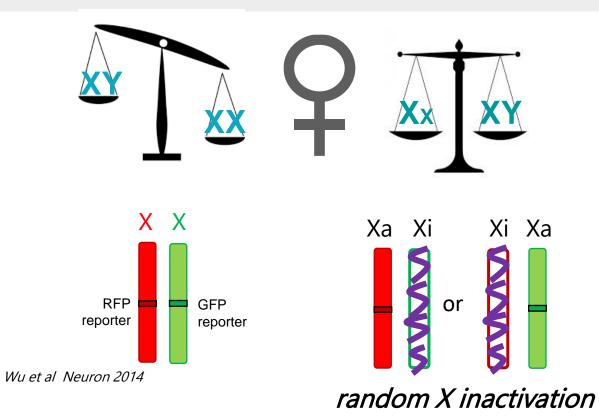




Sex dosage compensation: X chromosome inactivation in female mammals

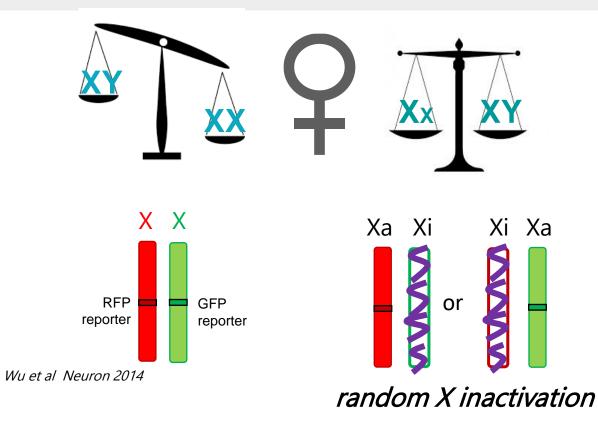


Sex dosage compensation: X chromosome inactivation in female mammals



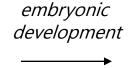
Part I

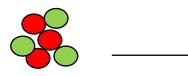
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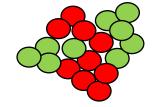


Part I





1- choice

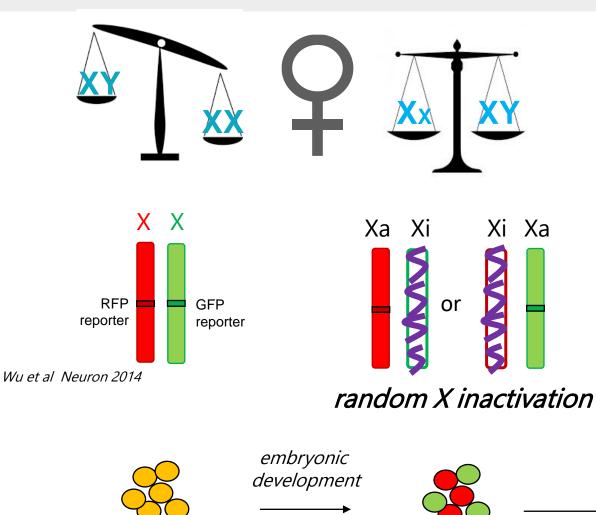


2- clonal propagation

Sex dosage compensation: X chromosome inactivation in female mammals

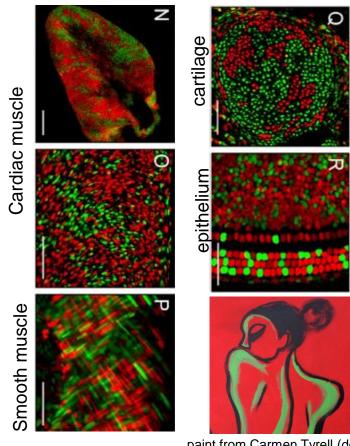
2- clonal

propagation



1- choice

Murine Tissues from Wu et al Neuron 2014

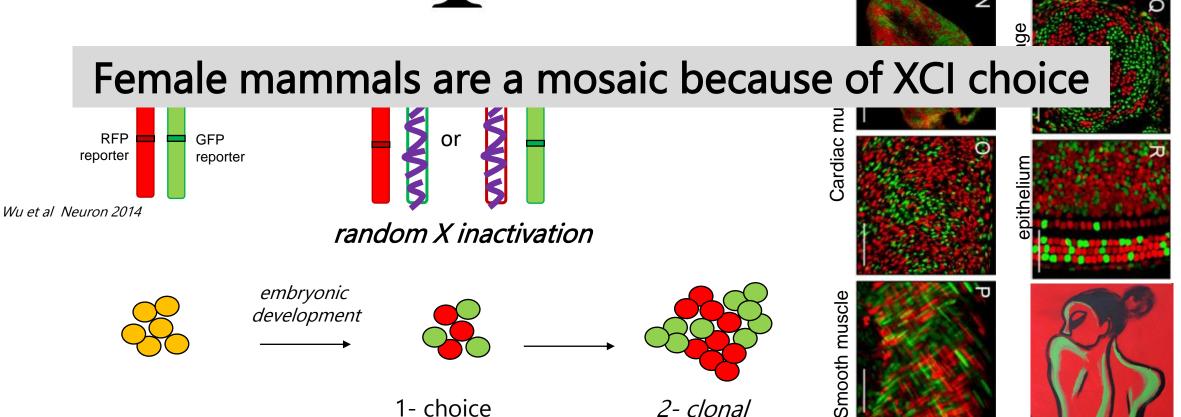


paint from Carmen Tyrell (detail)

Sex dosage compensation: X chromosome inactivation in female mammals



Murine Tissues from Wu et al Neuron 2014

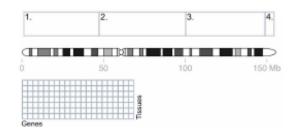


propagation

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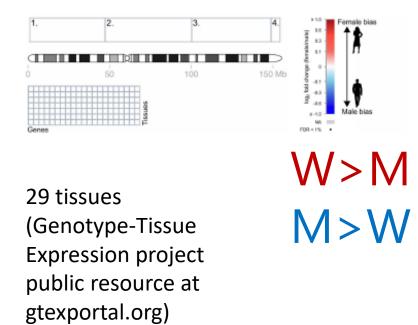
Part I Variability of X linked gene expression across tissues

Landscape of X chromosome inactivation across human tissues *Tukiainen et al Nature 2017*



