CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2022-2023

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

Cours I - Introduction : les maladies ont-elles un sexe ? *6 mars*

Cours II - Biais liés au sexe : comment distinguer les effets dus aux chromosomes sexuels, hormones ou mode de vie ? *13 mars*

Cours III - L'impact de l'expression des gènes liés aux chromosomes X inactif et Y sur les différences entre les sexes. *20 mars*

Cours IV - L'importance de la régulation du dosage des gènes sur le chromosome X dans la susceptibilité à certaines maladies. *27 mars*

Colloque – en lien avec le sujet du cours, le **21 avril, 2023**

COLLÈGE DE FRANCE 1530

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Image : *La chute de l'homme* (détail), Cornelis Cornelisz van Haarlem, 1592. © Rijksmuseum

COLLOQUE

The Genetic and Epigenetic Basis of Sex Bias in Disease

21 avril 2023

COLLÈGE DE FRANCE 1530

Thomas Römer
Administrateur du Collège de France
11, place Marcelin-Berthelot, 75005 Paris
www.college-de-france.fr

Année académique 2022/2023

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 required

COLLÈGE DE FRANCE 1530

E. Heard, March 20th 2023

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2022-2023

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

20 mars, 2023

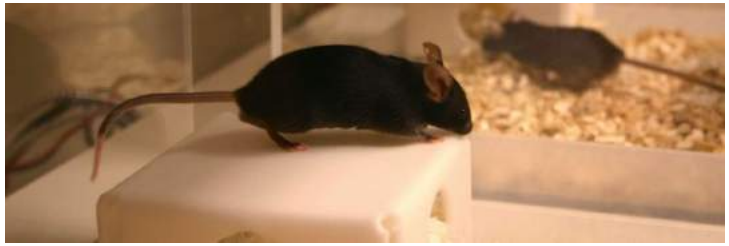
Cours III

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

COLLÈGE DE FRANCE 1530

SUMMARY of LAST WEEK

- Sex bias in disease: overcoming bias in *practices* and *perceptions*
- Exclusion of females in experimental research and of women in clinical trials
- Females are not more variable than males (on the contrary – latest studies on mouse behaviour!)
- Defining the causes of sex bias in disease – distinguishing between sex hormones, genetics and environment
- Sex Hormones – presence at different levels and with different variation throughout life of men and women
- Impact of hormones on the brain and other non-gonadal tissues
- Cell autonomous sex identity from conception throughout life - extreme example of bird ***gynandromorphs***
- Sex chromosomes modulate phenotypes throughout human body indirectly through their primary role in sex determination and the subsequent organizing effects of gonadal hormones.
- Sex chromosomes directly affect non-gonadal tissues with or without gonadal hormones (activational effects).
- Methods for distinguishing effects of sex chromosome number from effects of sex hormones



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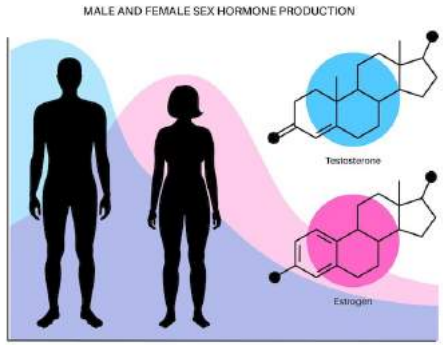
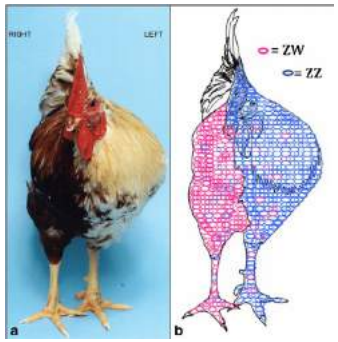
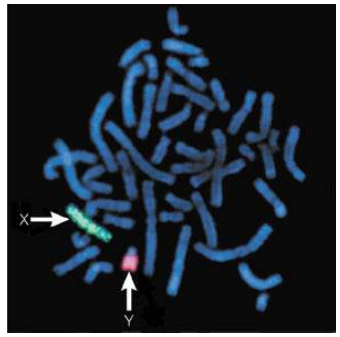






