Sex as a Biological Variable in preclinical assays

Katia ANCELIN & Virginie DANGLES-MARIE

WG CEEA-IC #118

Why this presentation?

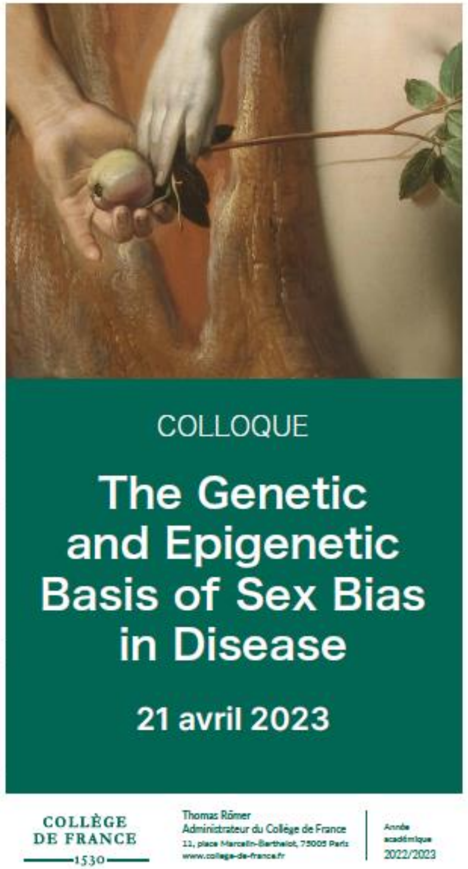
- DAP / **3.4.12. Indicate the sex of the animals used and why**

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...



21 avril 2023 de 9 h à 18 h
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

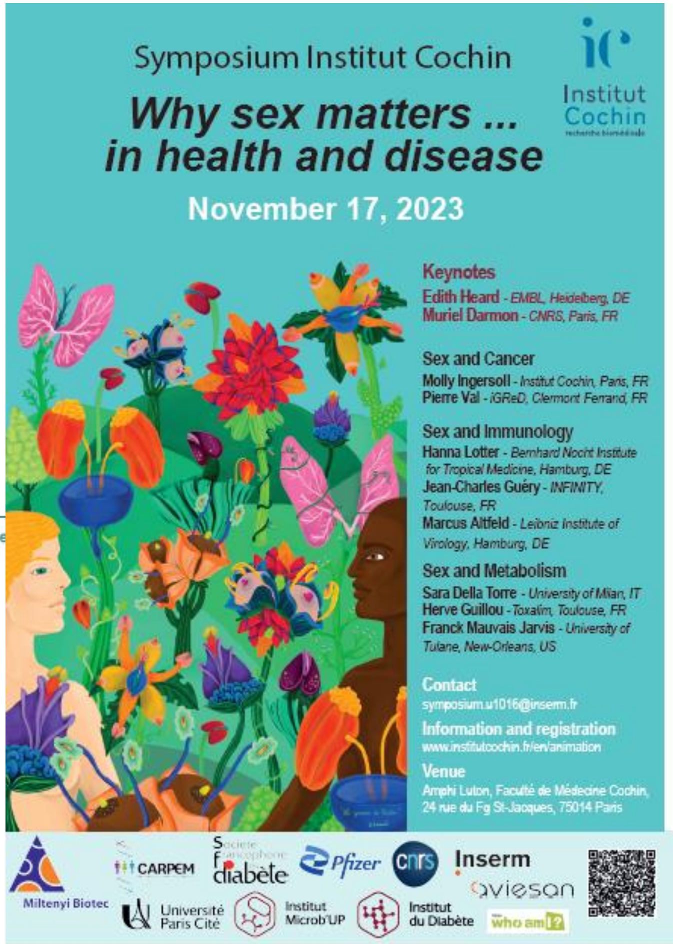
Irene Miguel-Aliaga
Imperial College, London, UK

Jessica Tollkuhn
Cold Spring Harbor Lab, New York, USA

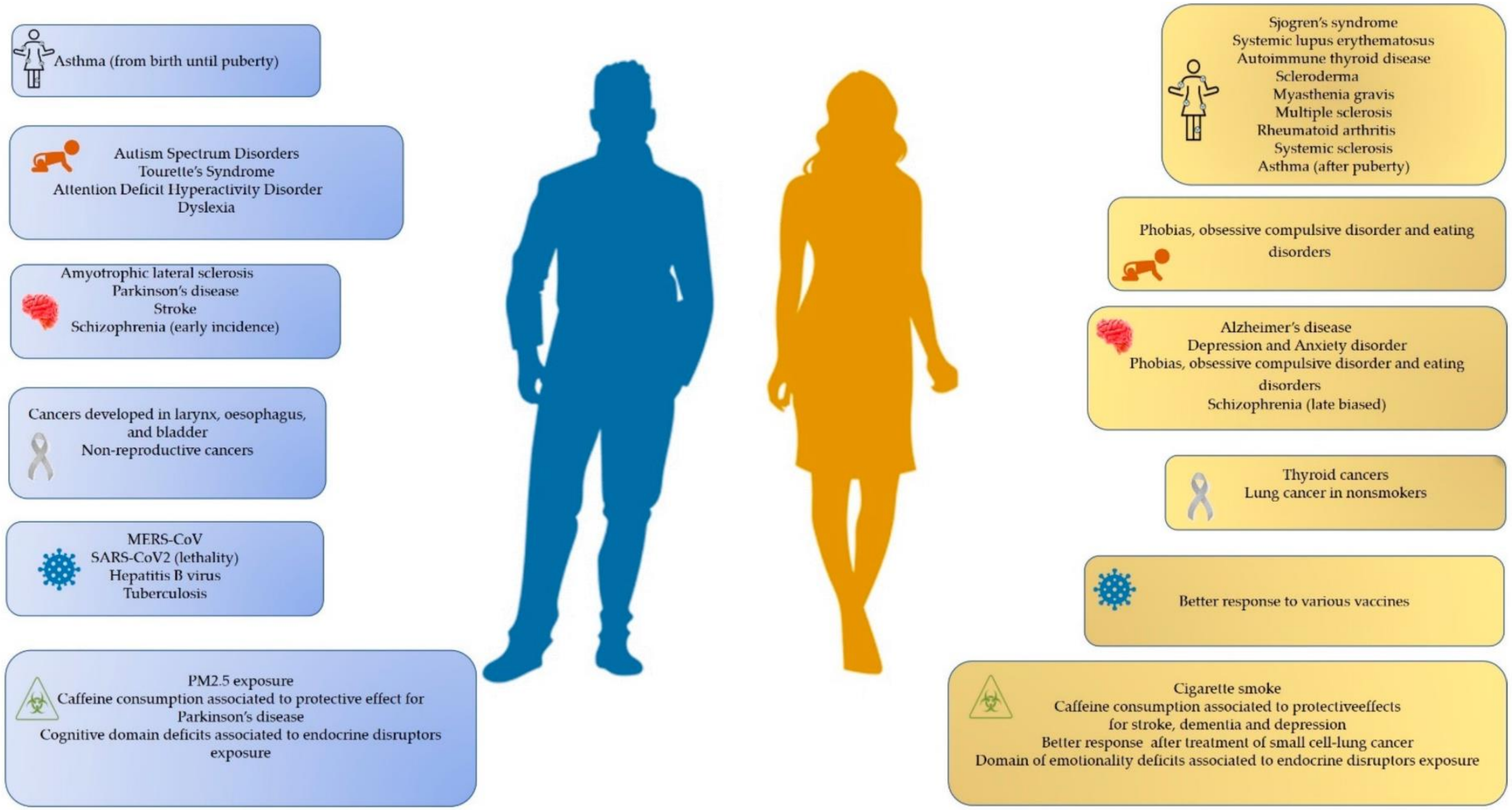
Taru Tukiainen
FIMM, Helsinki, Finland

Judith Zaugg
EMBL Heidelberg, Germany

Colloquium in English, free entry, no registration require



Sex specific differences in disease susceptibility



From Migliore et al Biomedicines 2021

Sex As a Biological Variable in animal research

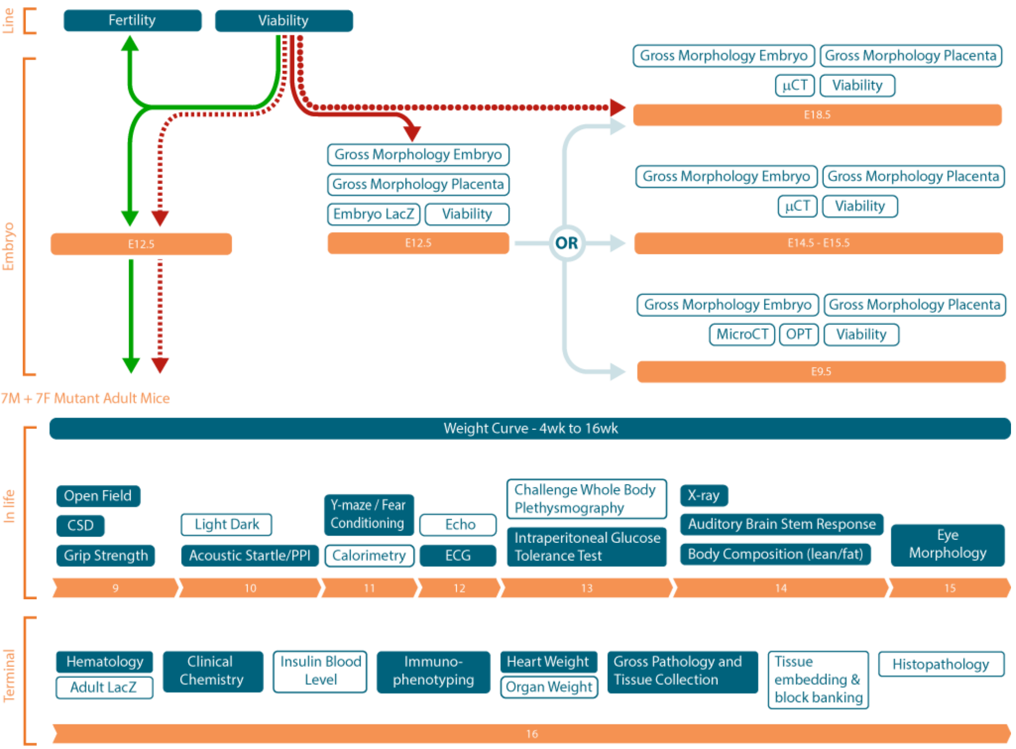
nature COMMUNICATIONS

ARTICLE

Received 27 Oct 2016 | Accepted 30 Mar 2017 | Published 26 Jun 2017 DOI: 10.1038/ncomms15475 OPEN

Prevalence of sexual dimorphism in mammalian phenotypic traits

IMPReSS pipeline www.mousephenotype.org/impress/index



- 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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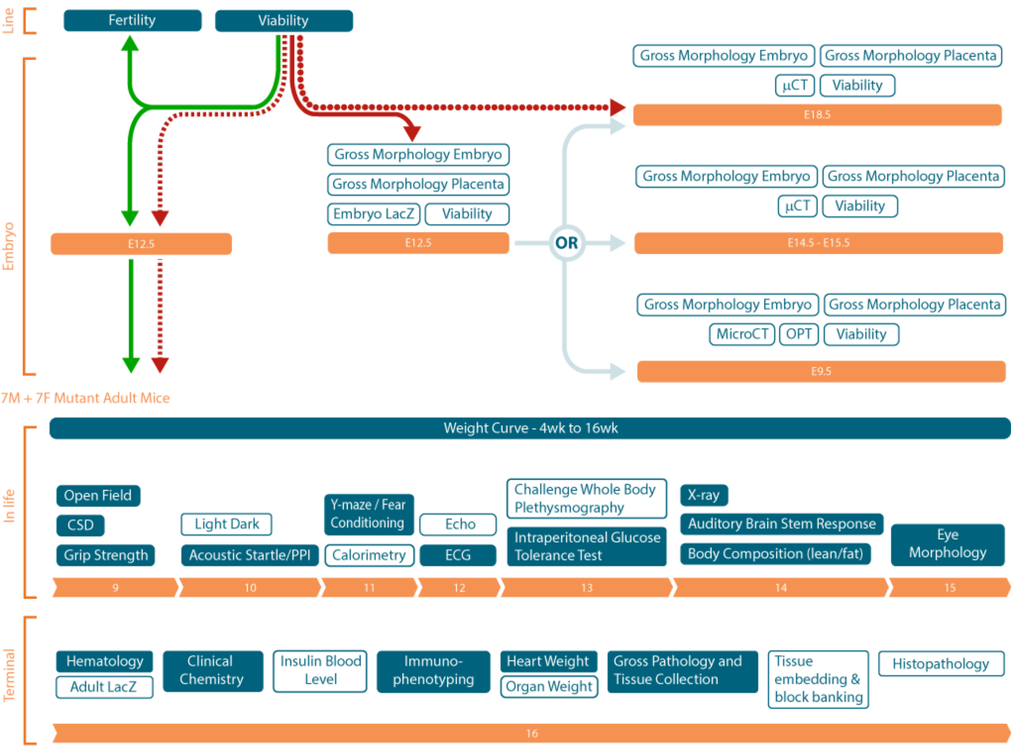

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- 14,250 wildtype animals + 40,192 mutant mice
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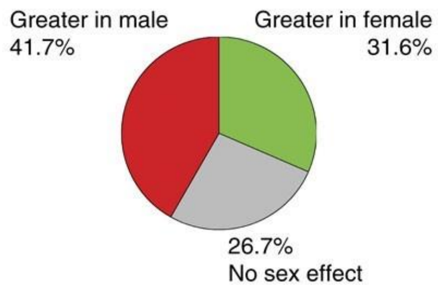
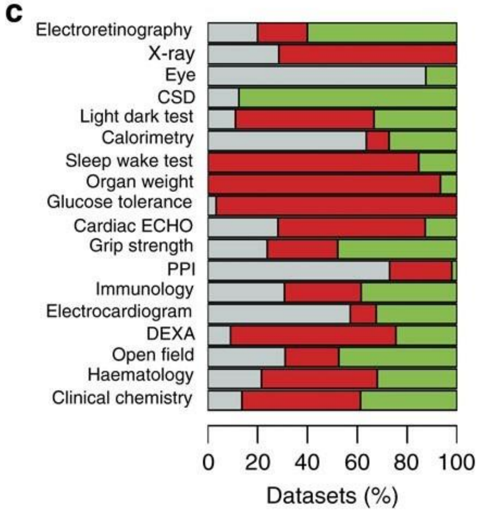
662/903 data sets



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



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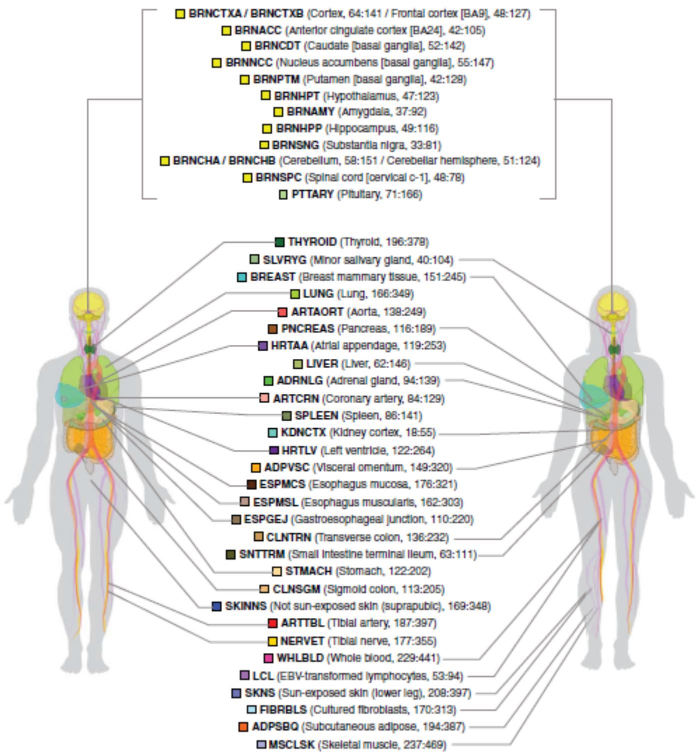
Oliva et al. *Science* 2020 **The impact of sex on gene expression across human tissues**

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



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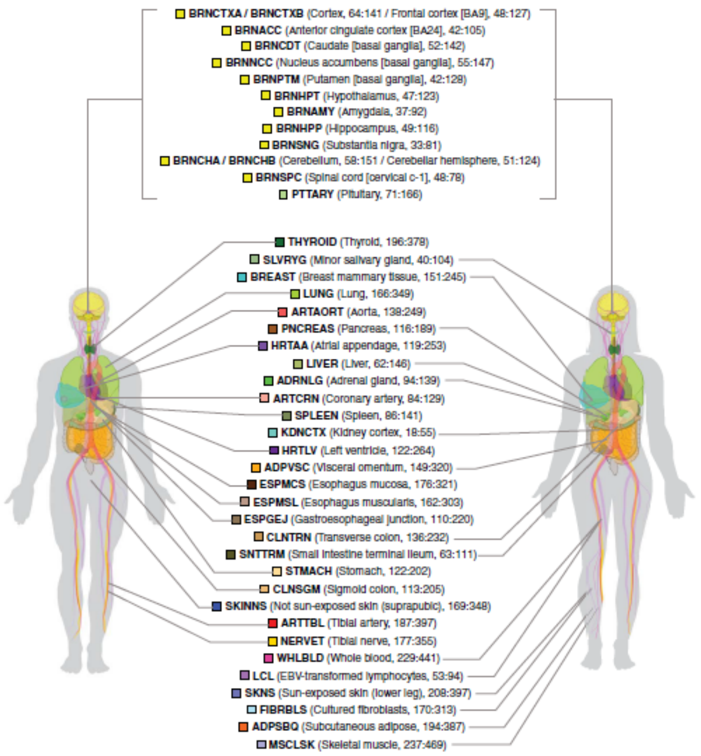