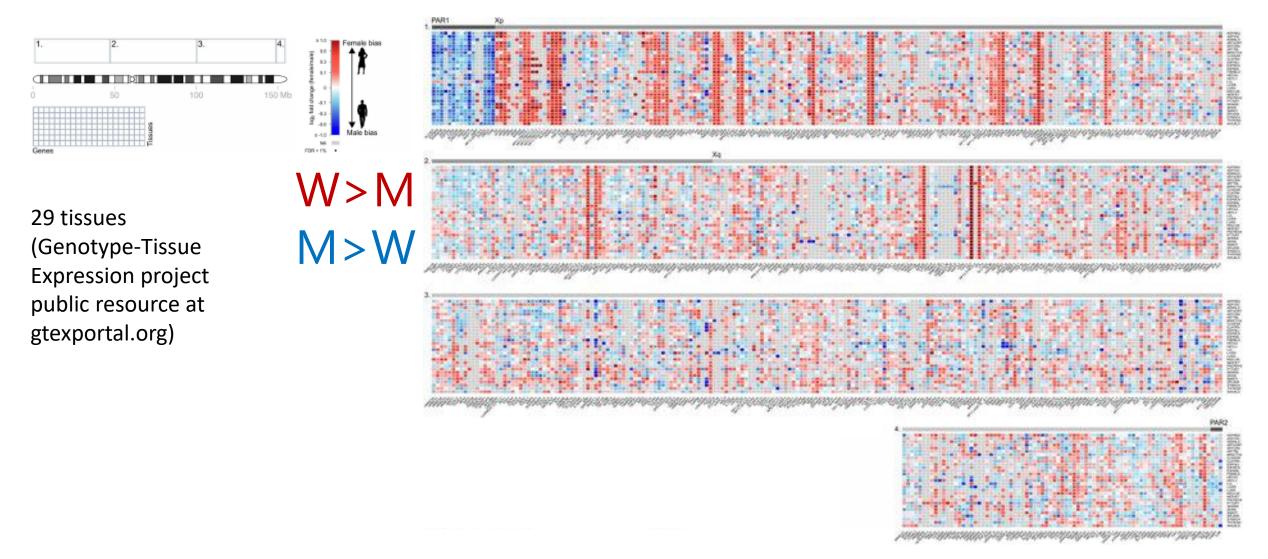
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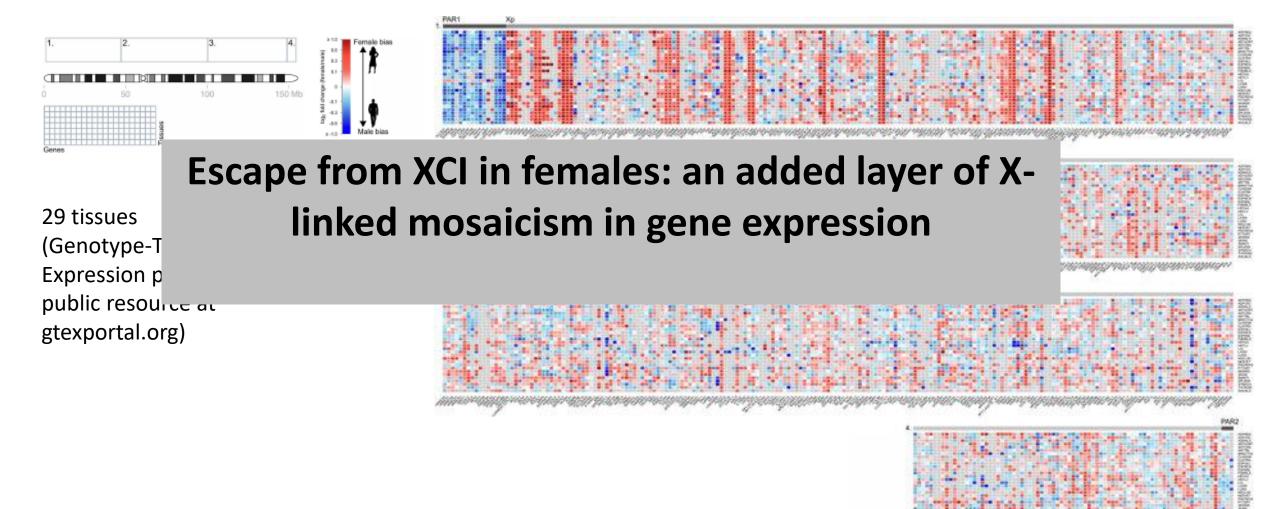
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Part | Variability of Y linked gene expression across tissues

index of X–Y gene pair Y Х PRKX NLGN4X VCX TBL1X AMELX TMSB4X] PAR1 \sim MSY 1 MI TXLNG EIF1AX ZFX 8 atin 9 USP9X 10 DDX3X 11 KDM6A 12 TSPYL2 13 KDM5C let PAR2 14 RPS4X b TGIF2LX c PCDH11X 10 Mb RBMX 15 SOX3 16 >99% sequence 17 HSFX identity X genes without Region of Y homologs X-Y crossovers (n ≈ 800) not shown during meiosis

Α

Alexander K. Godfrey et al. Genome Res. 2020;30:860-873



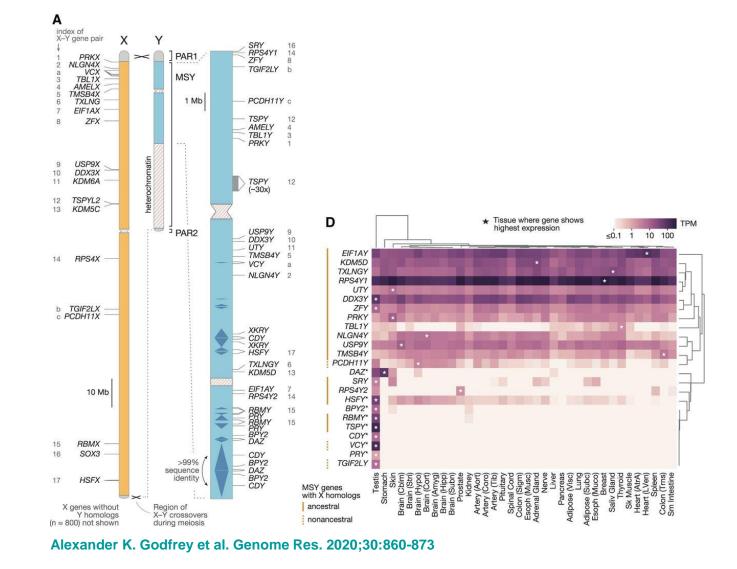
Variability of Y linked gene expression across tissues

Α index of X–Y gene pair Х Y SRY RPS4Y1 ZFY 16 14] PAR1 \sim PRKX 8 NLGN4X VCX TBL1X AMELX TMSB4X TGIF2LY b MSY 1 Mb PCDH11Y c TXLNG EIF1AX 7 TSPY 12 ZFX 8 AMELY 4 TBL1Y 3 PRKY atin 9 USP9X 10 DDX3X 11 KDM6A TSPY (~30x) 12 12 TSPYL2 13 KDM5C het .44 USP9Y DDX3Y UTY TMSB4Y ³ PAR2 10 11 5 14 RPS4X VCY a NLGN4Y 2 b TGIF2LX c PCDH11X XKRY CDY XKRY HSFY 17 TXLNGY KDM5D 6 13 EIF1AY RPS4Y2 10 Mb 14 RBMY PRY RBMY PRY BPY2 DAZ 15 15 RBMX 15 16 SOX3 CDY BPY2 DAZ BPY2 >99% sequence identity 17 HSFX CDY × X genes without Region of Y homologs X-Y crossovers (n ≈ 800) not shown during meiosis