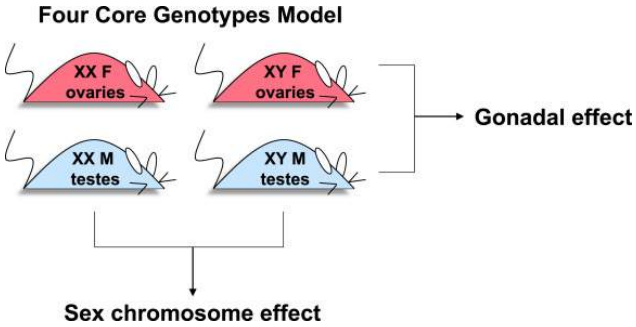
COLLÈGE DE FRANCE 1530

E. Heard, March 20th 2023

SUMMARY of LAST WEEK

Four Core Genotypes (FCG) model, and the **XY* model** provide tools to ask **if a phenotypic sex difference in mice is caused by differential action of sex chromosome genes, or by gonadal hormones.** (Burgoyne and Arnold, 2016; Burgoyne et al., 1998; De Vries et al., 2002; Eicher et al., 1991; Mahadevaiah et al., 1998)

The model separates the effects of sex chromosome complement by fixing the gonadal status: XX vs. XY with ovaries; XX vs. XY with testes, from the effects of gonads by fixing the sex chromosome type (ovaries vs. testes with XX genotype; ovaries vs. testes with XY genotype).



Paul Burgoyne
(1946-2020)
Mouse Geneticist
Specialist of sex chromosomes
Developed the FCG model together with Art Arnold and Robin Lovell-Badge

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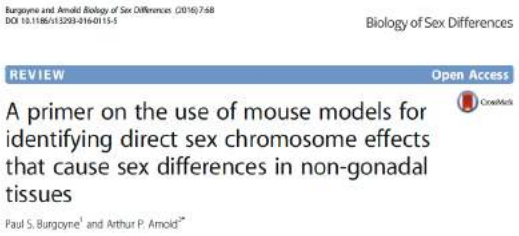
When a sex chromosome effect (XX not equal to XY) is detected in FCG mice, it could be due to
- the number of X chromosomes (including X dose, X imprint or indirect effects of X inactivation),
- or the presence / absence of the Y chromosome

(Arnold, 2017a; Burgoyne and Arnold, 2016)

The XY* model is then useful to discriminate between these possibilities (Eicher et al., 1991).
- XY* mice have an aberrant pseudoautosomal region on the Y chromosome, which recombines abnormally with the X chromosome (Burgoyne and Arnold, 2016; Burgoyne et al., 1998).
- XY* fathers, mated to XX females, produce mice that are very similar to XX and XO gonadal females, and XY and XXY gonadal males (Burgoyne and Arnold, 2016) .
- The effects of one vs. two X chromosomes is measured by comparing XO vs. XX females, or XY vs. XXY males.
- The effects of one vs. no Y chromosome is measured by comparing XY vs. XO, and XXY vs. XX. In the XY* model, mice with a Y chromosome are gonadal males.

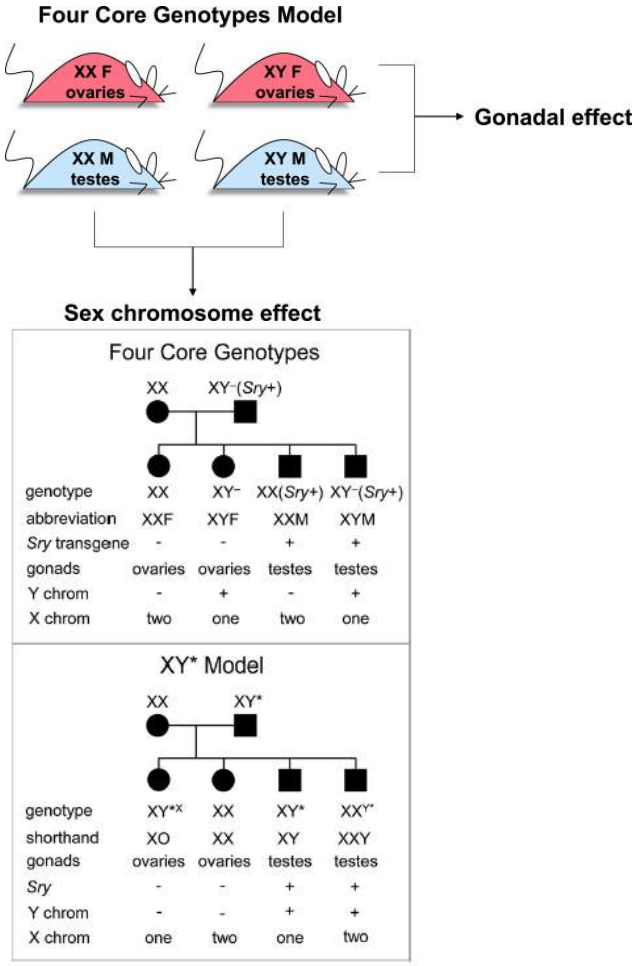

Burgoyne and Arnold *Biology of Sex Differences* (2016) 7:68
DOI 10.1186/s13293-016-0115-5