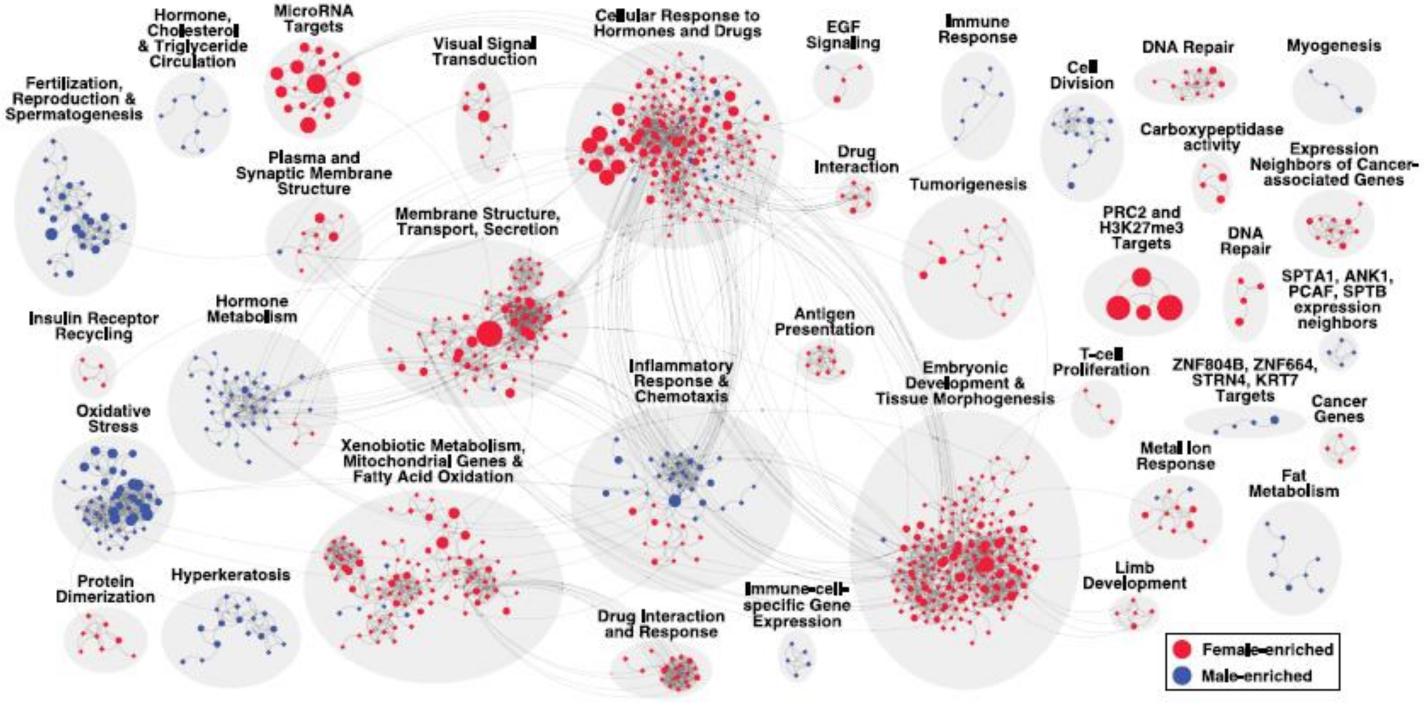

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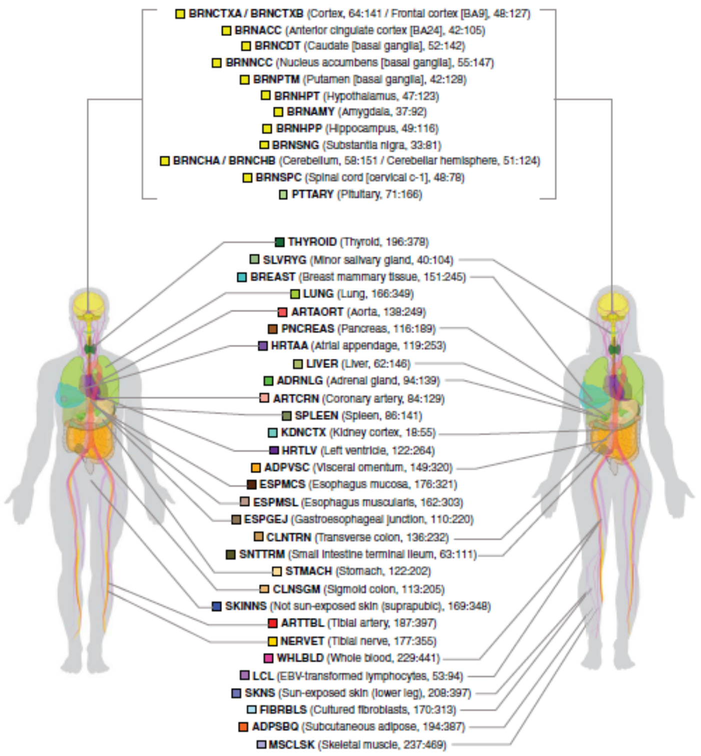
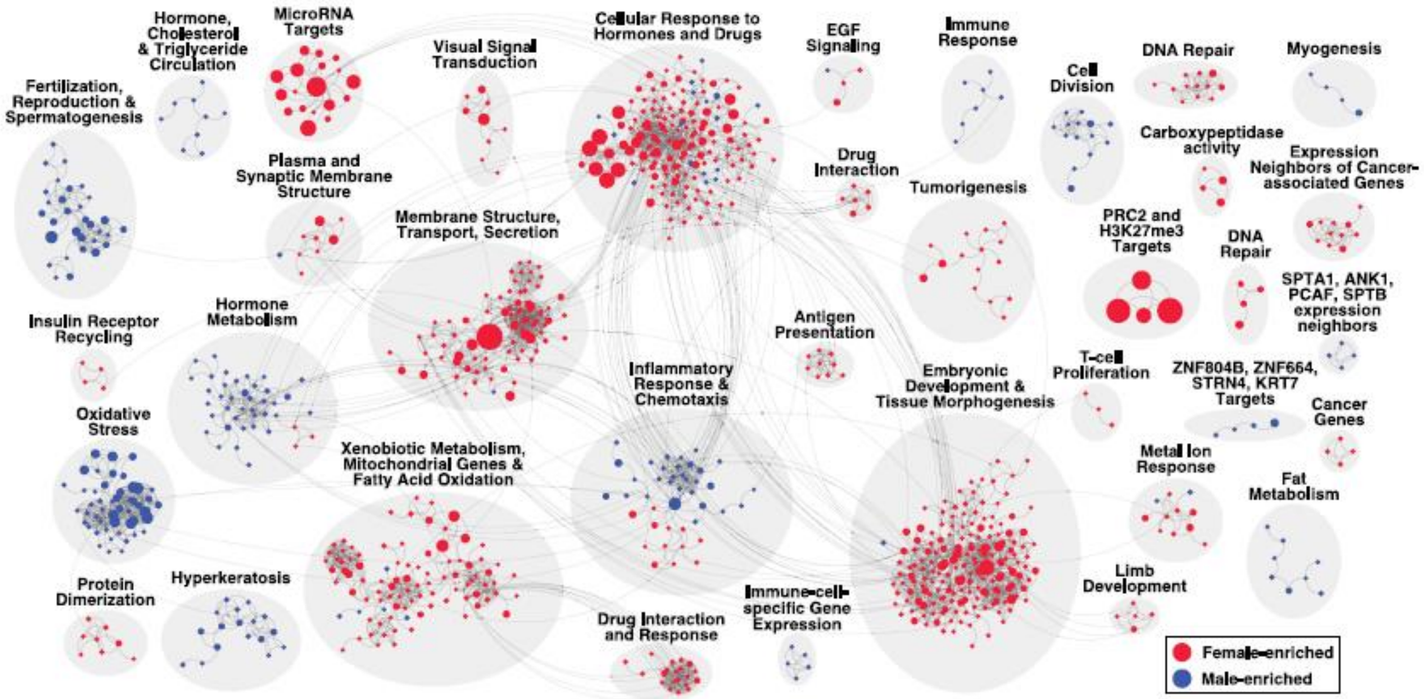
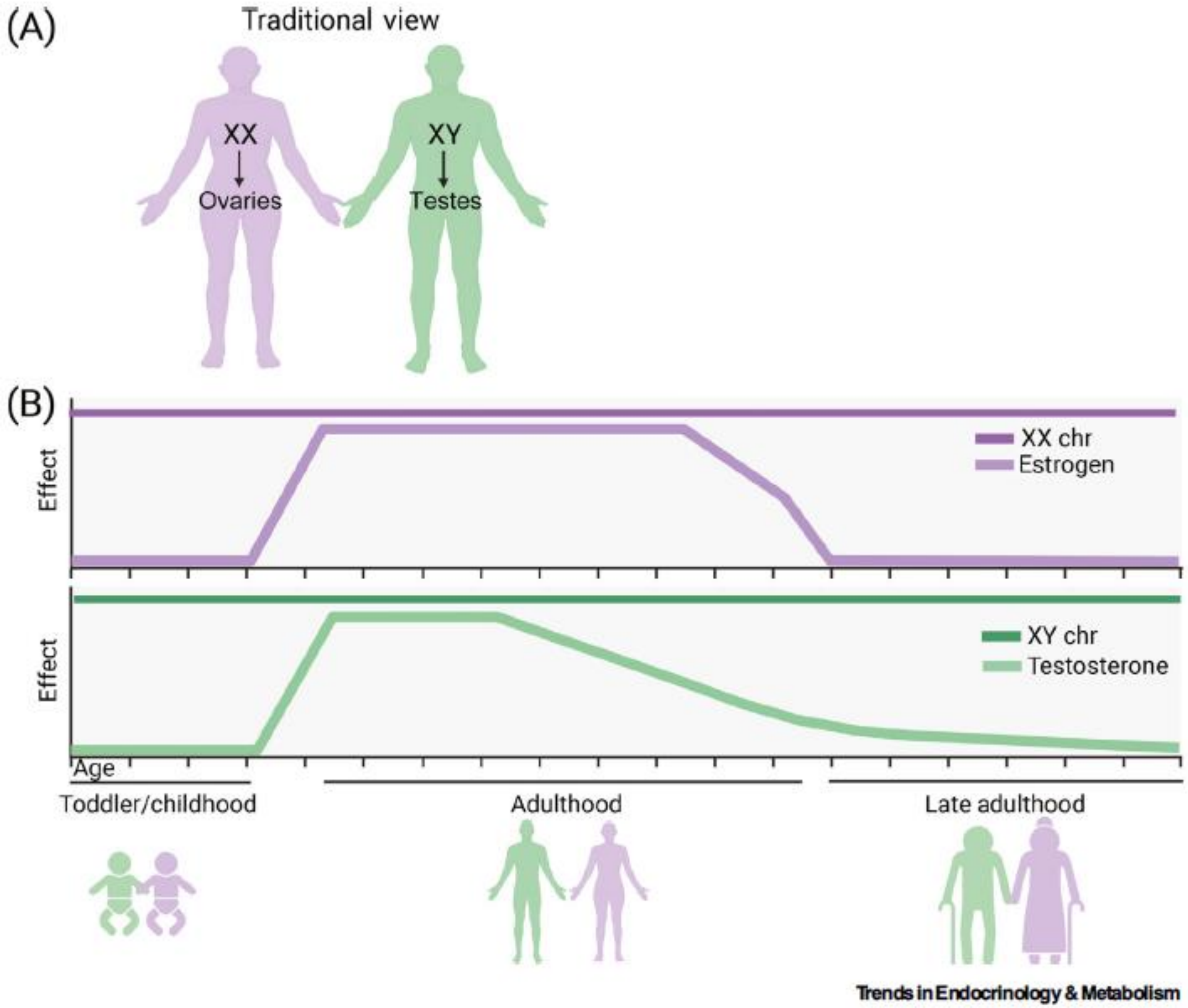




37,5% (13 294/35 431 genes; protein coding, lncRNA, & transcribed but less characterized genes)
of the human transcriptome was differently expressed in at least one tissue.

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

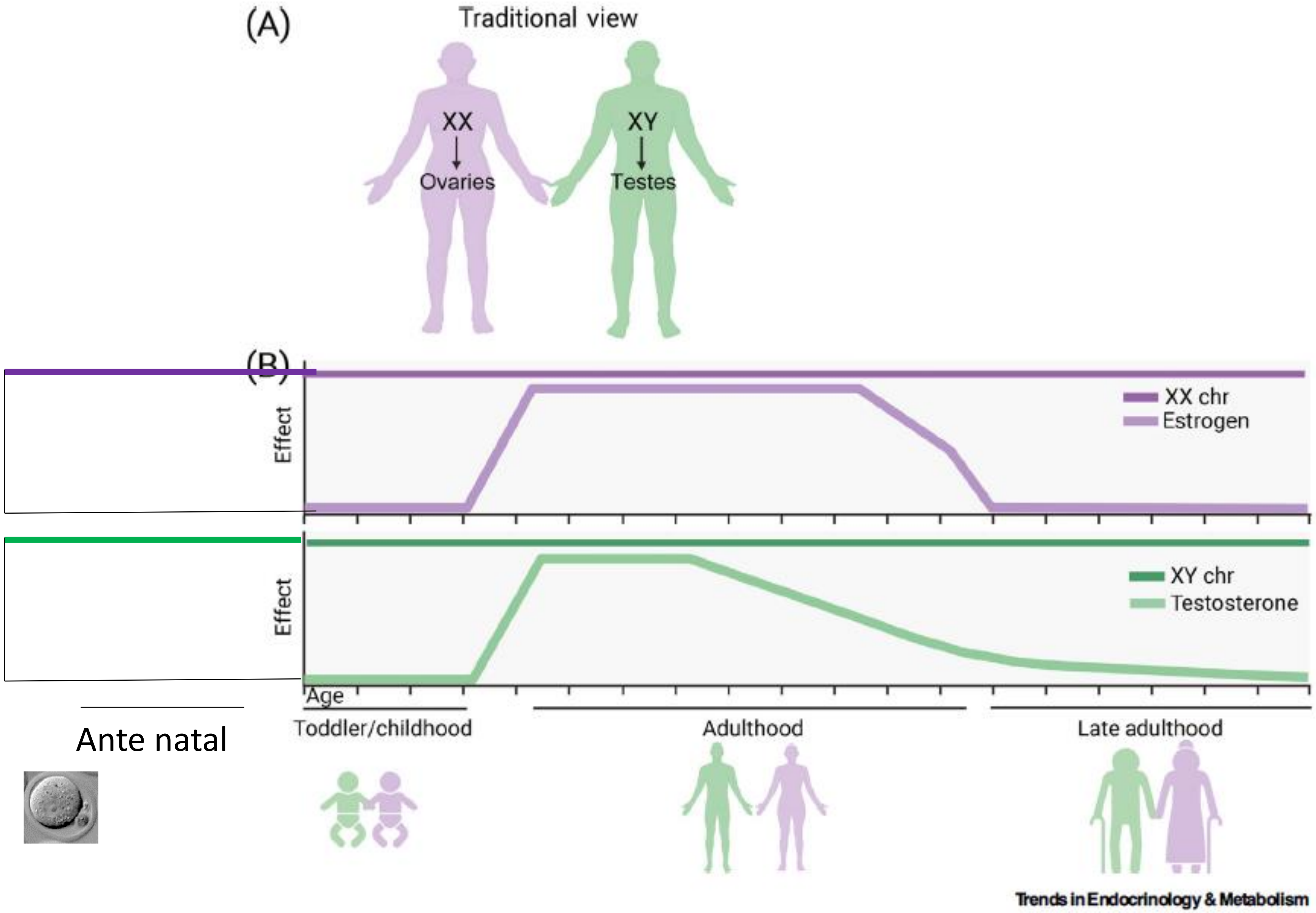
Sex differences in phenotype



- Sex chromosome content
- Circulating hormones
- Sex chromosome content
- Circulating hormones