Alexander K. Godfrey et al. Genome Res. 2020;30:860-873



Part | Variability of Y linked gene expression across tissues





Part | Sex differences in phenotype: sex chromosome content





-> « epigenetic effect » of the sex chromosomes

- the inactive X as a « sink » for chromatin factors

- factors that can influence the dosage of autosomal genes

-> differences in dosage

- XCI escapees
 - parental imprints
 maternal X ≠ paternal X

Y genes with no X homologtransregulation between Xs

Part I Sex differences in phenotype: sex chromosome content



-> « epigenetic effect » of the sex chromosomes

- the inactive X as a « sink » for chromatin factors

- factors that can influence the dosage of autosomal genes

Article

https://doi.org/10.1038/s41590-023-01463-8

The X-linked epigenetic regulator UTX controls NK cell-intrinsic sex differences

Received: 27 April 2022 Accepted: 14 February 2023 Published online: 36 March 2023

Mandy I. Cheng @¹², Joey H. Li¹², Luke Riggan¹²³, Bryan Chen O¹, Rana Yakhshi Tafti^{1,2}, Scott Chin¹, Feiyang Ma^{0,1,4}, Matteo Pellegrini^{1,4}, Haley Hmcir⁵, Arthur P. Arnold⁵, Timothy E. O'Sullivan @¹² & Maureen A. Su@126

Nature Immuno 2023 UTX (X linked) / no role for UTY (Y linked)



-> differences in dosage

- XCI escapees

- parental imprints maternal X \neq paternal X - Y genes with no X homolog - transregulation between Xs

Article

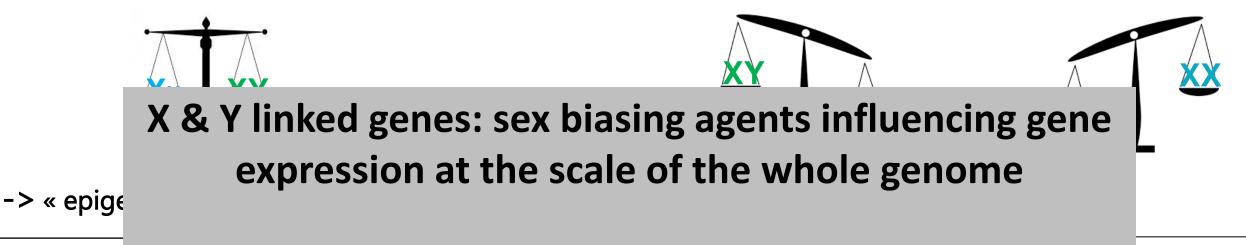
Histone demethylase KDM5D upregulation drives sex differences in colon cancer

https://doi.org/10.1038/s41586-	023-06254
Received: 18 October 2021	
Accepted: 24 May 2023	
Published online: 21 June 2023	

Jiexi Li¹, Zhengdao Lan¹, Wenting Liao^{1,2}, James W. Horner³, Xueping Xu³, Jielin Liu⁴, Yohei Yoshihama¹, Shan Jiang³, Hong Seok Shim¹, Max Slotnik¹, Kyle A. LaBella¹, Chang-Jiun Wu⁵, Kenneth Dunner Jr.¹, Wen-Hao Hsu¹, Rumi Lee¹, Isha Khanduri⁶, Christopher Terranova⁵, Kadir Akdemir^{5,7}, Deepavali Chakravarti¹, Xiaoying Shang¹, Denise J. Spring¹, Y. Alan Wang^{1,8} & Ronald A. DePinho¹

Nature 2023 Kdm5d (Ylinked)/ no role for 5c (X linked)

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 -parental imprints
 maternal X ≠ paternal X

-Y genes with no X homolog -transregulation between Xs

Article

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Nature Immuno 2023 UTX (X linked) / no role for UTY (Y linked) Nature 2023 Kdm5d (Ylinked)/ no role for 5c (X linked)

Sex As a Biological Variable in animal research

- Evidences of sex impacting biology: an overview
- Specific illustrations in cancer and immunology
- Tools: How to apply Sex As a Biological Variable?

Sex-bias in colorectal cancer

✤ iKAP (mouse) model:

KRas^{G12D} + conditional null alleles of *Apc* and *Trp53* (villin-CreERT2)

Article	
Histone demethylase KDM5D upregulatio	n
drives sex differences in colon cancer	

https://doi.org/10.1038/s41586-023-06254-7	Jiexi Li ¹ , Zhengdao Lan ¹ , Wenting Liao ¹² , James W. Horner ³ , Xueping Xu ³ , Jielin Liu ⁴ ,	
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Sex-bias in colorectal cancer

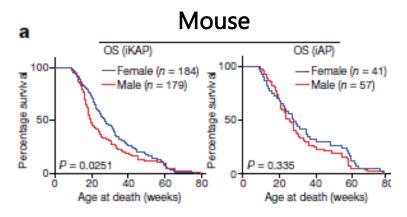
Article Histone demethylase KDM5D upregulation drives sex differences in colon cancer

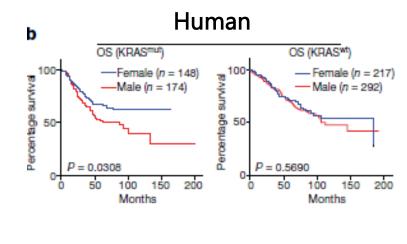


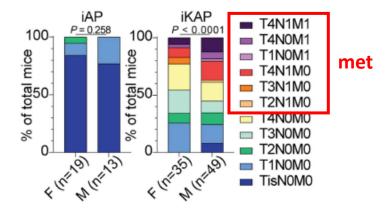
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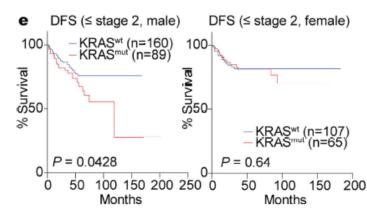
KRas^{G12D} + conditional null alleles of Apc and Trp53 (villin-CreERT2)

Sex differences tumor aggressiveness is related to KRAS*









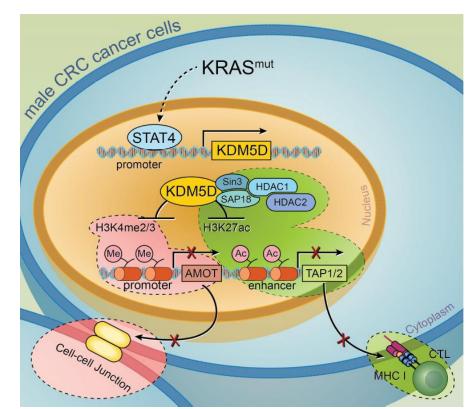


Nature 619, 632–639 (2023)