Biology of Sex Differences

REVIEW — Open Access

A primer on the use of mouse models for identifying direct sex chromosome effects that cause sex differences in non-gonadal tissues

Paul S. Burgoyne[1] and Arthur P. Arnold[2*]



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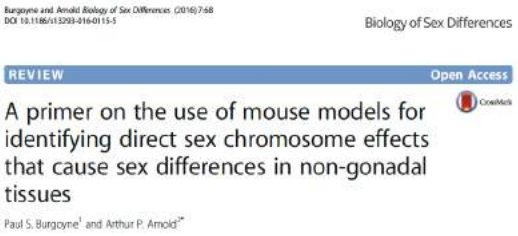
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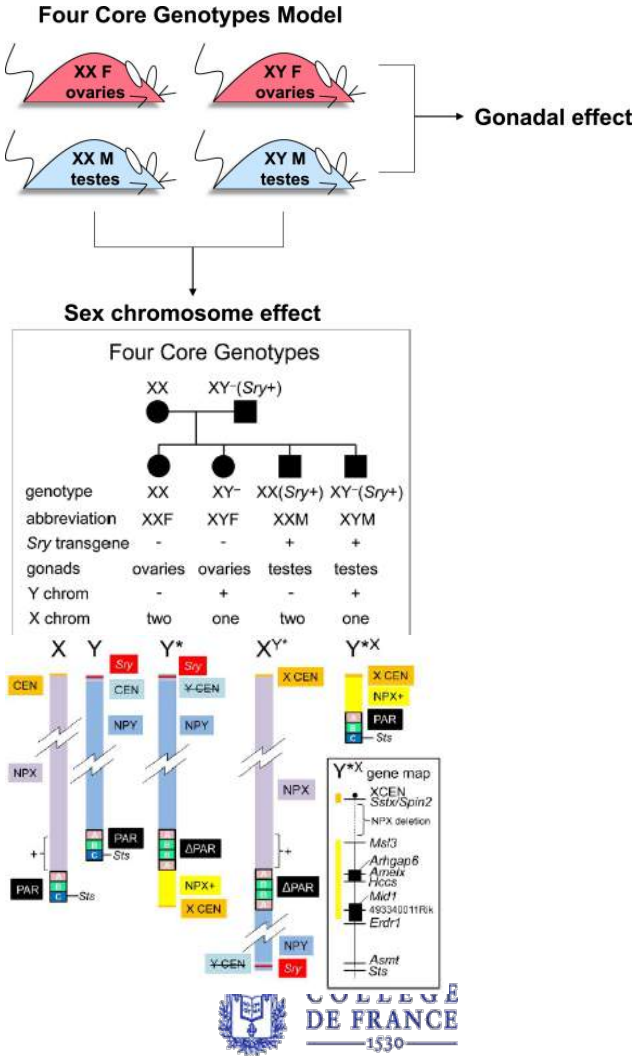