Wiese et al 2024

Sex differences in phenotype



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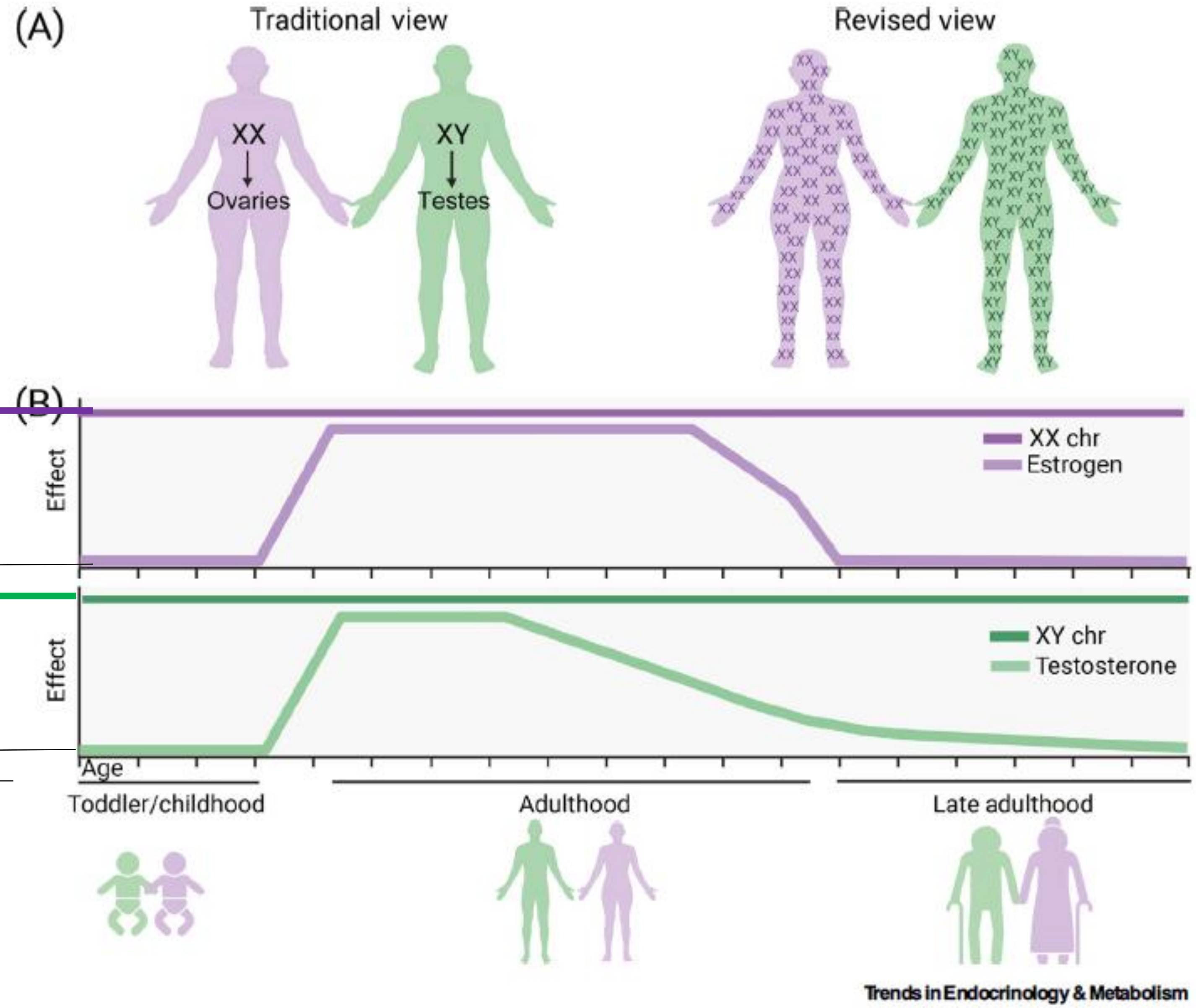

- Sex chromosome content
- Circulating hormones
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Ante natal

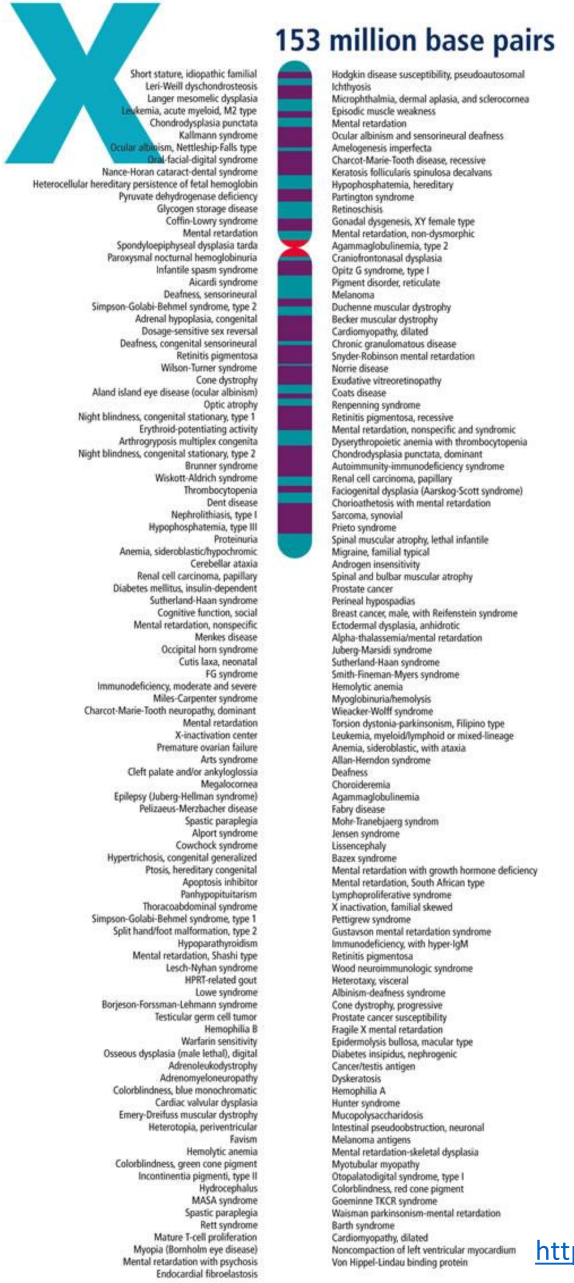
Wiese et al 2024

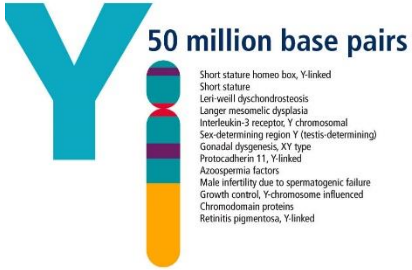
Sex As a Biological Variable in animal research

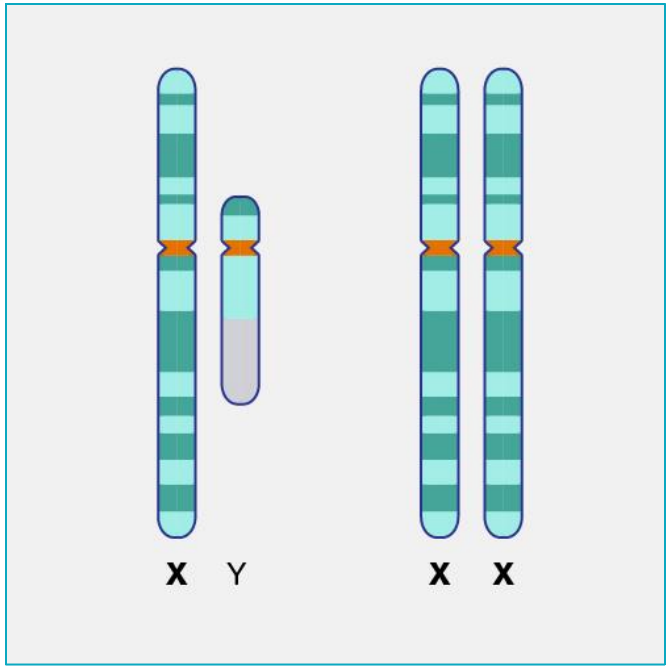
- Evidences of sex impacting biology: an overview about sex chromosomes
- Specific illustrations in cancer and immunology
- Tools: How to apply Sex As a Biological Variable?

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

Part I X & Y sex chromosomes are very different







https://public.ornl.gov/site/gallery/highres/GenomePoster2009.pdf

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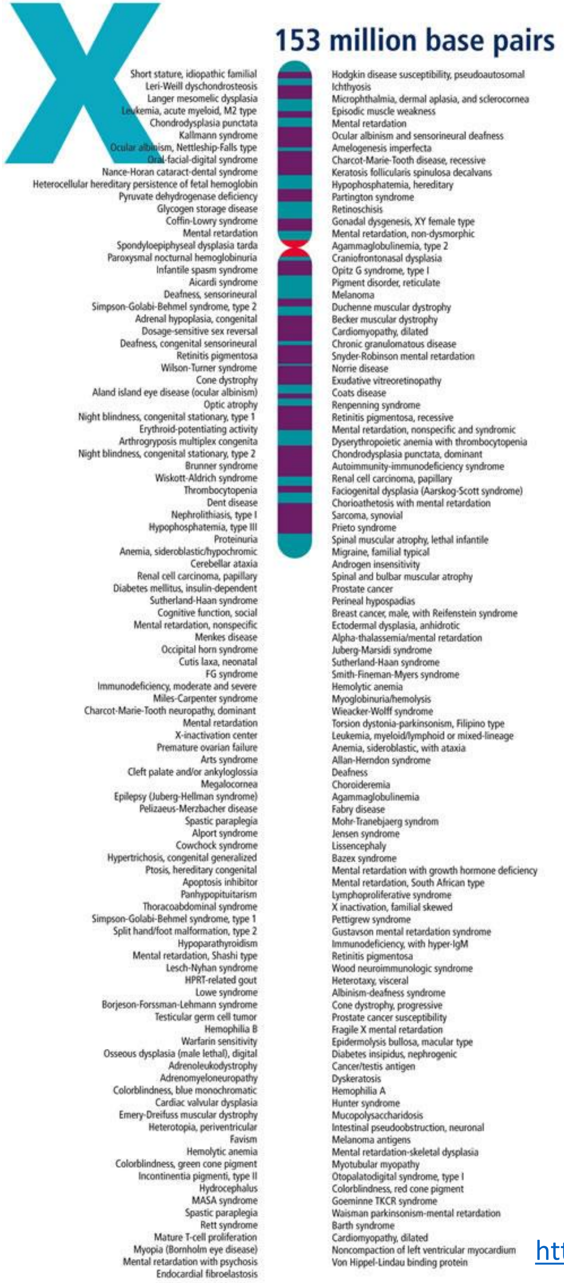

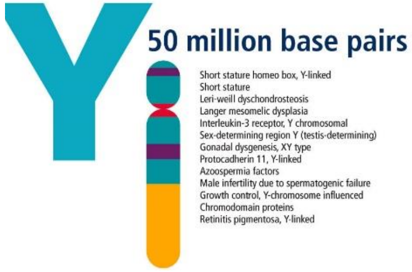

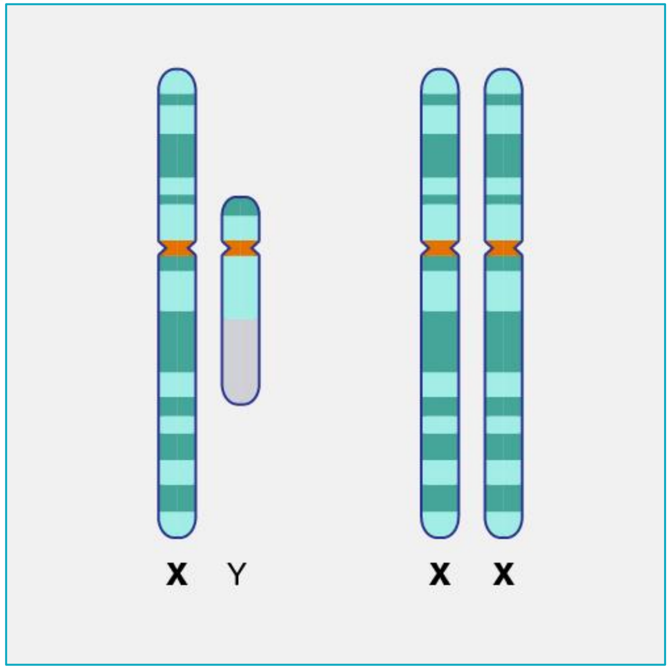

- XX individual: X chr =5 % genome ; XY individuals : X=2.6% & Y=0.97%
- X > **1000 genes** ; 884 are protein coding genes (NEXPROT)
- **Y ~ 100 genes** ; 58 protein coding genes (NEXPROT) but see the 2023 genome release

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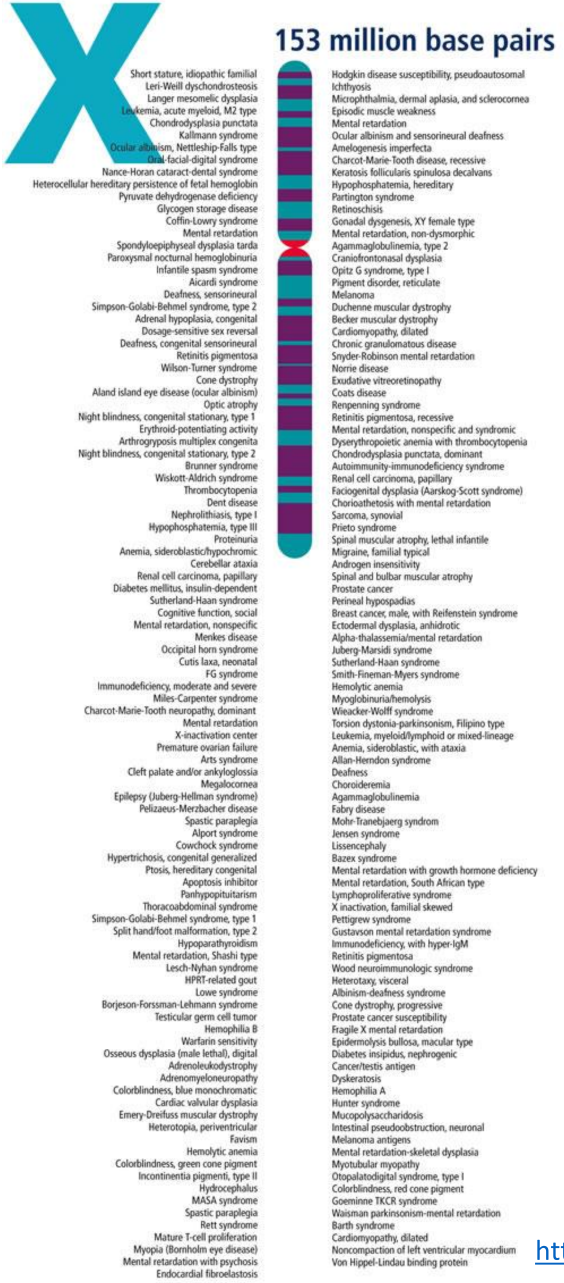

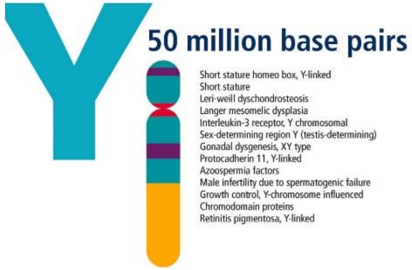

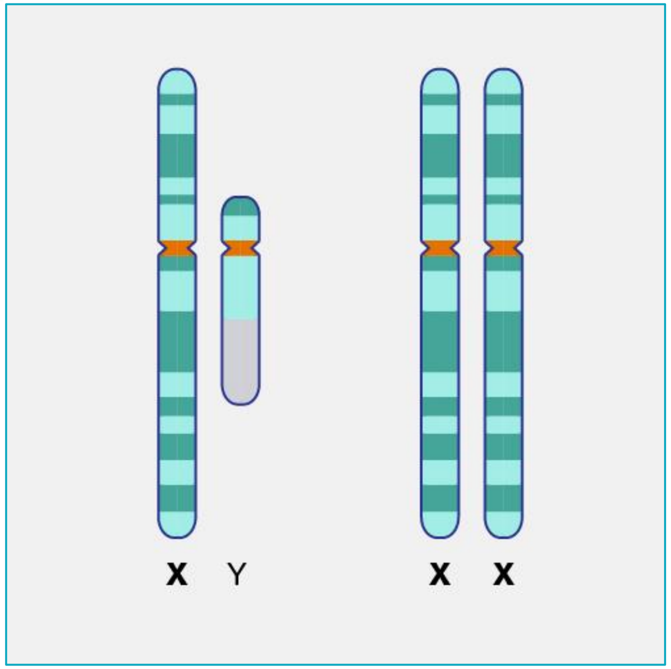

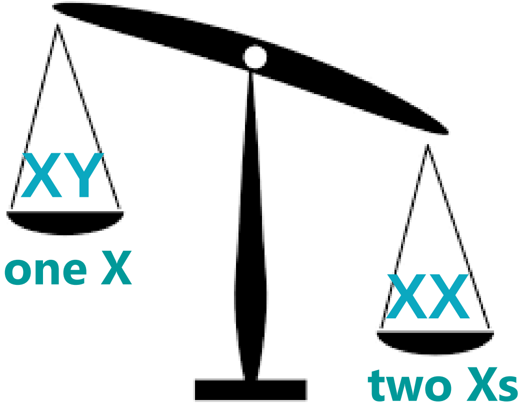

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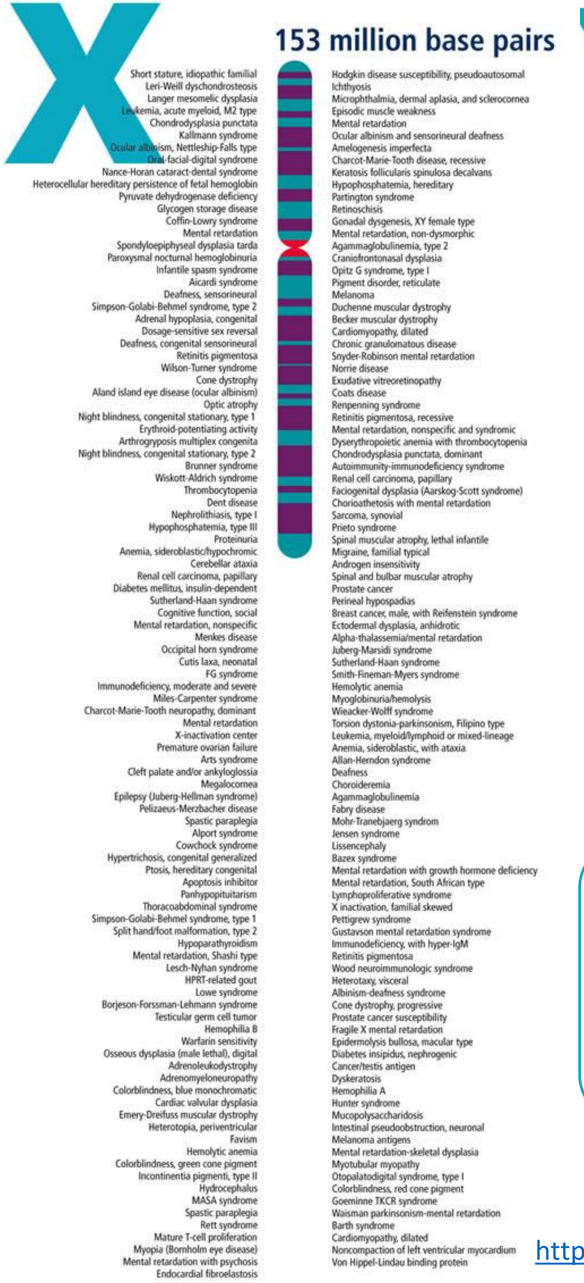