Sex-bias in colorectal cancer

> *KDM5D*: the sole Y-chromosome gene with differential expression

Primary vs metastatic iKAP tumours from males + KRAS* on vs off



> \uparrow Dissemination and immune escape

Nature 619, 632–639 (2023)

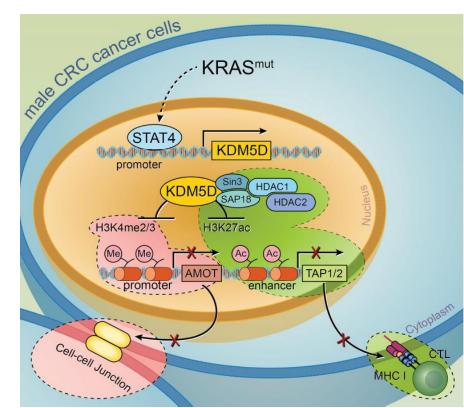




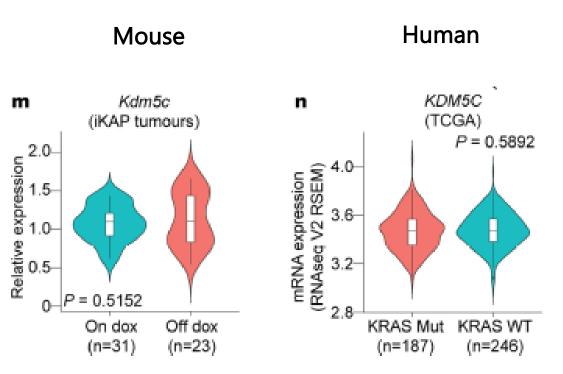
Sex-bias in colorectal cancer

> *KDM5D*. the sole Y-chromosome gene with differential expression

Primary vs metastatic iKAP tumours from males + KRAS* on vs off



KDM5C: the X-chromosome paralogue is not regulated



↑ Dissemination and immune escape

.

Nature 619, 632–639 (2023)

Sex-bias in bladder cancer

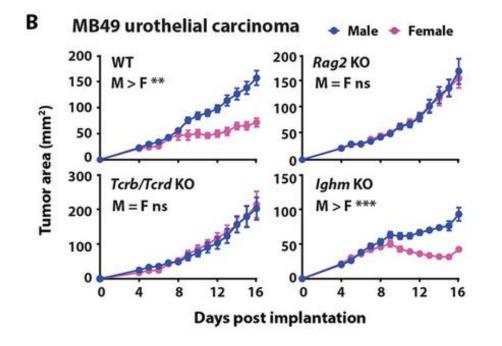


SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

CANCER IMMUNOLOGY

Androgen conspires with the CD8⁺ T cell exhaustion program and contributes to sex bias in cancer

MB49 cells: *in vitro* carcinogenesis of male mouse urothelial cells, with loss of Y



Lower tumor growth in females than in males

- Sexual dimorphism driven by:
 - endogenous antitumor T cell immunity (exhaustion)
 - T cell–intrinsic AR signaling

Sex-bias in [bladder] cancer



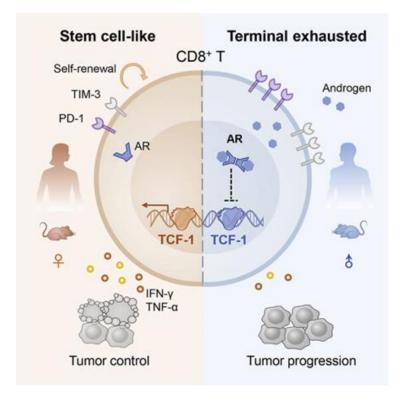
Immunity



Volume 55, Issue 7, 12 July 2022, Pages 1268-1283.e9

Article

Androgen receptor-mediated CD8⁺ T cell stemness programs drive sex differences in antitumor immunity



Immunity, Vol 55, Issue 7, 12 1268-1283.e9, 2022 Nature, 2022 Jun;606(7915):791-796. Clin & Trans Imm, Vol 11, Issue: 8, 2022

Article Androgen receptor activity in T cells limits checkpoint blockade efficacy

https://doi.org/101038/s41586-022-04522-6
Xiangnan Guan^{13 X31}, Fanny Polesso¹³², Chaoje Wang^{18, 21}, Archana Sehrawat³,
Received: 12 August 2020
Received: 12 August 2020
Received: 4 February 2022
Unlikedno dnine 23 March 2022
Unlikedno dnine 23 March 2022

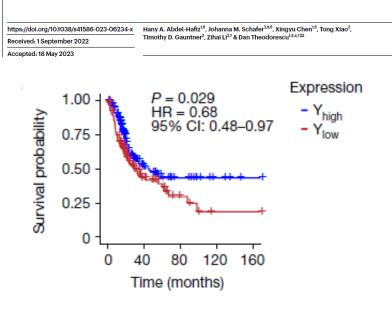
- High expression of AR in tumour infiltrating CD8+T
- AR deficiency (KO mice) increased the expansion, proliferation potential and anti-tumour functions of CD8⁺ T cells and led to the expansion of stem-like TPEX cells
- Human CRC and skin cutaneous melanoma: Positive correlation between AR signalling genes and expression of exhaustion markers of CD8⁺TIL ; lower frequencies of T cells in males



Part II But... Y chromosome could also be linked to antitumor role

Article

Y chromosome loss in cancer drives growth by evasion of adaptive immunity

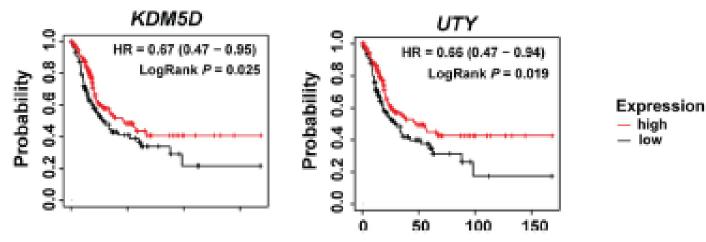


Patient data + vitro/vivo mouse data with MB49 cell sublines Y chromosome RNA expression signature score 300 men with locally advanced muscle-invasive bladder Cancer (TCGA)

- LOY (Loss of Chromosome Y) associated with a worse patient outcome
- Involvement of KDM5D and UTY

high

low





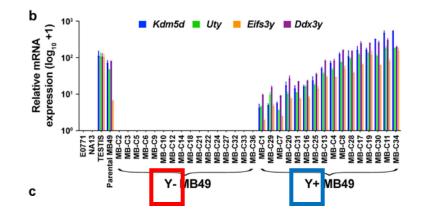
Part II But... Y chromosome could also be linked to <u>antitumor</u> role