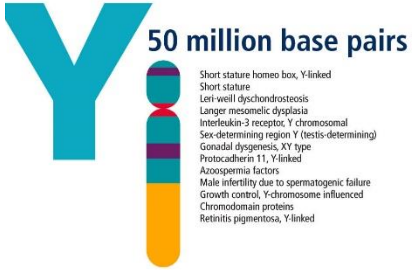

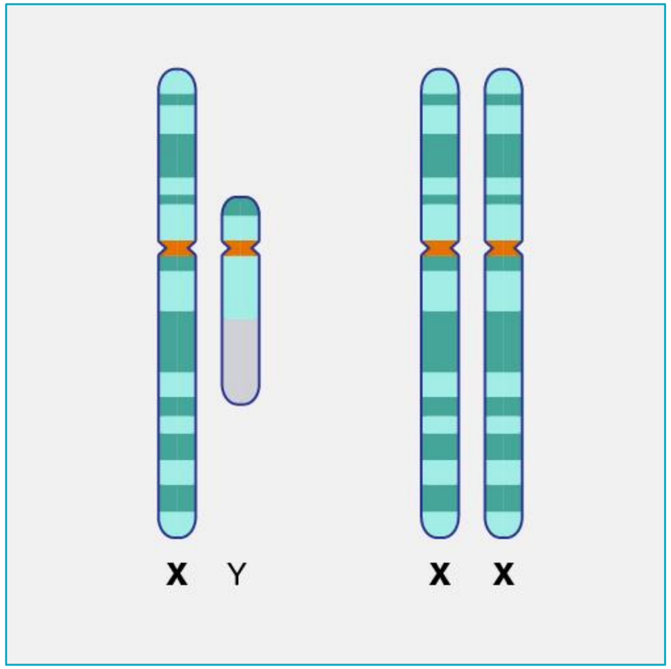

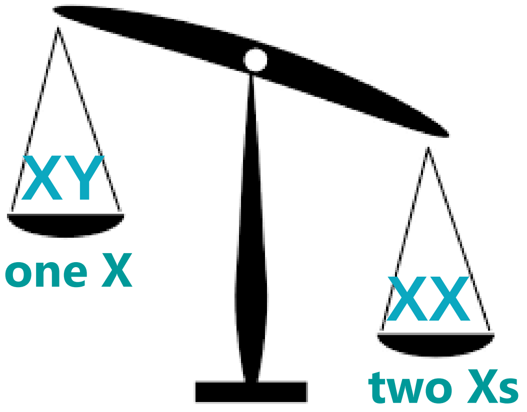

differences in sex chromosome content & expression

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Part I

Sex dosage compensation:
X chromosome inactivation in female mammals

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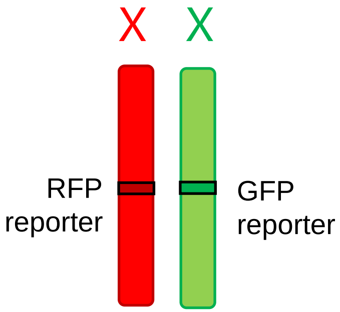
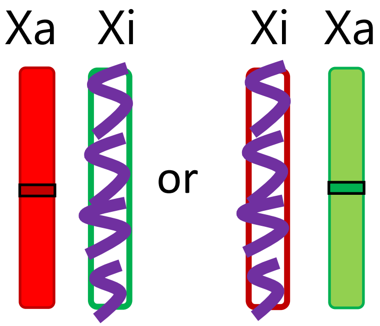


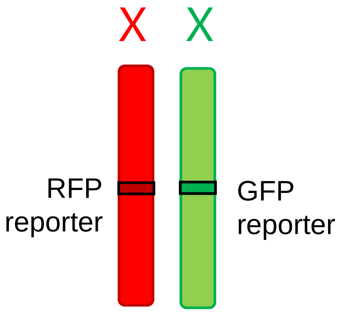
Wu et al Neuron 2014



random X inactivation

Part I

Sex dosage compensation: X chromosome inactivation in female mammals



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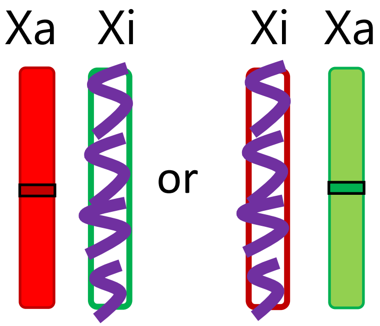

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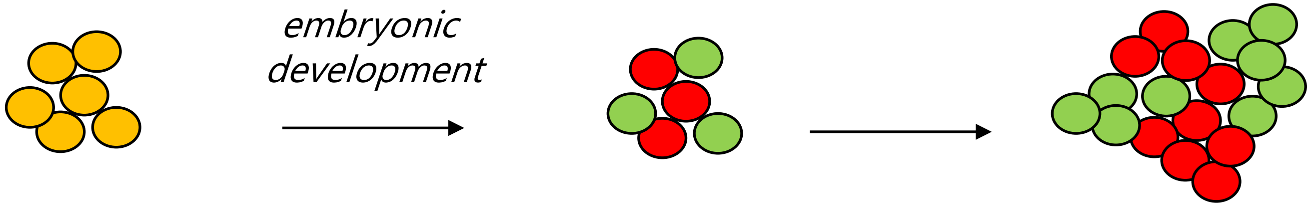
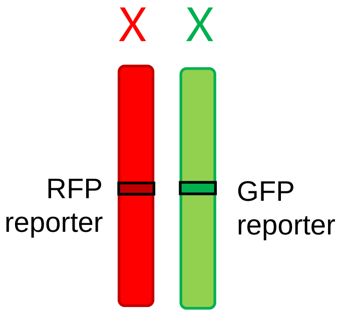


1- choice

2- clonal propagation

Part I

Sex dosage compensation: X chromosome inactivation in female mammals



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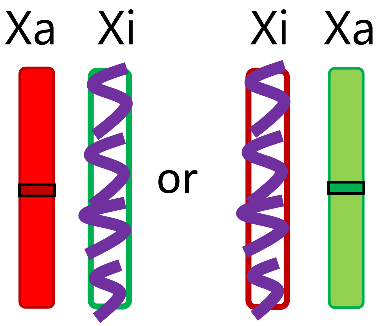

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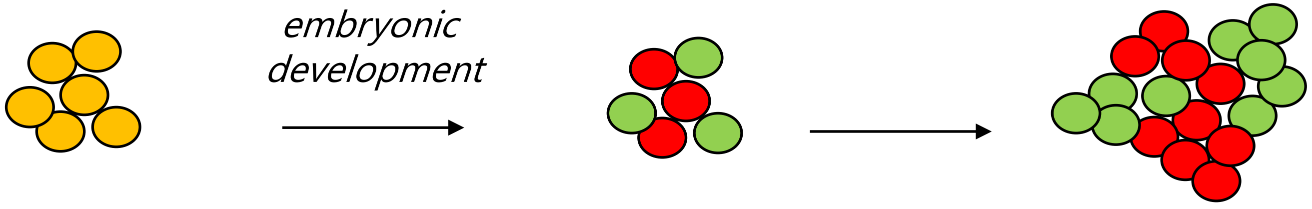
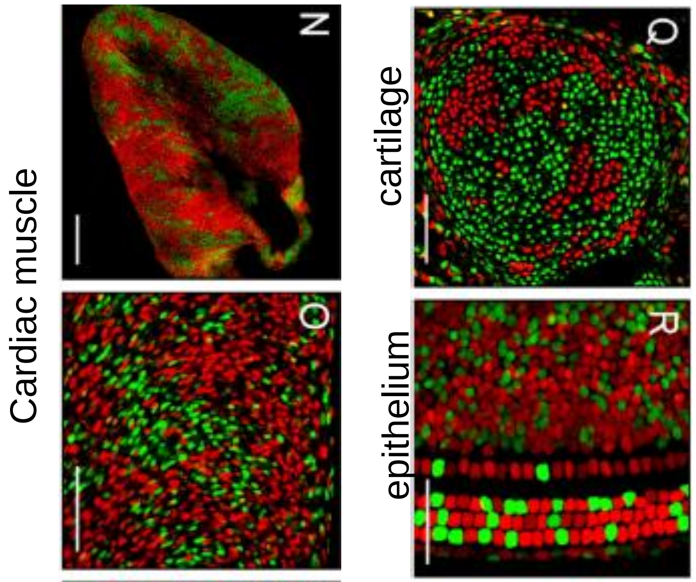
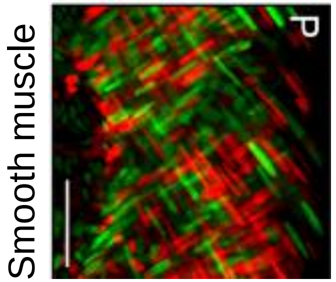


Murine Tissues from Wu et al Neuron 2014



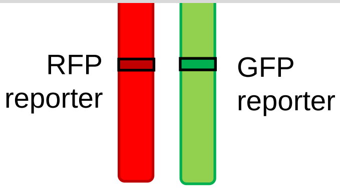


paint from Carmen Tyrell (detail)

Part I

Sex dosage compensation: X chromosome inactivation in female mammals

Female mammals are a mosaic because of XCI choice



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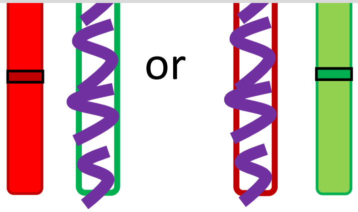
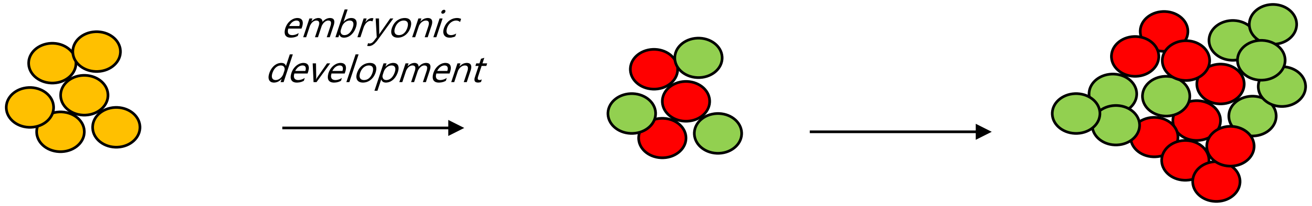


random X inactivation

embryonic development



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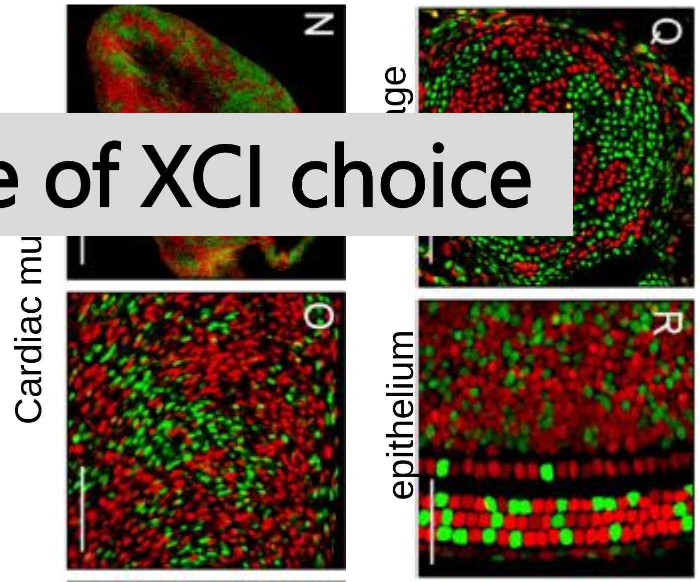

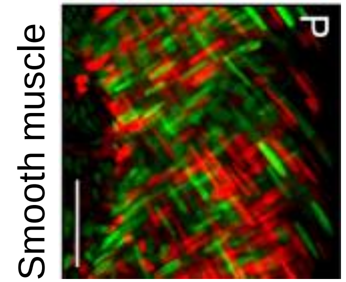

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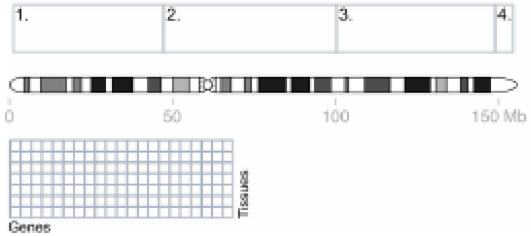
Part I Variability of X linked gene expression across tissues

Landscape of X chromosome inactivation across human tissues

Tukiainen et al Nature 2017



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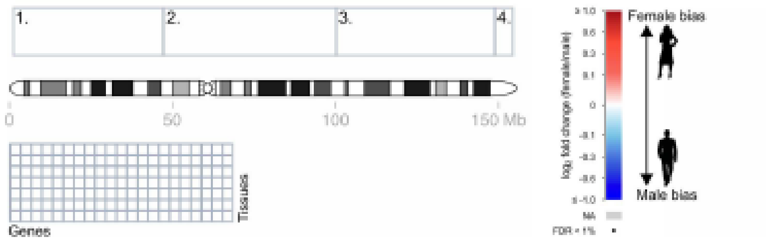

W > M

M > W

29 tissues (Genotype-Tissue Expression project public resource at gtexportal.org)

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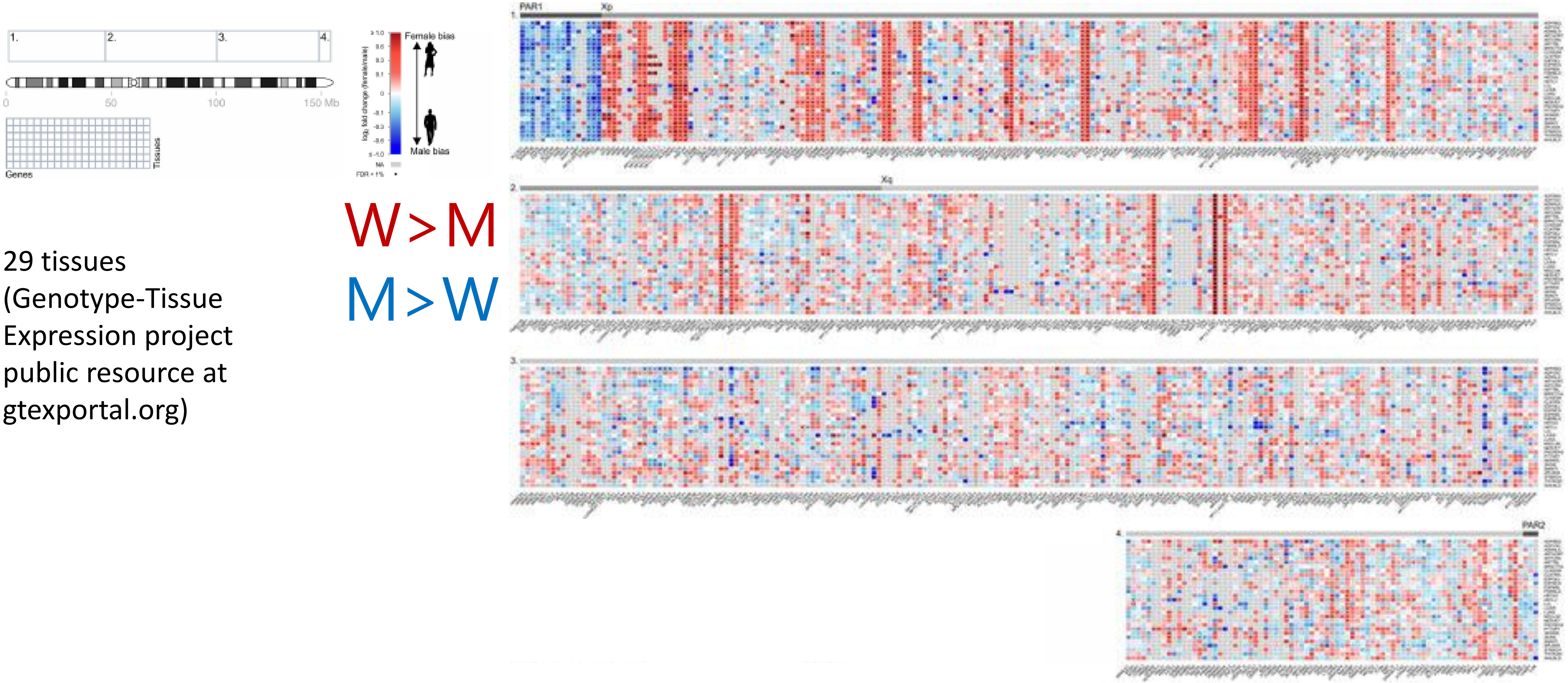

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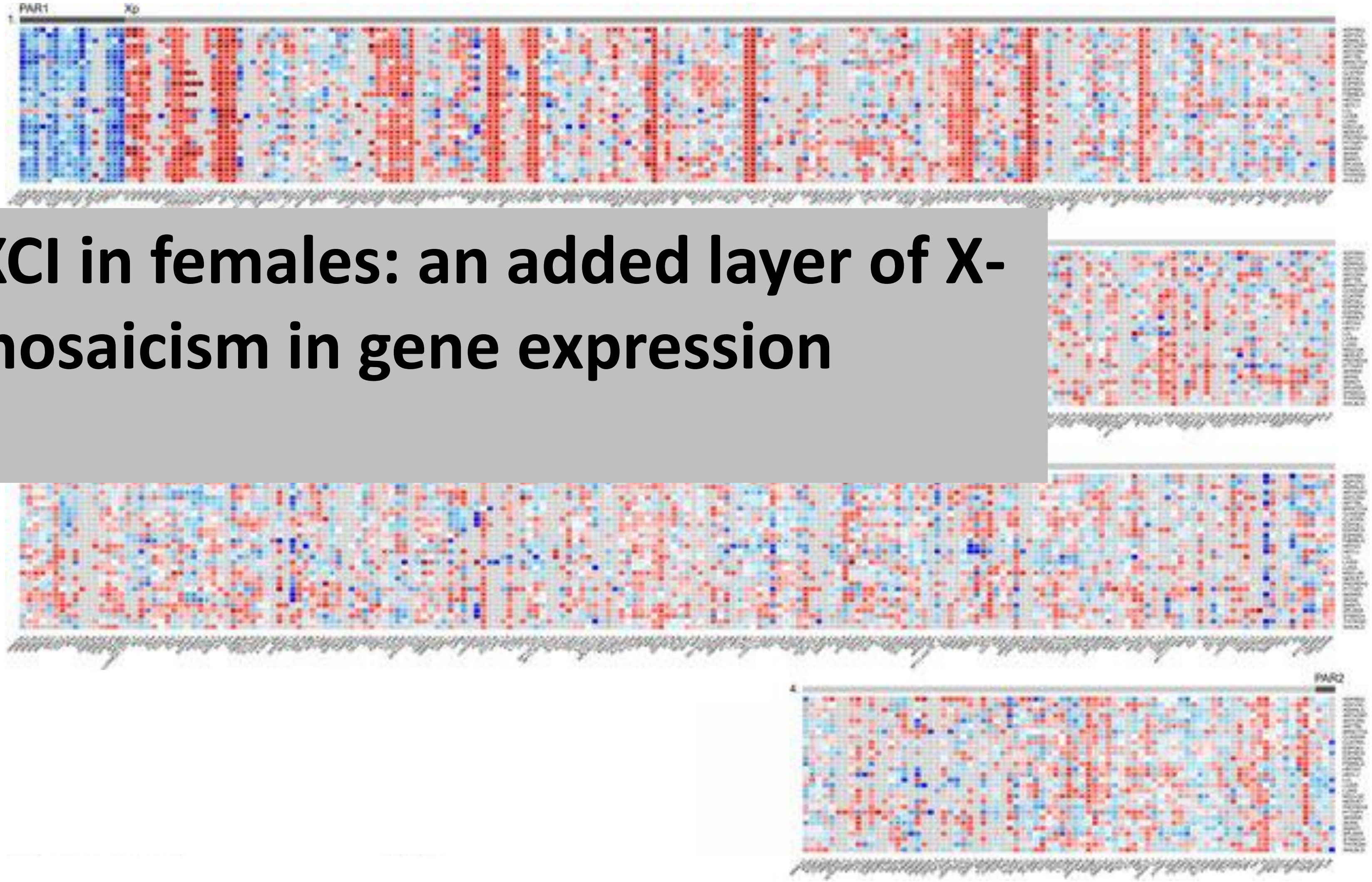

Escape from XCI in females: an added layer of X-linked mosaicism in gene expression

29 tissues
(Genotype-T
Expression p
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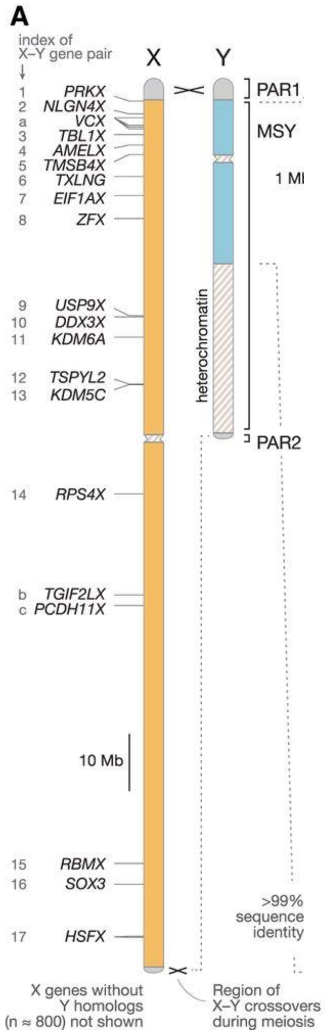

Alexander K. Godfrey et al. Genome Res. 2020;30:860-873

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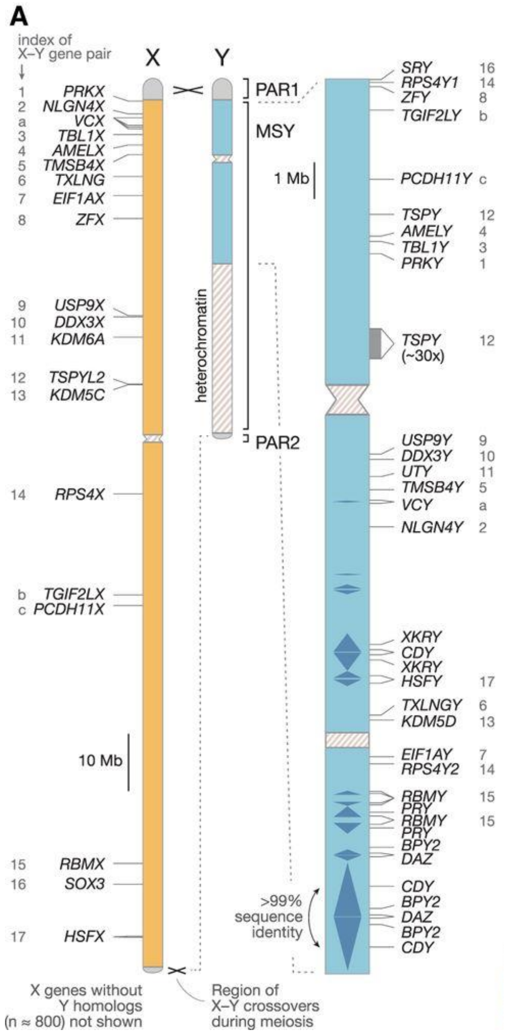

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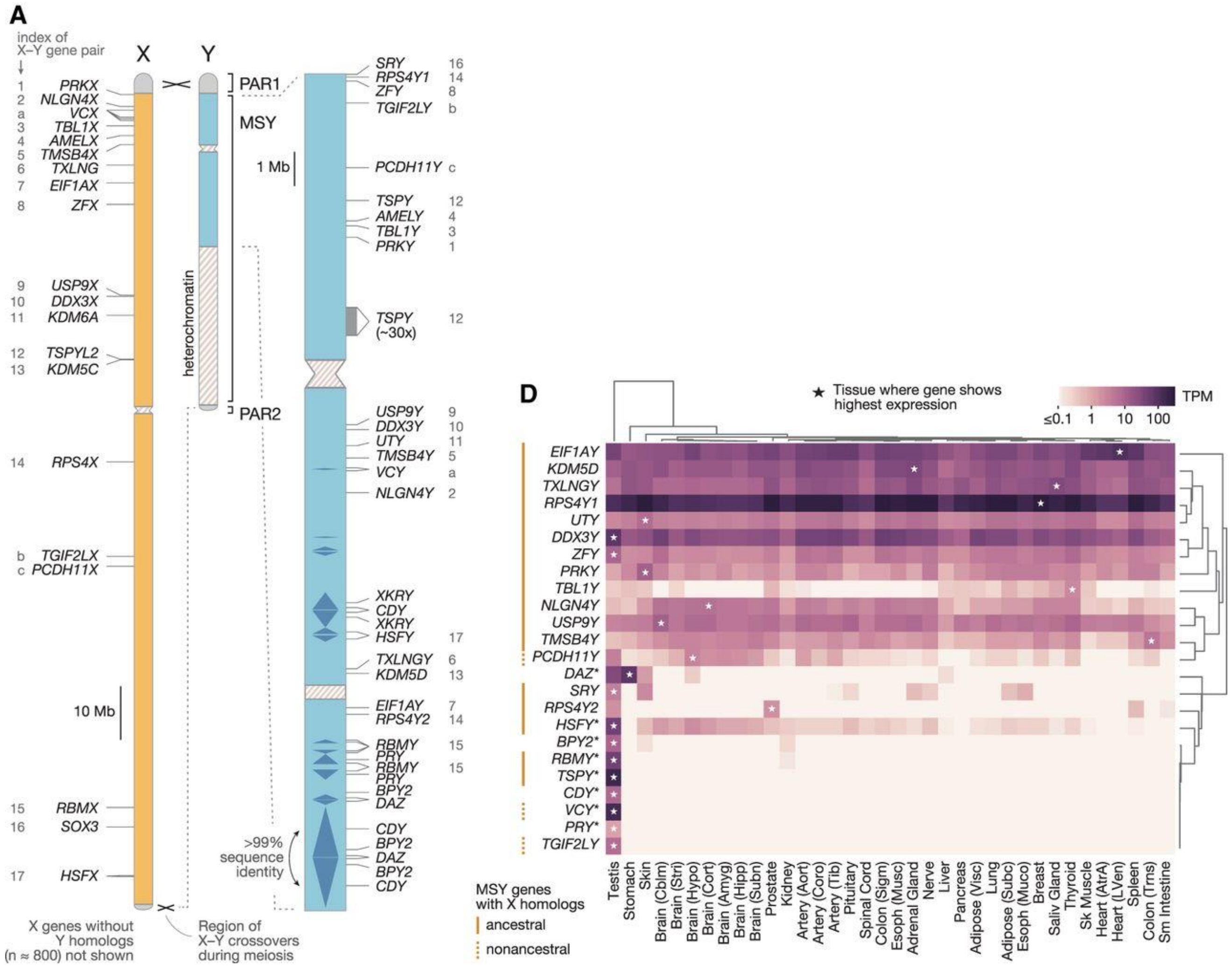

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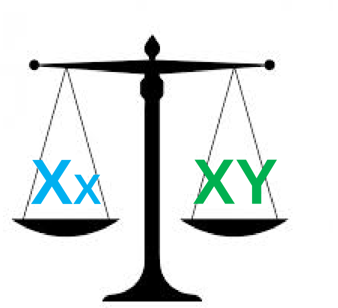
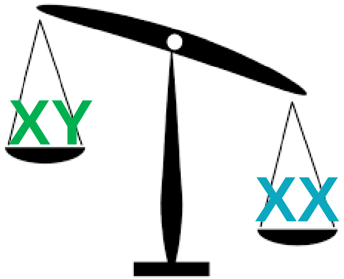
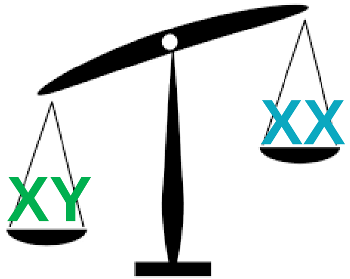
Part I Sex differences in phenotype: sex chromosome content



-> « epigenetic effect » of the sex chromosomes

- the inactive X as a « sink » for chromatin factors
- factors that can influence the dosage of autosomal genes



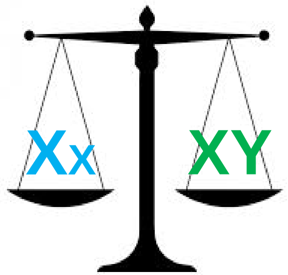


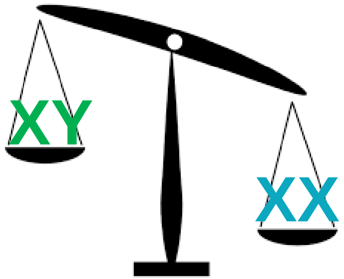
-> differences in dosage

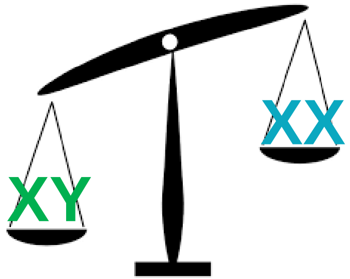
- XCI escapees
- parental imprints
maternal X ≠ paternal X

- Y genes with no X homolog
- transregulation 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

-> differences in dosage

- XCI escapees
- parental imprints maternal X ≠ paternal X

- Y genes with no X homolog
- transregulation between Xs

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[1,2], Joey H. Li[1,2], Luke Riggan[1,2,3], Bryan Chen[1], Rana Yakhshi Tafti[1,2], Scott Chin[1], Feiyang Ma[3,4], Matteo Pellegrini[3,4], Haley Hrncir[5], Arthur P. Arnold[5], Timothy E. O'Sullivan[1,2] & Maureen A. Su[1,2,6]

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

Article

Histone demethylase KDM5D upregulation drives sex differences in colon cancer

https://doi.org/10.1038/s41586-023-06254-7
Received: 18 October 2021
Accepted: 24 May 2023
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Jiexi Li[1], Zhengdao Lan[1], Wenting Liao[1,2], James W. Horner[3], Xueping Xu[3], Jielin Liu[4], Yohei Yoshihama[1], Shan Jiang[3], Hong Seok Shim[1], Max Slotnik[1], Kyle A. LaBella[1], Chang-Jiun Wu[5], Kenneth Dunner Jr.[1], Wen-Hao Hsu[1], Rumi Lee[1], Isha Khanduri[6], Christopher Terranova[5], Kadir Akdemir[5,7], Deepavali Chakravarti[1], Xiaoying Shang[1], Denise J. Spring[1], Y. Alan Wang[1,8] & Ronald A. DePinho[1]

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

Sex differences in phenotype: sex chromosome content

X & Y linked genes: sex biasing agents influencing gene expression at the scale of the whole genome

-> « epige

- the inactive X as a « sink » for chromatin factors
- factors that can influence the dosage of autosomal genes

- XCI escapees
-parental imprints
maternal X ≠ paternal X

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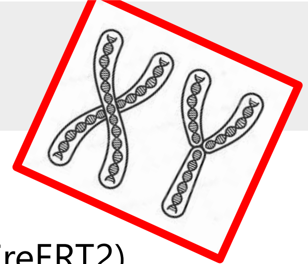
Nature 2023 Kdm5d (Ylinked)/ no role for 5c (X linked)

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?