Article

Y chromosome loss in cancer drives growth by evasion of adaptive immunity

https://doi.org/10.1038/s41586-023-06234-x	Hany A. Abdel-Hafiz ^{1,6} , Johanna M. Schafer ^{2,5,6} , Xingyu Chen ^{1,6} , Tong Xiao ² ,	
Received: 1 September 2022	Timothy D. Gauntner², Zihai Li²² & Dan Theodorescu ^{13,4,7}	
Accepted: 18 May 2023		

Patient data + vitro/vivo mouse data with MB49 cell sublines



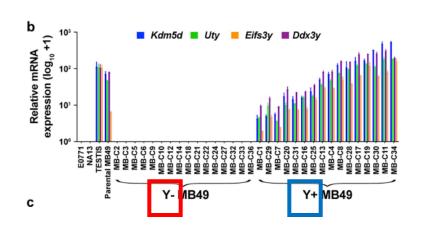


Part II But... Y chromosome could also be linked to antitumor role

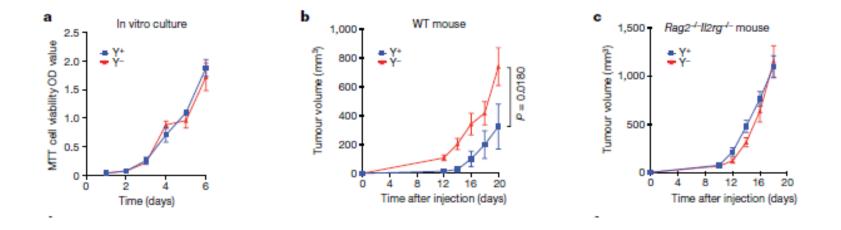
Article

Y chromosome loss in cancer drives growth by evasion of adaptive immunity





Patient data + vitro/vivo mouse data with MB49 cell sublines



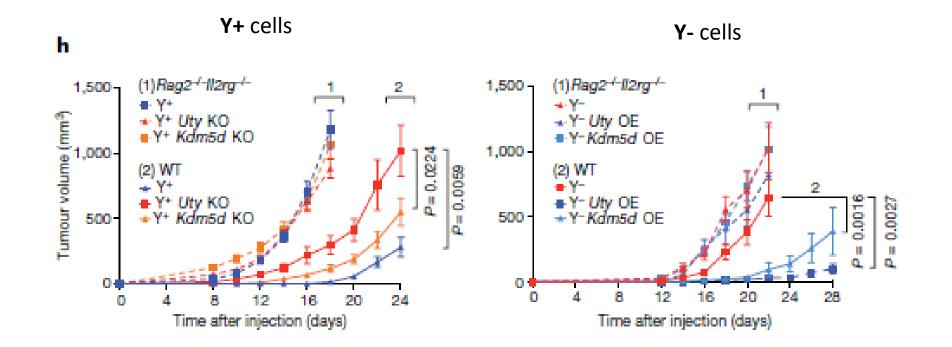
> No effect on *in vitro* growth between Y+/Y-

> Y- more aggressive in immunocompetent mice



Part II But... Y chromosome could also be linked to <u>antitumor</u> role

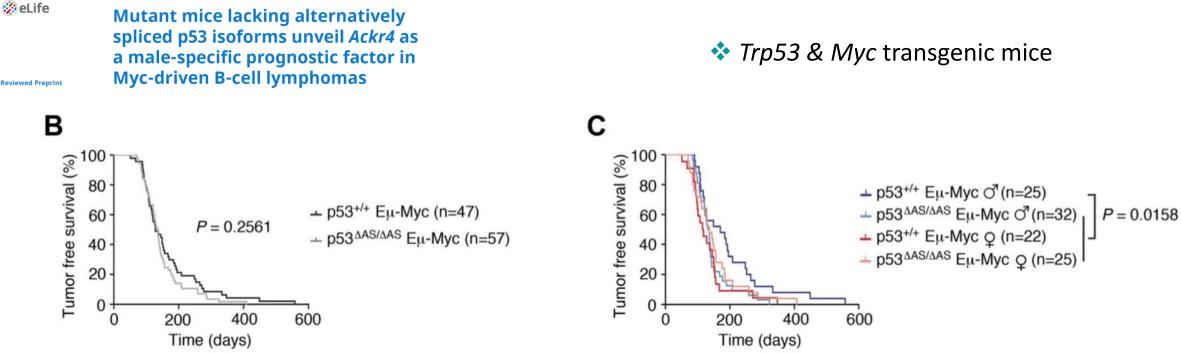
Craft in immunodeficient (RagγC) or immunocompetent mice of:



> Molecular drivers lost in Y – tumours that contribute to immune evasion: UTY, KDM5D

Sex-bias in B-lymphomas





> Similar TFS curves for $E\mu$ -Myc/Tp53wt vs μ -Myc/Tp53^{ΔAS}KO when sexes were not considered > $E\mu$ -Myc/Tp53 wt less aggressive in males



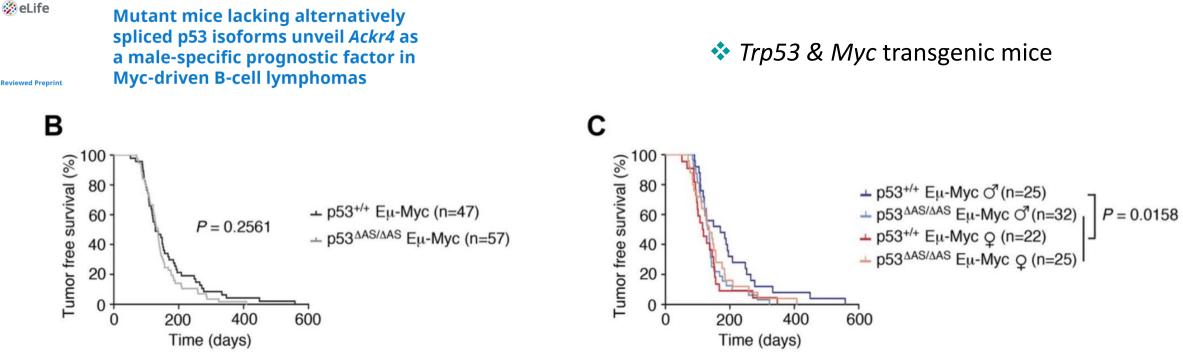
2024 eLife. https://doi.org/10.7554/eLife.92774.1

Part II

Cancer Biology

Sex-bias in B-lymphomas





> Similar TFS curves for $E\mu$ -Myc/Tp53wtvs μ -Myc/Tp53^{ΔAS}KO when sexes were not considered

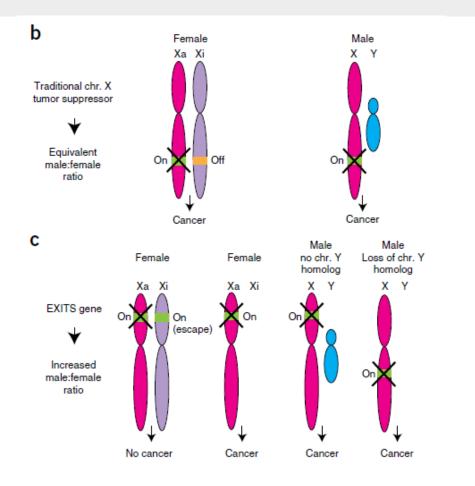
- > *Eµ-Myc/Tp53 wt* less aggressive in males
- Role of Ackr4 in tumor aggressiveness in females than in males



Part II

Cancer Biolog

Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias



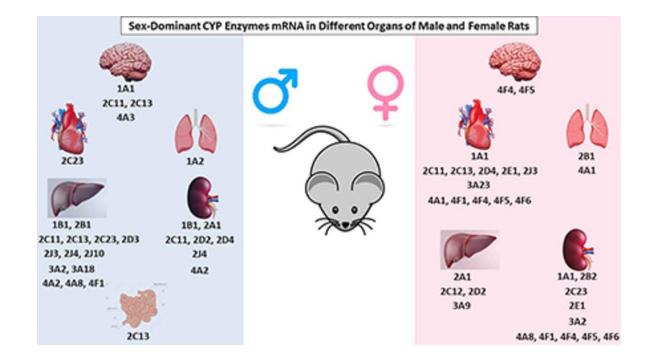
Putative X-linked tumor suppressor genes in human cancers

FOXP3	Xp11.23	Mouse, Rat, Dog
RBBP7	Xp22.2	Mouse, Rat, Dog
CD99	Xp22.32 a	and Yp11.3 Dog, (but not in Mouse, Rat)
FAM123B	Xq11.1	Mouse, Rat, Dog
EDA2R	Xq12	Mouse, Rat, but unknow in Dog
RPS6KA6	Xq21	Mouse, Rat, Dog
ATRX	Xq21.1	Mouse, Rat, Dog
ELF4	Xq26.1	Mouse, Rat, Dog
PHF6	Xq26.3	Mouse, Dog, but unknow in Rat
LDOC1	Xq27	Mouse, Rat, Dog
RPL10	Xq28	Mouse, Rat, Dog
DKC1	Xq28	Mouse, Rat, Dog

Biallelic expression of 'Escape from X-inactivation tumor-suppressor' (EXITS) genes in females: reduced cancer incidence in females vs males

That's not all:

Efficacy assays: Sexual dimorphism in the expression and/or activity levels of P450 enzymes in different organs





Genes on the X with the potential to influence immunocompetence

IGBP1

IGSFI

CD99

MTCP1

PFC

TIMP1

CD40L

Z39IG

ITGB1BP2

B Descriptions 0 according to the description

SCIENCE & SOCIETY

The X-files in immunity: sex-based differences predispose immune responses

Eleanor N. Fish

nature immunology

Article

https://doi.org/10.1038/s41590-023-01463-8

The X-linked epigenetic regulator UTX controls NK cell-intrinsic sex differences

Received: 27 April 2022

Accepted: 14 February 2023

Published online: 16 March 2023

Mandy I. Cheng ©¹², Joey H. Li¹², Luke Riggan^{12,3}, Bryan Chen ©¹, Rana Yakhshi Tafti¹², Scott Chin¹, Feiyang Ma ©^{3,4}, Matteo Pellegrini^{3,4}, Haley Hrncir⁵, Arthur P. Arnold⁵, Timothy E. O'Sullivan ©¹²⊠ & Maureen A. Su ©^{12,6}⊠

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. Voskuhl¹

¹Department of Neurology, David Geffen School of Medicine, UCLA, Los Angeles, California, USA.²Molecular Biology Institute, UCLA, Los Angeles, California, USA.³Department of Integrative Biology and Physiology, UCLA, Los Angeles, California, USA.

a Receptors & associated proteins		
AR	Androgen receptor	
AGTR2	Angiotensin receptor 2	
CSF2RA	Colony-stimulating factor 2 receptor α (granulocyte-macrophage)	
GPCR	G-protein coupled receptors 23, 50, 101, 112, 119, 174 and CX-chemokine receptor 3	
CYSLTR1	Cysteinyl leukotriene receptor 1	
IL-1RAP1	Interleukin-1 (IL-1) receptor accessory protein-like 1	
IL-1RAP2	IL-1 receptor accessory protein-like 2	
IL-2RG	IL-2 receptor γ-chain	
IL-3RA	IL-3 receptor α-chain	
IL-9R	IL-9 receptor	
IL-13RA1	IL-13 receptor α1-chain	
IL-13RA2	IL-13 receptor α2-chain	
IRAK	IL-1 receptor-associated kinase	
NGFRAP1	Nerve-growth-factor receptor associated protein 1	
TLR7	Toll-like receptor 7	
TLR8	Toll-like receptor 8	
o Immune-re	esponse related proteins	
XSCID	X-linked severe combined immunodeficiency	
ELK1	Involved in B-cell development	
EPAG	Early lymphoid activation protein	
GATA1	GATA-binding protein 1	
GTD	Gonadotropin deficiency	
IDDMX	X-linked susceptibility to insulin-dependent diabetes	
LCDD1	CD704 receive a debute breaks a sector 1	

CD79A, immunoglobulin binding protein 1

Also known as MIC2; associated with T-cell function

Immunoglobulin superfamily member 1

Tissue inhibitor of metalloproteinase 1

An immunoglobulin superfamily protein

Integrin-β,-binding protein 2

Mature T-cell proliferation 1 Properdin P factor, complement

CD40 ligand

c Transcriptional & translational control effectors

e manscriptiona	a translational control effectors
RHOGAP	RAS homologue (RHO) GTPase activating proteins 4, 6
CDC42GEF	Cell-division cycle 42 guanine-nucleotide-exchange factors 6, 9
ETK	Also known as BMX
BTK	Bruton agammaglobulinaemia tyrosine kinase
CDX4	Caudal homeobox transcription factor 4
TRAP170	A co-factor for SP1 transcription factor activation
DUSP	Dual specificity phosphatases 9, 21
EEF	Eukaryotic translation elongation factors 1α13, β4
EIF	Eukaryotic translation initiation factor 1A*, 2a
FOXP3	Forkhead box P3 (associated with the development and function of regulatory T cells)
GAB3	Growth-factor-receptor-bound protein 2-associated binding protein 3
HDAC	Histone deacetylases 6, 8
ΙΚΚγ	IκB kinase; also known as NEMO
MAPKKK15	Mitogen-activated protein kinase kinase kinase 15
NFKBRF	Nuclear factor-κB (NF-κB) repressing factor
NRK	NF-κB-inducing kinase-related kinase
NXF	Nuclear RNA export factors 2, 3, 4, 5
PAK3	p21 (also known as CDKN1A)-activated kinase 3
PPP	Protein phosphatases 1, 2*, 6
PRKCI	Protein kinase Ci
S6K	Ribosomal protein S6 kinase
SWI/SNF	SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin
STK9	Serine/threonine kinase 9
TAFI	TATA-box-binding protein-associated factor 1, TFIID subunit
UBEI	Ubiquitin-activating enzyme El
UBE2A	Ubiquitin-conjugating enzyme E2A
USP	Ubiquitin-specific proteases 9*, 11, 26, 27, 511
WASP	Wiskott–Aldrich syndrome protein