Part II Sex-bias in colorectal cancer



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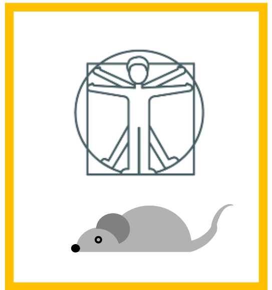
❖ **iKAP** (mouse) model:

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

Nature ***619***, *632–639 (2023)*



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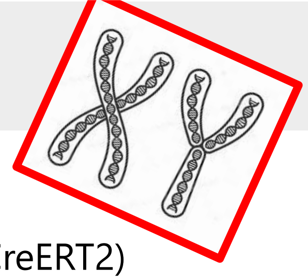

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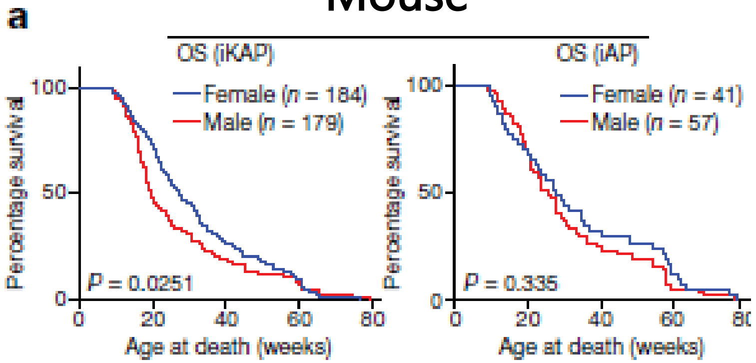
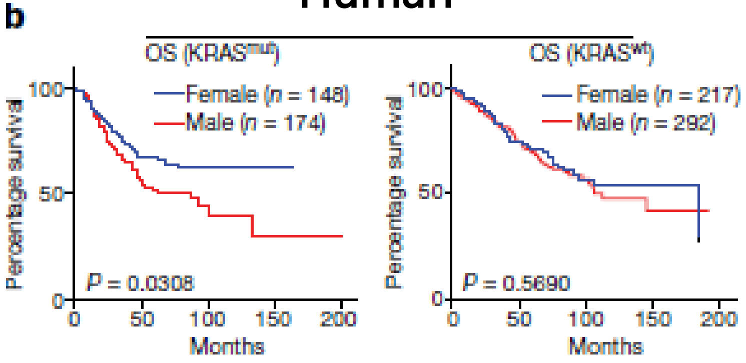
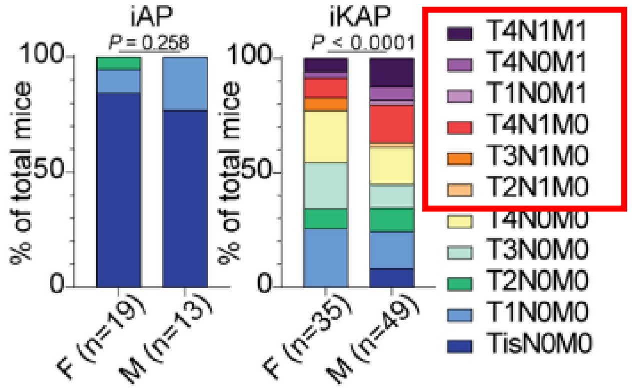
➢ Sex differences tumor aggressiveness is related to *KRAS**

Mouse

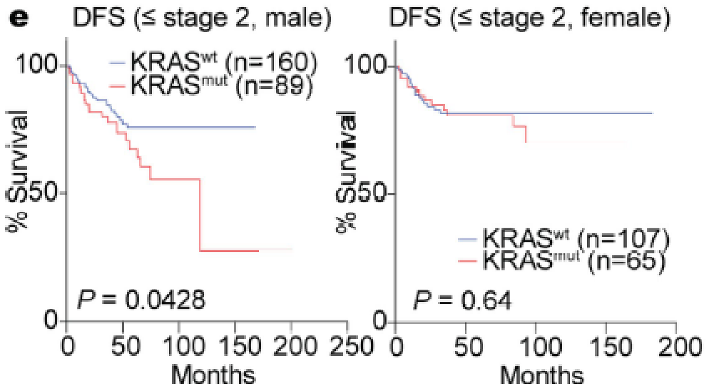


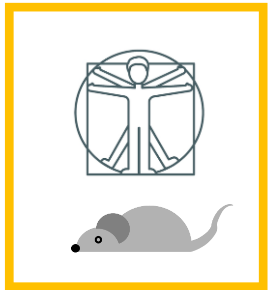
Human





met



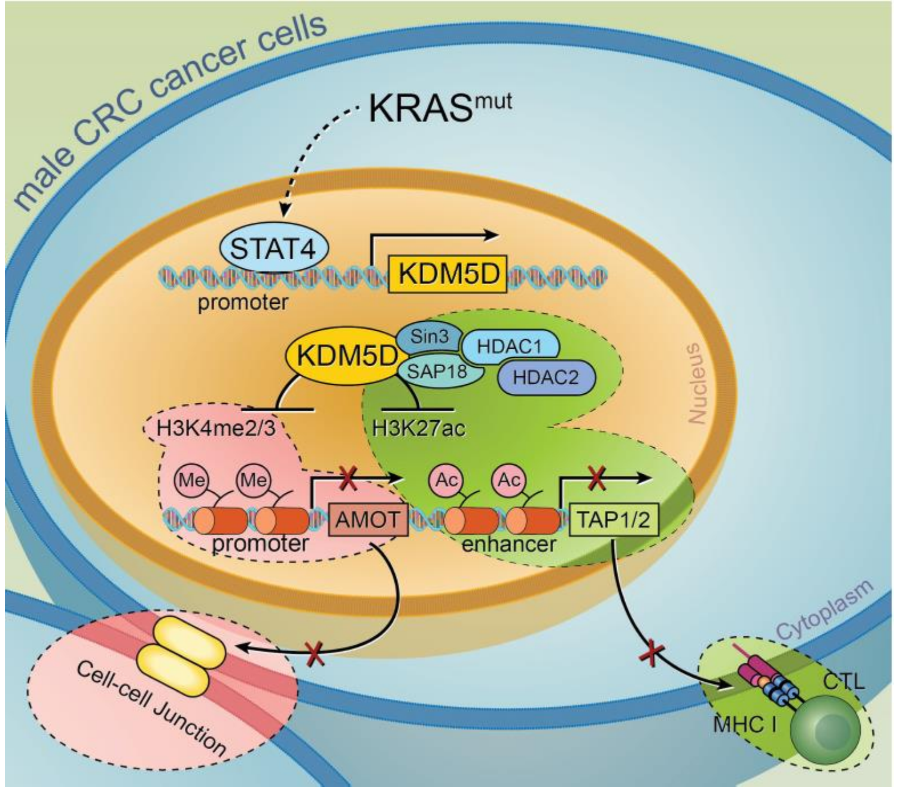


*Nature **619**, 632–639 (2023)*

Part II 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



- ↑ Dissemination and immune escape

Nature ***619***, *632–639 (2023)*

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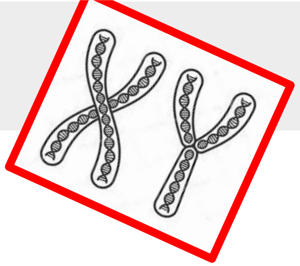

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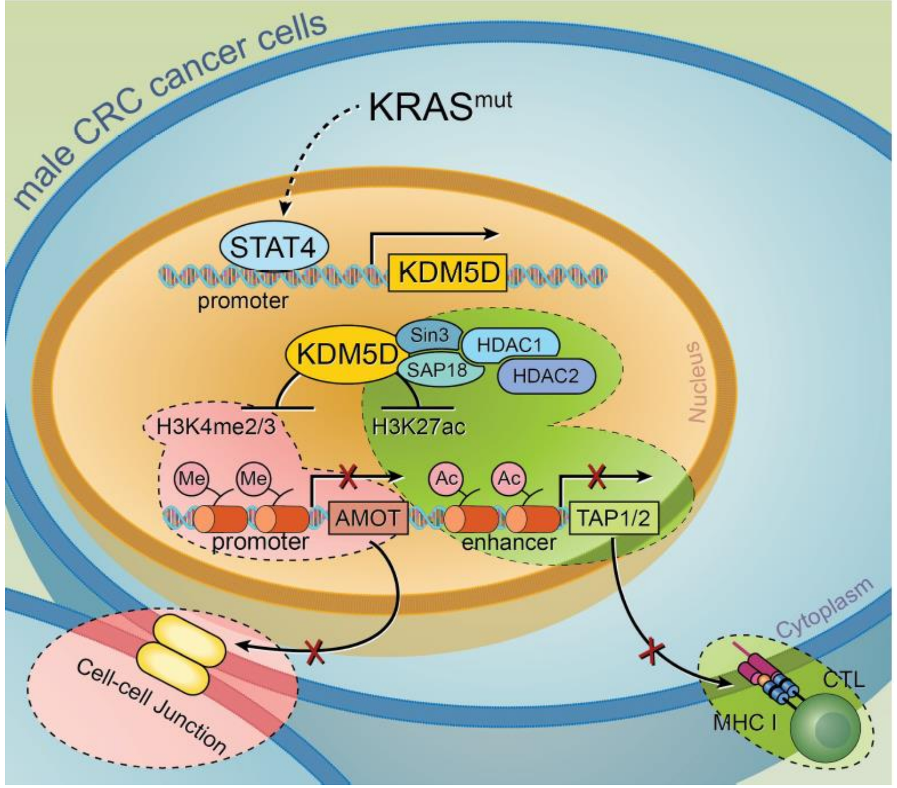
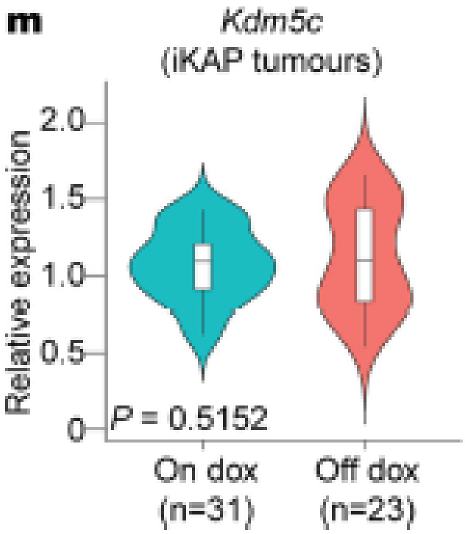


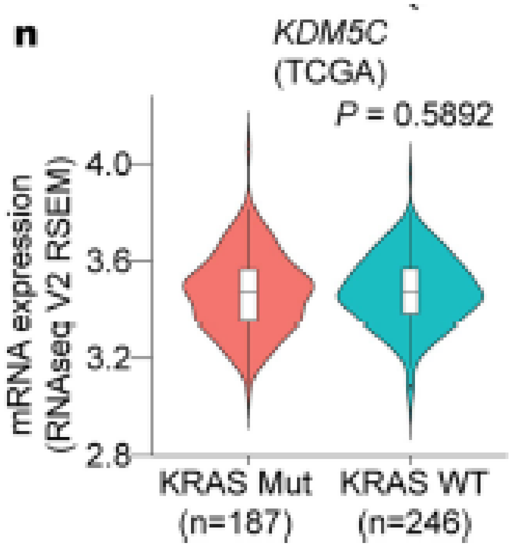
➢ ↑ Dissemination and immune escape

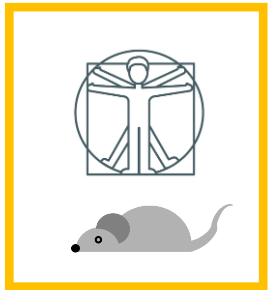
➢ *KDM5C:* the X-chromosome paralogue is not regulated

Mouse



Human





Nature ***619***, 632–639 (2023)

Sex-bias in bladder cancer

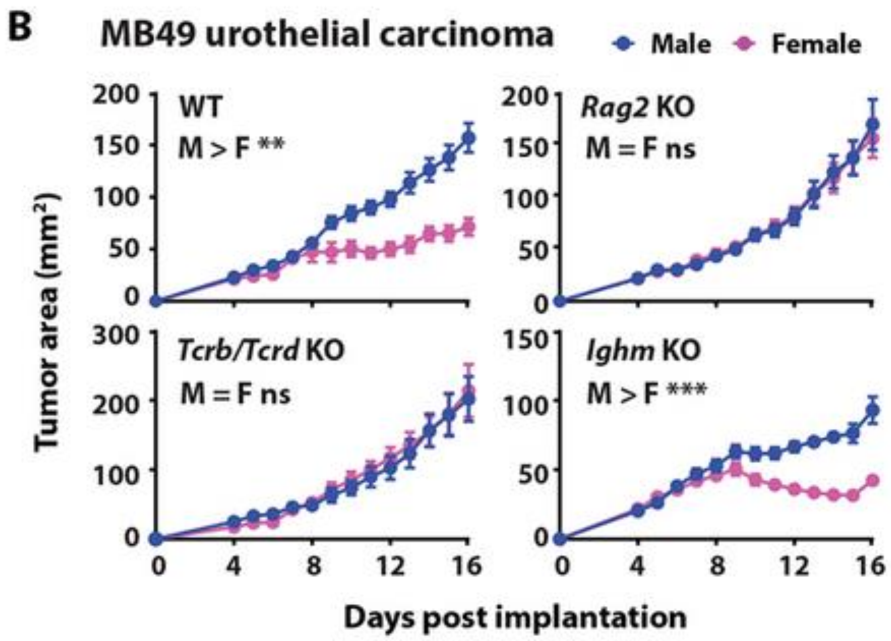
Hormon

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

Sci Immunol. 2022 ; 7(73): eabq2630.

Part II

Sex-bias in [bladder] cancer

Hormon

Immunity

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

Article

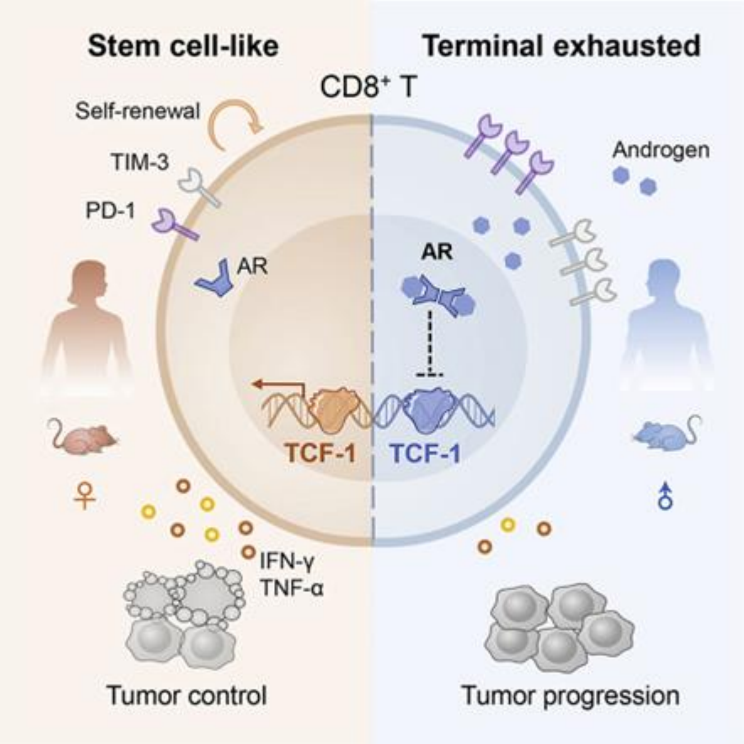
Androgen receptor-mediated CD8$^+$ T cell stemness programs drive sex differences in antitumor immunity

Article

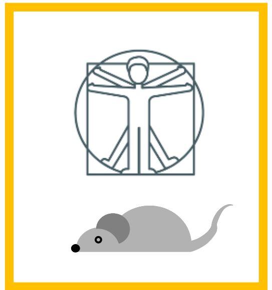
Androgen receptor activity in T cells limits checkpoint blockade efficacy

https://doi.org/10.1038/s41586-022-04522-6
Received: 12 August 2020
Accepted: 4 February 2022
Published online: 23 March 2022

Xiangnan Guan[1,2,10,12], Fanny Polesso[3,12], Chaojie Wang[3,11,12], Archana Sehrawat[3], Reed M. Hawkins[3], Susan E. Murray[2,4], George V. Thomas[5,6], Breanna Caruso[3], Reid F. Thompson[1,5,7,8], Mary A. Wood[8], Christina Hipfinger[3], Scott A. Hammond[9], Julie N. Graff[5,8], Zheng Xia[1,2,5,13] & Amy E. Moran[3,5,13]✉

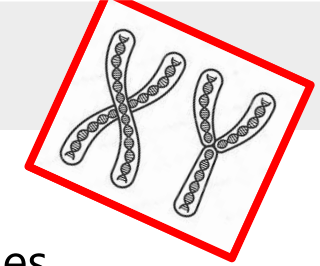


- High expression of AR in tumour infiltrating CD8+ T
- AR deficiency (KO mice) increased the expansion, proliferation potential and anti-tumour functions of CD8$^+$ T cells and led to the expansion of stem-like TPEX cells
- Human CRC and skin cutaneous melanoma: Positive correlation between AR signalling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



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

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

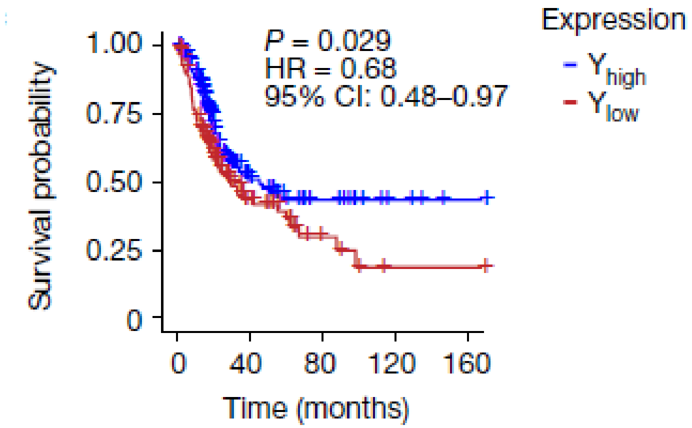


Article

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

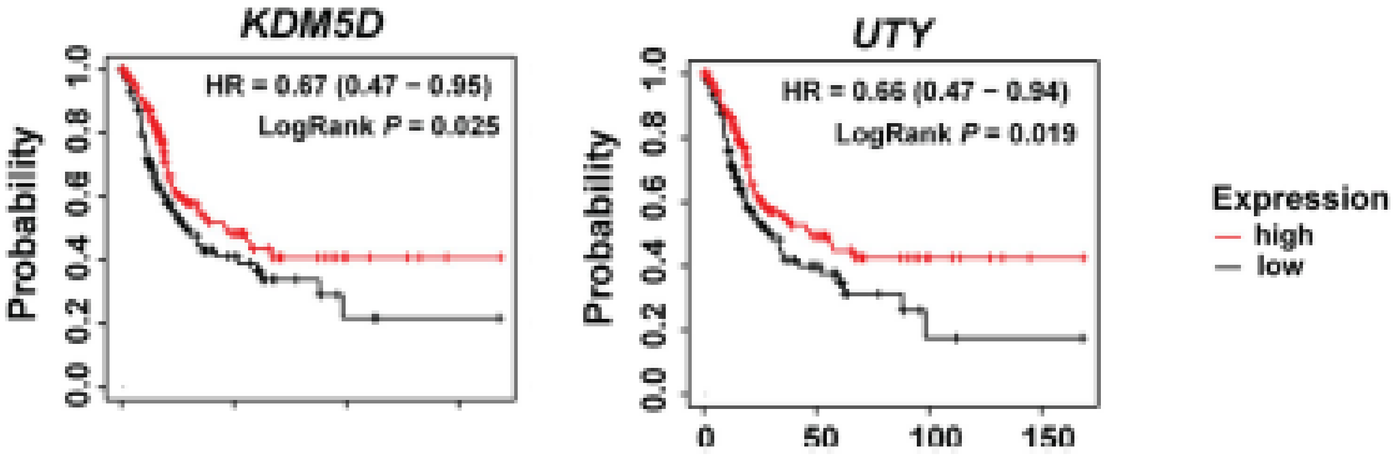
https://doi.org/10.1038/s41586-023-06234-x
Received: 1 September 2022
Accepted: 18 May 2023