Sex-bias in NK number and function

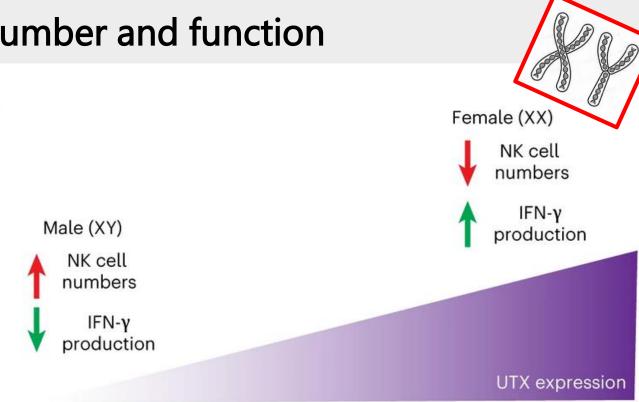


nttps://doi.org/10.1038/s41590-023-01463-8 The X-linked epigenetic regulator UTX controls NK cell-intrinsic sex differences

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- \succ Sex differences in NK cell numbers and IFN- γ production are independent of gonadal
- > X-linked UTX displays sexually dimorphic gene expression independent of sex hormones.





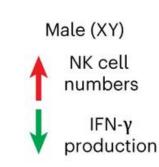
Sex-bias in NK number and function



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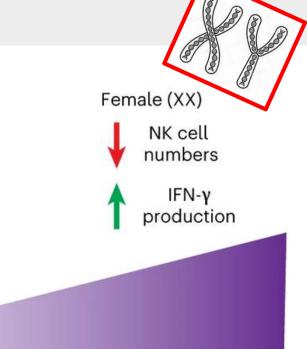
Received: 27 April 2022	
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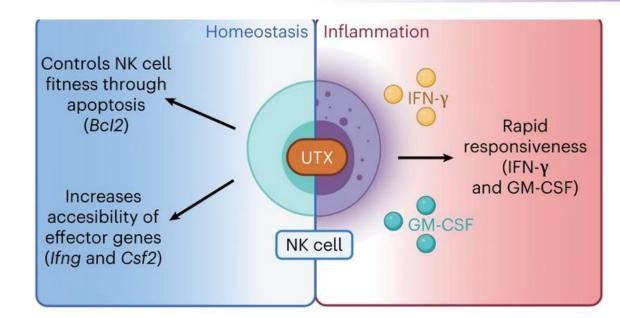


 \succ Sex differences in NK cell numbers and IFN- γ production are independent of gonadal

> X-linked UTX displays sexually dimorphic gene expression independent of sex hormones.



UTX expression





Sex As a Biological Variable in animal research

- Evidences of sex impacting biology: an overview
- Specific illustrations in cancer and immunology
- Tools: How to apply Sex As a Biological Variable?

Part III How to apply Sex As a Biological Variable?

Pubmed: systematically check 'sex' 'male and female' 'sex bias' 'X or Y chromosome' to your literature search

Pubmed your candidate gene (X and Y linked genes?)

Funding & International guidelines

Biostatistics

Courses

[Four core genotype (FCG) mouse model]

Part III How to apply Sex As a Biological Variable?

Pubmed: systematically check 'sex' 'male and female' 'sex bias' 'X or Y chromosome' to your literature search

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- Funding & International guidelines
- Biostatistics
- Courses
- [Four core genotype (FCG) mouse model]

All steps are concerned





Design studies that take sex into account, or explain why it isn't incorporated



Collect

Tabulate sex-based data



Characterize

Analvze

sex-based data



Communicate

Report and publish sex-based data

International guidelines & Funding





When can experiments be done in only one sex?

- When studying a sex-specific phenomenon, such as ovarian cancer or prostate cancer.
- To address inadequate published data for one sex in a particular area.
- Where there is statistically robust evidence that sex does not influence a trait or outcome.

In diseases where one sex predominates, such as breast cancer, both sexes may still need to be included, but researchers may choose not



European Commission

> GENDERED INNOVATIONS 2: How Inclusive Analysis Contributes to Research and Innovation

Policy Review

Research and Innovation

Nature. 2014;509(7500):282-3.

REVIEW

Heidari et al. Research Integrity and Peer Review (2016) 1:2 DOI 10.1186/s41073-016-0007-6

SAGER Guidelines

Research Integrity and Peer Review

Open Access



Sex and Gender Equity in Research: rationale for the SAGER guidelines and recommended use

Shirin Heidari¹, Thomas F. Babor^{2*}, Paola De Castro³, Sera Tort⁴ and Mirjam Curno⁵

nature > editorials > article

EDITORIAL | 18 May 2022

Nature journals raise the bar on sex and gender reporting in research

Authors will be prompted to provide details on how sex and gender were considered in study design.

Instructions for authors

- ✓ Cell
- ✓ Nature
- ✓ Springer

Deringer

Subjects Services
About Us

Sex and Gender in Research (SAGER Guidelines)

We encourage our authors to follow the <u>'Sex and Gender Equity in Research – SAGER –</u> <u>guidelines'</u> and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/ or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full <u>guidelines</u> before submission.

SAGER Guidelines: Checklist

			-
General			
	1	The terms sex/gender used appropriately	
Title			
	2a	Title specifies the sex of animals or any cells, tissues, and other material derived from these	T L I r
	2b	In applied sciences (technology, engineering, etc.), the title indicates if the study model was based on one sex/gender or the application was considered for the use of one specific sex/gender	
Abstract			
	3a	Abstract specifies sex of animals or any cells, tissues, and other material derived from these	
	3b	In applied sciences (technology, engineering, etc.), the abstract indicates if the study model was based on one sex/gender or the application was considered for the use of one specific sex/gender	
Introductio	on		
	4a	If relevant, previous studies that show presence or lack of sex or gender differences or similarities are cited	
	4b	Mention of whether sex/gender might be an important variant and if differences might be expected	

Methods		
5a		In cell biological, molecular biological, or biochemical experiments, the origin and sex chromosome constitutions of cells or tissue cultures are stated. If unknown, the reasons are stated
	5b	For studies testing devices or technology, explanation of whether the product will be applied or used by all genders and if it has been tested with a user's gender in mind
	5с	If relevant, description of how sex/gender was considered in the design
	5d	For in-vivo and in-vitro studies using primary cultures of cells, or cell lines from humans or animals, or ex-vivo studies with tissues from humans or animals, the sex of the subjects or source donors

is stated (except for immortalized cell lines, which are highly transformed)

Results		
	6	For studies using animal models, present a sex breakdown of the
	0	animals*
Discussior	ı	
		If relevant, potential implications of sex/gender on the study
	7	results and analyses, including the extent to which the findings
		can be generalized to all sexes/genders in a population
Adapted from SAGER guidelines. Sex and gender equity in research: rationale for the SAGER		
guidelines and recommended use. Research Integrity and Peer Review 1, Article number: 2 (2016)		
1		

https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6. *These points extend beyond the original SAGER table.