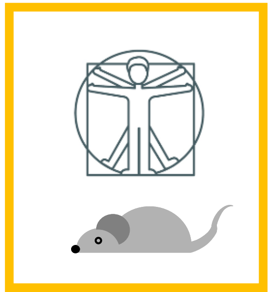
Hany A. Abdel-Hafiz[1,6], Johanna M. Schafer[2,5,6], Xingyu Chen[1,6], Tong Xiao[2], Timothy D. Gauntner[2], Zihai Li[2,7] & Dan Theodorescu[1,3,4,7]



- **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)

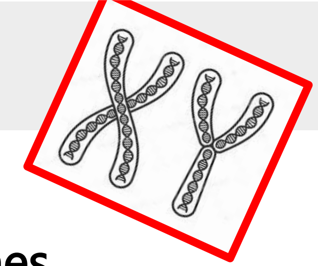
- **LOY (Loss of Chromosome Y) associated with a worse patient outcome**
- **Involvement of *KDM5D* and *UTY***

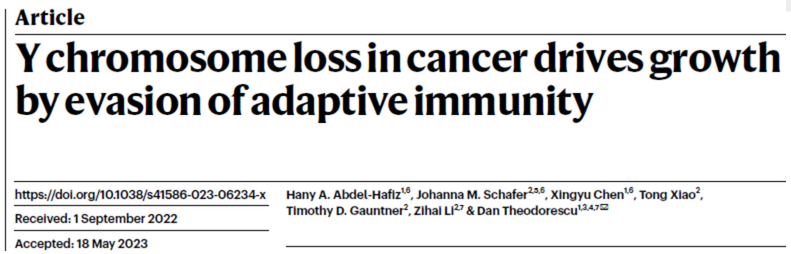




Nature 2023;619(7970):624-631

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

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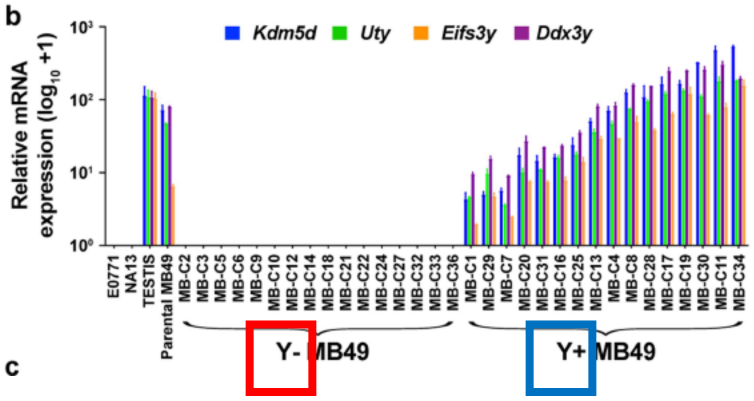

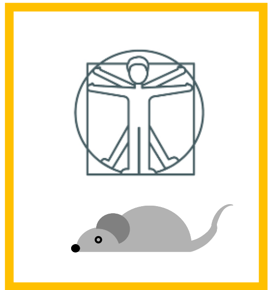

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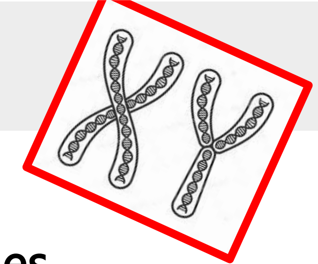

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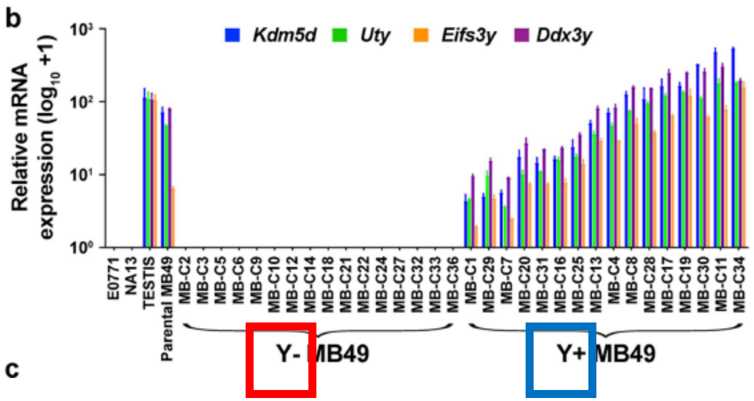
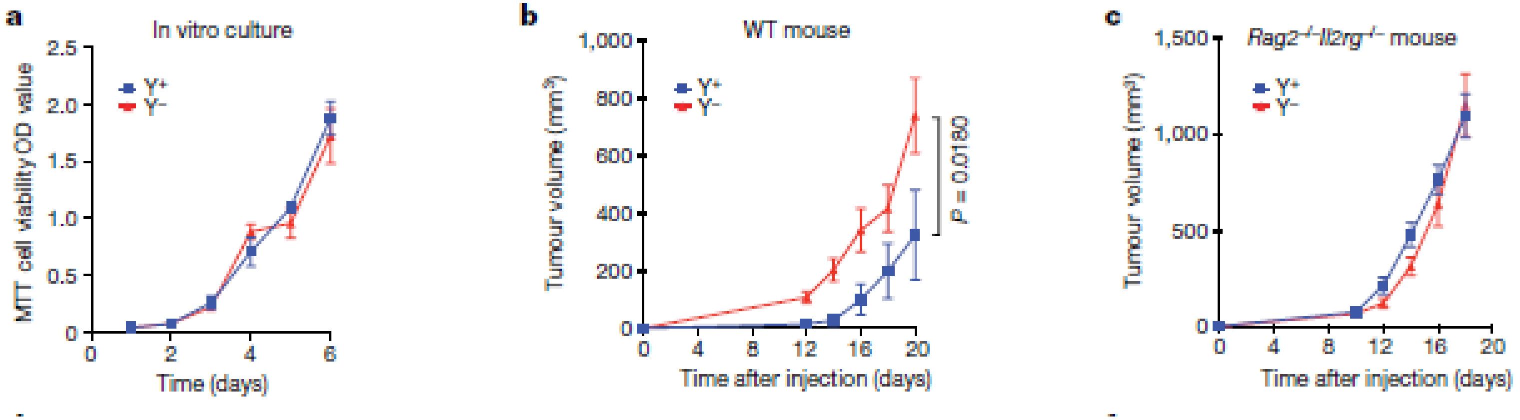
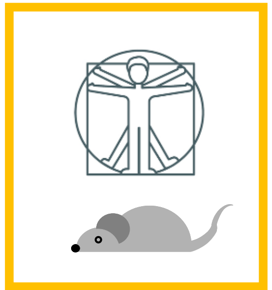




➢ No effect on *in vitro* growth between Y+/Y-

➢ Y- more aggressive in immunocompetent mice



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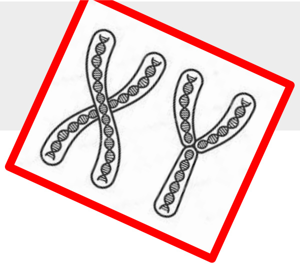
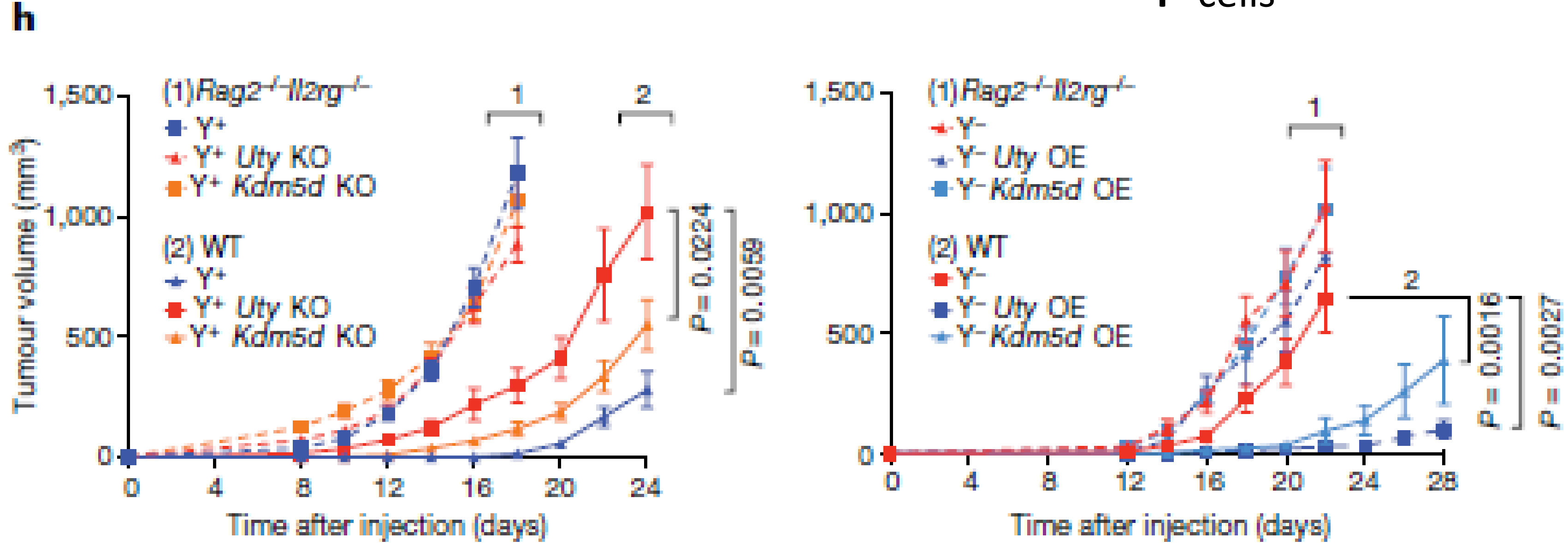


- Graft in immunodeficient (RagγC) or immunocompetent mice of:

Y+ cells **Y-** cells



- Molecular drivers lost in Y− tumours that contribute to immune evasion: *UTY, KDM5D*

Nature 2023;619(7970):624-631

Part II Sex-bias in B-lymphomas

Hormon

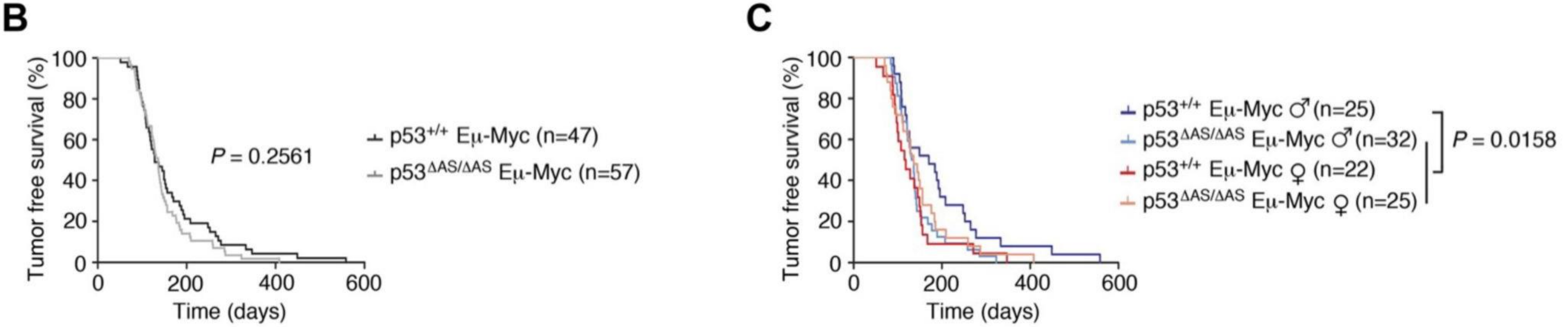
eLife

Cancer Biology

Mutant mice lacking alternatively spliced p53 isoforms unveil *Ackr4* as a male-specific prognostic factor in Myc-driven B-cell lymphomas

Reviewed Preprint

❖ *Trp53 & Myc* transgenic mice



- Similar TFS curves for *Eμ-Myc/Tp53wt* vs *μ-Myc/Tp53$^{\Delta AS}$* KO when sexes were not considered
- *Eμ-Myc/Tp53 wt* less aggressive in males

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

Part II

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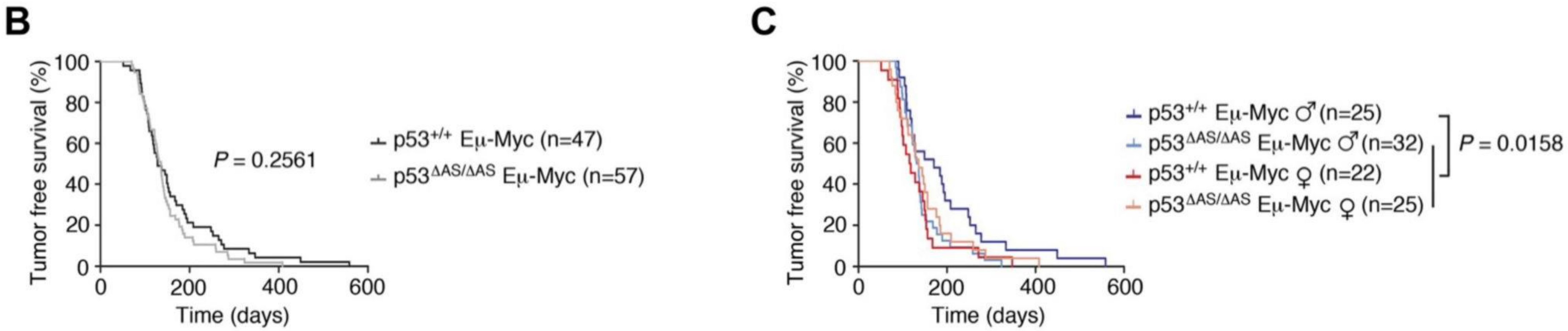
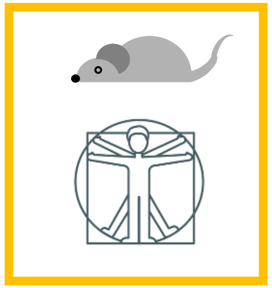
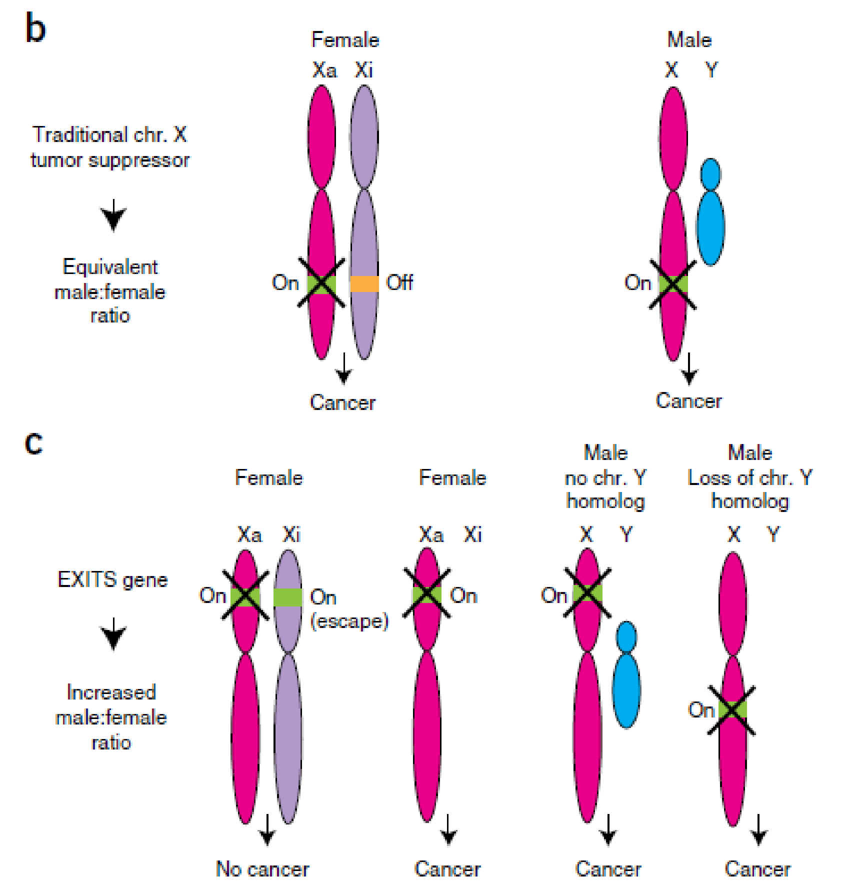


- Similar TFS curves for *Eμ-Myc/Tp53wt* vs *μ-Myc/Tp53*$^{\Delta AS}$ KO when sexes were not considered
- *Eμ-Myc/Tp53 wt* less aggressive in males
- Role of *Ackr4* in tumor aggressiveness in females than in males



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

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 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

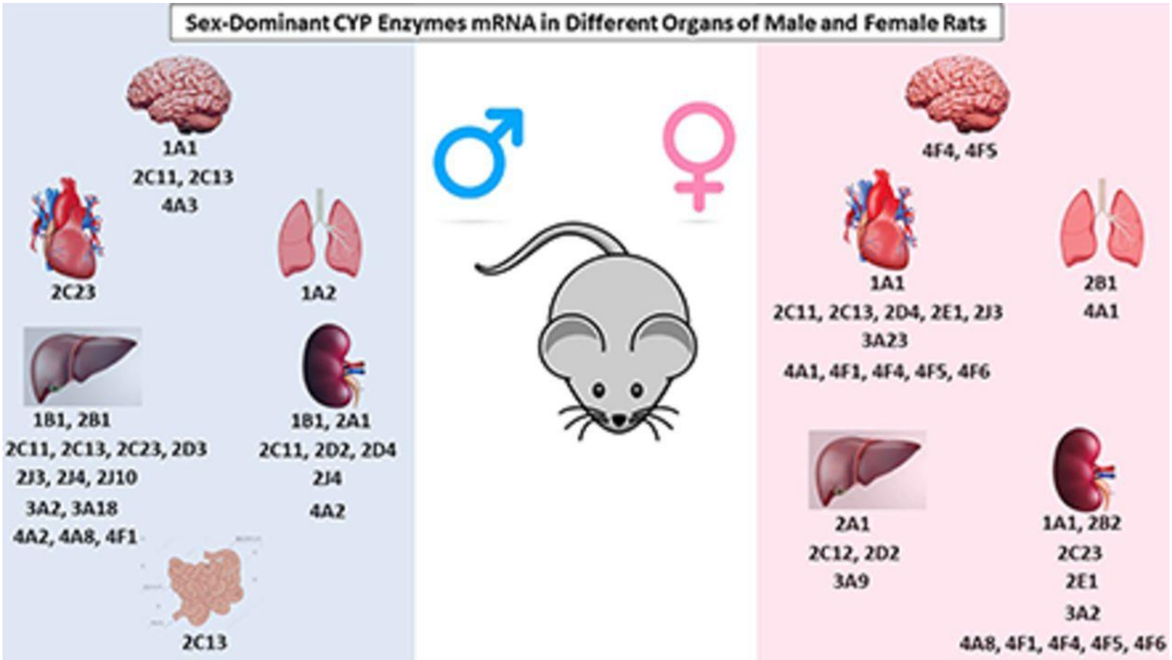
Nature Genetics 2017,49, 10-19

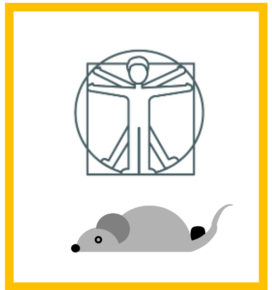
Future Oncol 2012;8(4):463-81

Part II

That's not all:

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





Drug Metabolism and Disposition 2023, 51 (1) 81-94;

Genes on the X with the potential to influence immunocompetence

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

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Mandy I. Cheng[1,2], Joey H. Li[1,2], Luke Riggan[1,2,3], Bryan Chen[1], Rana Yakhshi Tafti[1,2], Scott Chin[1], Feiyang Ma[3,4], Matteo Pellegrini[3,4], Haley Hrncir[5], Arthur P. Arnold[5], Timothy E. O'Sullivan[1,2] & Maureen A. Su[1,2,6]

RESEARCH ARTICLE The Journal of Clinical Investigation

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

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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

b Immune-response related proteins

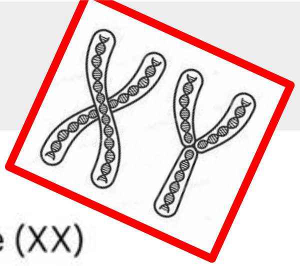
XSCID	X-linked severe combined immunodeficiency
ELK1	Involved in B-cell development
EPAG	Early lymphoid activation protein
GATA1	GATA-binding protein 1
GTD	Gonadotropin deficiency
IDDMX	X-linked susceptibility to insulin-dependent diabetes
IGBP1	CD79A, immunoglobulin binding protein 1
IGSF1	Immunoglobulin superfamily member 1
ITGB1BP2	Integrin-β_1-binding protein 2
CD99	Also known as MIC2; associated with T-cell function
MTCP1	Mature T-cell proliferation 1
PFC	Properdin P factor, complement
TIMP1	Tissue inhibitor of metalloproteinase 1
CD40L	CD40 ligand
Z39IG	An immunoglobulin superfamily protein

c Transcriptional & 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
IKKγ	IκB kinase; also known as NEMO
MAPKKK15	Mitogen-activated protein kinase kinase kinase 15
NFκBRF	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 Cι
S6K	Ribosomal protein S6 kinase
SWI/SNF	SWI/SNF-related, matrix associated, actin-dependent regulator of chromatin
STK9	Serine/threonine kinase 9
TAF1	TATA-box-binding protein-associated factor 1, TFIID subunit
UBE1	Ubiquitin-activating enzyme E1
UBE2A	Ubiquitin-conjugating enzyme E2A
USP	Ubiquitin-specific proteases 9*, 11, 26, 27, 511
WASP	Wiskott–Aldrich syndrome protein

Nature Rev Immunol, 2008, 8, 737-744

Part II   Sex-bias in NK number and function



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
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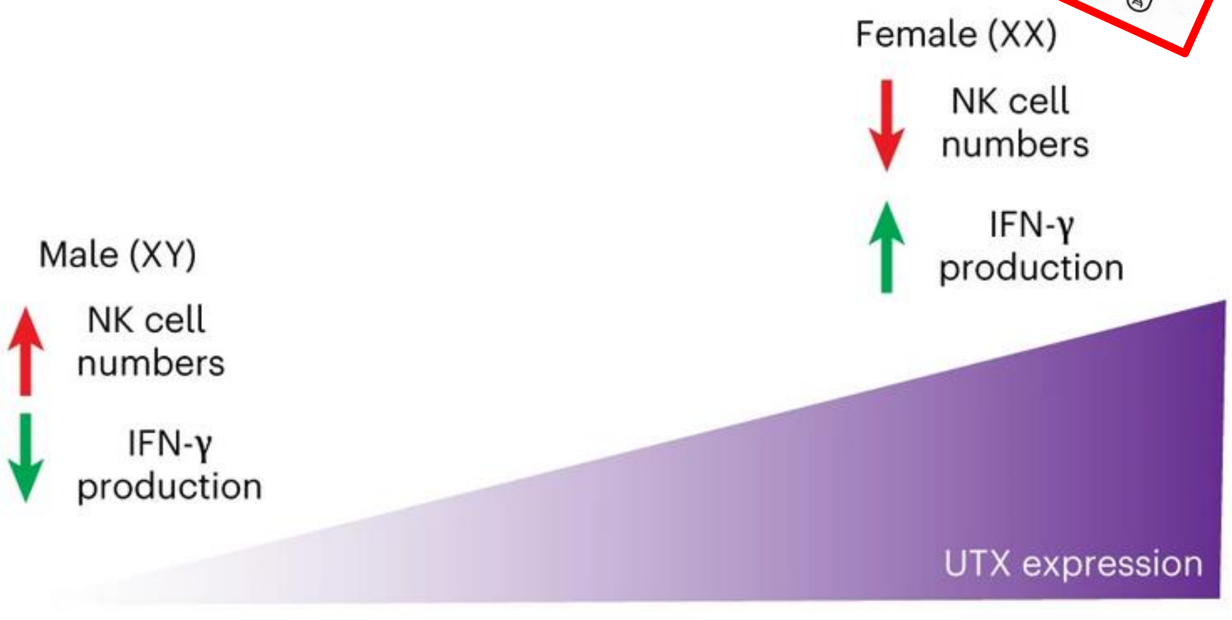
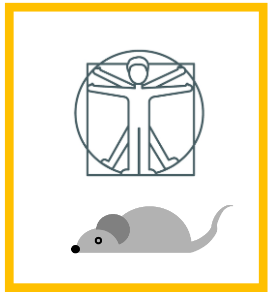
Mandy I. Cheng[1,2], Joey H. Li[1,2], Luke Riggan[1,2,3], Bryan Chen[1], Rana Yakhshi Tafti[1,2], Scott Chin[1], Feiyang Ma[3,4], Matteo Pellegrini[3,4], Haley Hrncir[5], Arthur P. Arnold[5], Timothy E. O'Sullivan[1,2] & Maureen A. Su[1,2,6]

- 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.





Nature Immunol, 2023, 24, 780-791

Part II

Sex-bias in NK number and function

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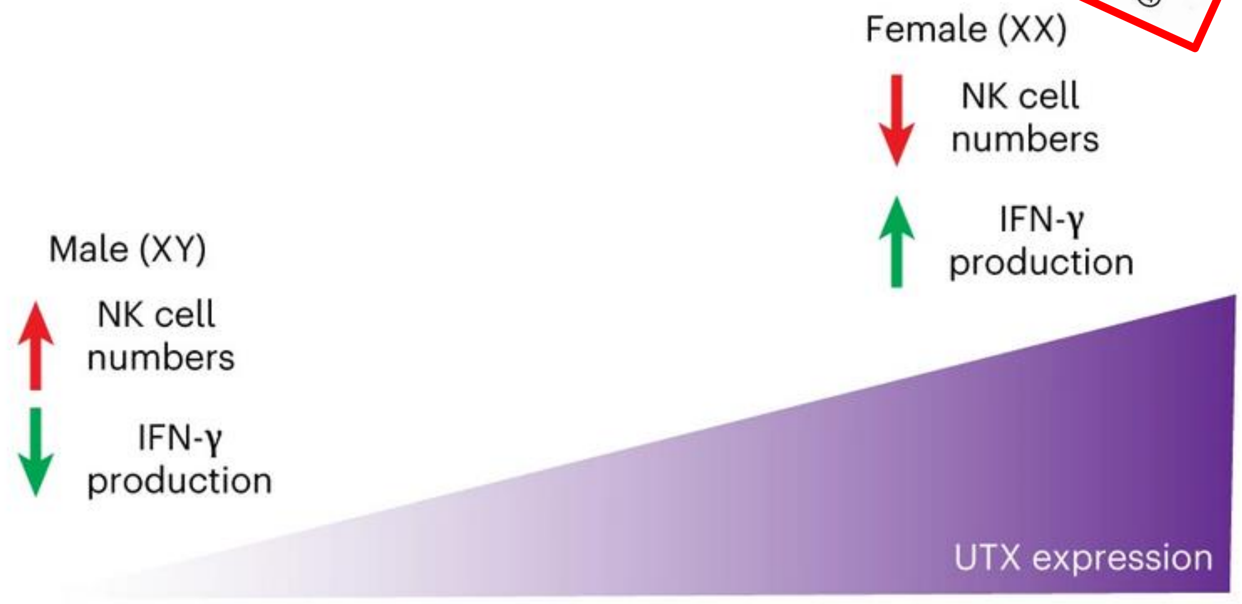

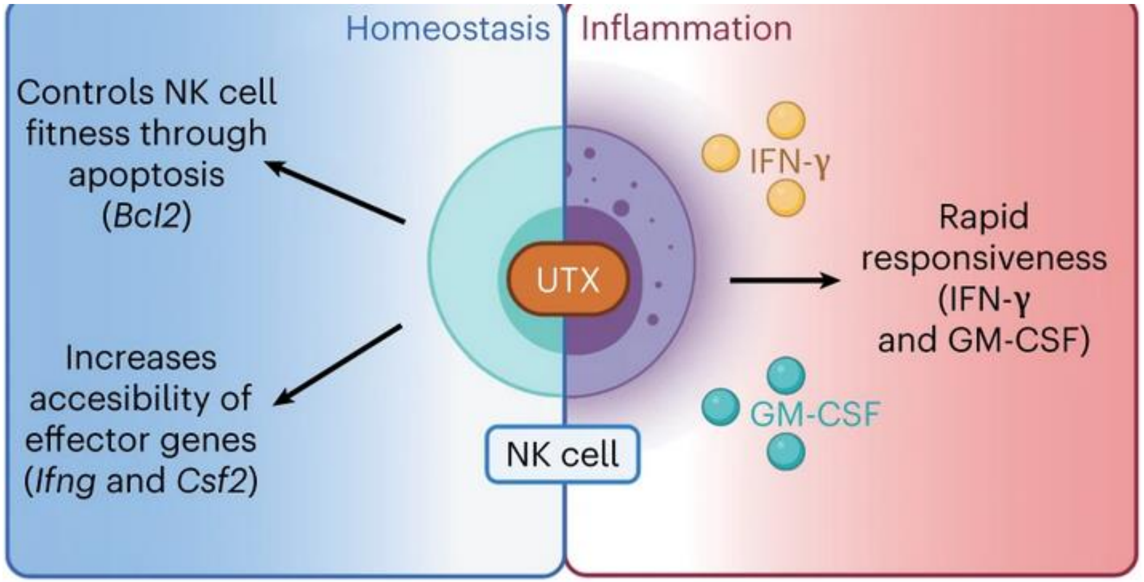

Nature Immunol, 2023, 24, 780-791

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*]

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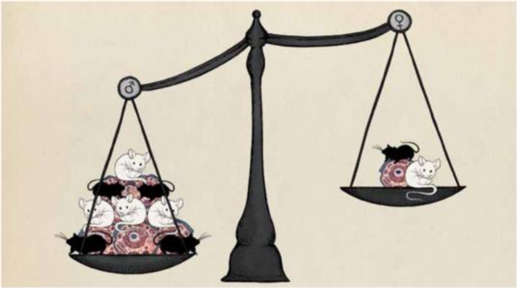
> All steps are concerned

International guidelines & Funding

National Institutes of Health



NIH to balance sex in cell and animal studies

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

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

Part III

SAGER Guidelines

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

Research Integrity and Peer Review

REVIEW **Open Access**

CrossMark

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

Shirin Heidari[1], Thomas F. Babor[2*], Paola De Castro[3], Sera Tort[4] and Mirjam Curno[5]

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

Springer

Subjects Services ▾ About Us

Sex and Gender in Research (SAGER Guidelines)

We encourage our authors to follow the 'Sex and Gender Equity in Research – SAGER – guidelines' 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 guidelines before submission.

Part III 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
	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
Introduction		
	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
	5c	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 animals*
Discussion		
	7	If relevant, potential implications of sex/gender on the study 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) 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?

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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 *in vivo* 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

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

- 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

Sex:
**could influence*
**should not influence*

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Part III

Courses

COLLÈGE DE FRANCE 1530

Enseignements Recherche Bibliothèq

Pr Edith Heard

Cours

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

Cours

Le lundi, de 10 h à 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

REPLAY
French

NIH National Institutes of Health
Office of Research on Women's Health

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

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
- Sex as a Biological Variable (SABV): A Primer
- SABV Primer: Train the Trainer
- Introduction to Sex and Gender: Core Concepts for Health-Related Research

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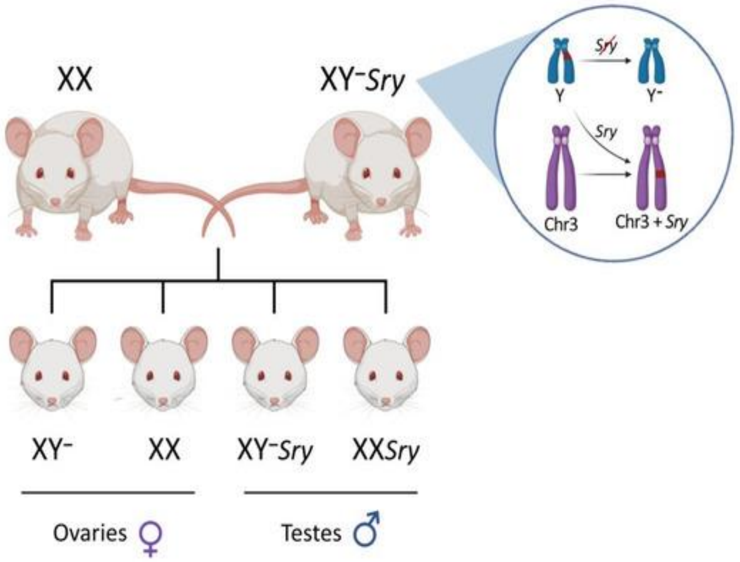

Perspectives

- 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

- 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

LETICIA RODRÍGUEZ-MONTES, SVETLANA OVCHINNIKOVA, XUEFEI YUAN, TANIA STUDER, IOANNIS SARROPOULOS, SIMON ANDERS, HENRIK KAESSMANN, AND MARGARIDA CARDOSO-MOREIRA Authors Info & Affiliations

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nature communications

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

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

Received: 3 August 2022
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Zaher ElBeck[1,2], Mohammad Bakhtiar Hossain[3], Humam Siga[1], Nikolay Oskolkov[4], Fredrik Karlsson[5], Julia Lindgren[6], Anna Walentinsson[7], Dominique Koppenhöfer[1], Rebecca Jarvis[8],

Thank you

Athanassia Sotiropoulos

Susana Gomez