Part III How to apply Sex As a Biological Variable?

Pubmed: systematically check 'sex' 'male and female' 'sex bias' 'X or Y chromosome' to your literature search

Pubmed your candidate gene (X and Y linked genes?)

Funding & International guidelines

Biostatistics

Courses

[Four core genotype (FCG) mouse model]

Biostatistics

	Basic	Updated
Replacement	Avoiding or replacing the use of animals in areas where they otherwise would have been used.	Accelerating the development and use of predictive and robust models and tools, based on the latest science and technologies, to address important scientific questions without the use of animals.
Reduction	Minimising the number of animals used consistent with scientific aims.	Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base.
Refinement	Minimising the pain, suffering, distress or lasting harm that research animals might experience.	Advancing research animal welfare by exploiting the latest <i>in vivo</i> technologies and by improving understanding of the impact of welfare on scientific outcomes.

- Absence of evidence regarding sex differences is not justification
- > Female variability is not sufficient justification
- Sex differences must be considered before they can be ruled out

NC 3R^s

Biostatistics: blocking experiments

Impact on animal numbers

https://eda.nc3rs.org.uk/experimental-design-animal-characteristics

- Males and females should be randomised separately to the experimental groups
- The sample size and the analysis method both depend on the purpose of the experiment:

Using sex as a blocking factor

Sex: *could influence *should not influence

- To determine the overall effect of an intervention
- Allows the variability introduced by sex to be taken into account
- Requires same number of animals as a single sex experiment

Using sex as a factor of interest

- To investigate whether the effect of the intervention depends on sex
- Requires increased number of animals compared to a single sex experiment



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Funding & International guidelines

- Biostatistics
- Courses

[Four core genotype (FCG) mouse model]

COLLÈGE **DE FRANCE** Enseignements Recherche Bibliothèa

Pr Edith Heard

Cours

Biais liés au sexe dans la susceptibilité aux maladies : causes génétiques et épigénétiques

Cours

Le lundi, de 10 hà 12 h30 — Amphithéâtre Maurice Halbwachs

6 mars 2023

Introduction : les maladies ont-elles un sexe ?

13 mars 2023

Biais liés au sexe : comment distinguer les effets dus aux chromosomes sexuels, hormones ou mode de vie?

20 mars 2023

L'impact de l'expression des gènes liés aux chromosomes X inactif et Y sur les différences entre les sexes

27 mars 2023

L'importance de la régulation du dosage des gènes sur le chromosome X dans la susceptibilité à certaines maladies



Courses



Putting science to work for the health of women

OUR WORK	RESOURCES & TRAINING	SEX & GENDER	WOMEN'S HEALTH EQUITY & INCLUSION
HOME > E-LEARNING			
E-Learning			
L Lourning			

OF

ORWH e-learning courses give users a thorough and up-to-date understanding of sex and gender influences on health and disease and NIH requirements on factoring sex as a biological variable into research designs. Users will be able to apply this knowledge when designing and conducting research or interpreting evidence for clinical practice. Offerings include Bench to Bedside: Integrating Sex and Gender to Improve Human Health (CME credits available), Sex as a Biological Variable: A Primer, Introduction: Sex- and Gender-Related Differences in Health, the SABV Primer: Train the Trainer, and Introduction to Sex and Gender: Core Concepts for Health-Related Research.

E-Learning Courses Flyer E-Learning Course Guide

The courses are open to the public, and registration is free.

Bench to Bedside: Integrating Sex and Gender to Improve Human Health

Ø Sex as a Biological Variable (SABV): A Primer

25 **SABV Primer: Train the Trainer**

Introduction to Sex and Gender: Core Concepts for Health-Related **F**+**F** EL-Ò Research

Part III How to apply Sex As a Biological Variable?

Pubmed: systematically check 'sex' 'male and female' 'sex bias' 'X or Y chromosome' to your literature search

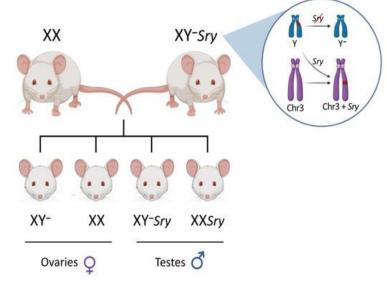
Pubmed your candidate gene (X and Y linked genes?)

Funding & International guidelines

Biostatistics

Courses

Four core genotype (FCG) mouse model : hormon vs chromosome]



Perspectives

> Of course 'sex' is not the only one variable to be taken into account:

Age, genetic background (stain and backcross), Experimental unit...(ARRIVE guidelines)

> Not only *in vivo* but **cells** too!

REVIEW

Did you forget your cell sex? An update on the inclusion of sex as a variable in *AJP-Cell Physiology*

Anthony Holland and © Neil A. Bradbury Department of Physiology and Biophysics, Chicago Medical School, North Chicago, Illinois, United States

 \succ Other topics, other species (mammals, birds, reptiles)

RESEARCH ARTICLE

IMMUNOLOGY

Sexual dimorphism in skin immunity is mediated by an androgen-ILC2-dendritic cell axis

Sex-biased gene expression across mammalian organ development and evolution

ticia rodríguez.Montes 🍈 . Svetlana ovchinnikova 🧿 . Xuefe vlar 🌍 . Tania studer. Ioannis Sarropoulos 🎒 . Simon anders 🌍 Enrik Kaessmann 💿 , and Margarida Cardoso-Moreira 🌍 . **Authors Info & Affiliations**

SCIENCE · 3 Nov 2023 · Vol 382, Issue 6670 · DOI: 10.1126/science.adf1046

nature communications

Article

https://doi.org/10.1038/s41467-024-46384-8

6

Epigenetic modulators link mitochondrial redox homeostasis to cardiac function in a sex-dependent manner

Received: 3 August 2022	Zaher ElBeck @ 1.2, Mohammad Bakhtiar Hossain @ 3, Humam Siga	
Accepted: 23 February 2024	Nikolay Oskolkov © ⁴ , Fredrik Karlsson ⁵ , Julia Lindgren © ⁶ , Anna Walentinsson © ⁷ , Dominique Koppenhöfer © ¹ , Rebecca Jarvis © ⁸	

Thank you



Athanassia Sotiropoulos

Susana Gomez