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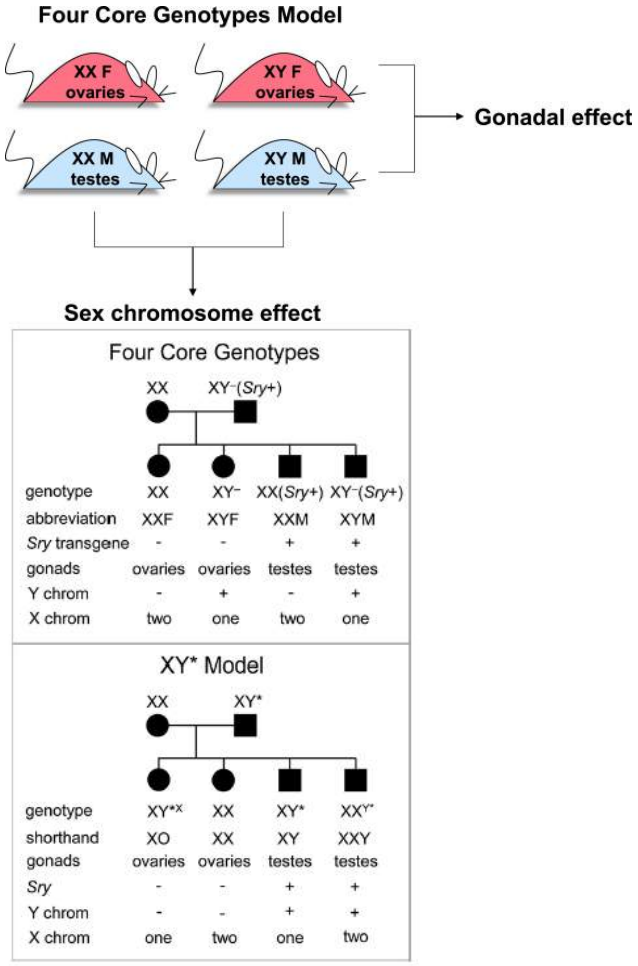
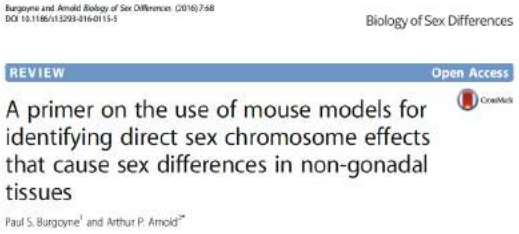
(Arnold, 2017a; Burgoyne and Arnold, 2016)

When a hormonal effect (ovaries not equal to testes) is detected, gonadectomy and different hormonal supplements can be applied

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Paul S. Burgoyne[1] and Arthur P. Arnold[2*]

E. Heard, March 20th 2023

COLLÈGE DE FRANCE 1530

Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in (non-gonadal) tissue gene regulation

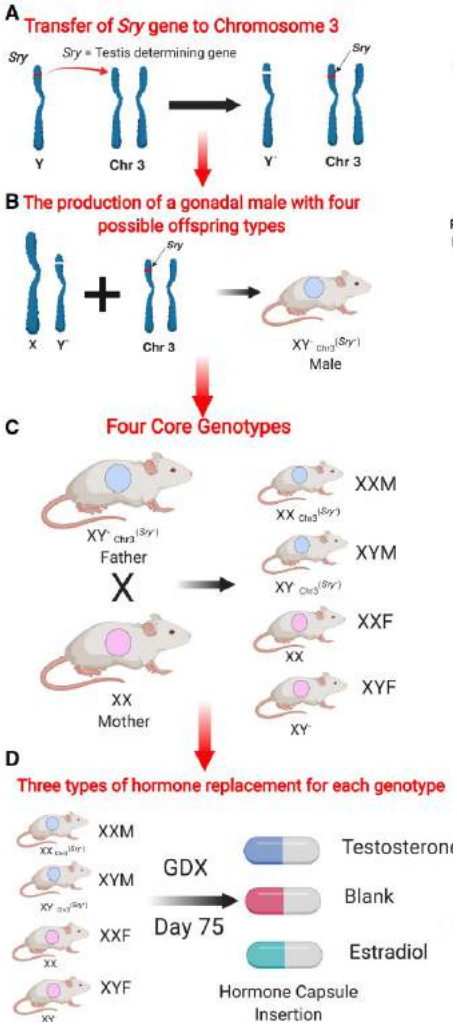
Research

Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in tissue gene regulation

Montgomery Blencowe,[1,2] Xuqi Chen,[1,3] Yutian Zhao,[1,2] Yuichiro Itoh,[1,3,4] Caden N. McQuillen,[1] Yanjie Han,[1] Benjamin L. Shou,[1] Rebecca McClusky,[1,3] Karen Reue,[5] Arthur P. Arnold,[1,2,3] and Xia Yang[1,2,5,6,7]

Use FCG mice and vary the gonadal hormone levels via gonadectomy and subsequent hormonal treatments:
=> assess how androgens and estrogens influence gene expression as a function of sex chromosome complement and gonadal sex. *NB microarrays not RNA-seq...*

The design allows comparison of the magnitude of effect of each sex-biasing factor and the interactions among different factors.



Blencowe et al, 2022, Genome Res. 32:807–824

Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in (non-gonadal) tissue gene regulation

- Activational hormone levels have the strongest influence on gene expression (circulating hormones, eliminated by gonadectomy);
- This is followed by the organizational gonadal sex effect (not reversed by gonadectomy and permanently established during development),
- Last, were the sex chromosomal effects
- Along with interactions among the three factors.
- Tissue specificity was prominent, with a major impact of estradiol on adipose tissue gene regulation and of testosterone on the liver transcriptome.

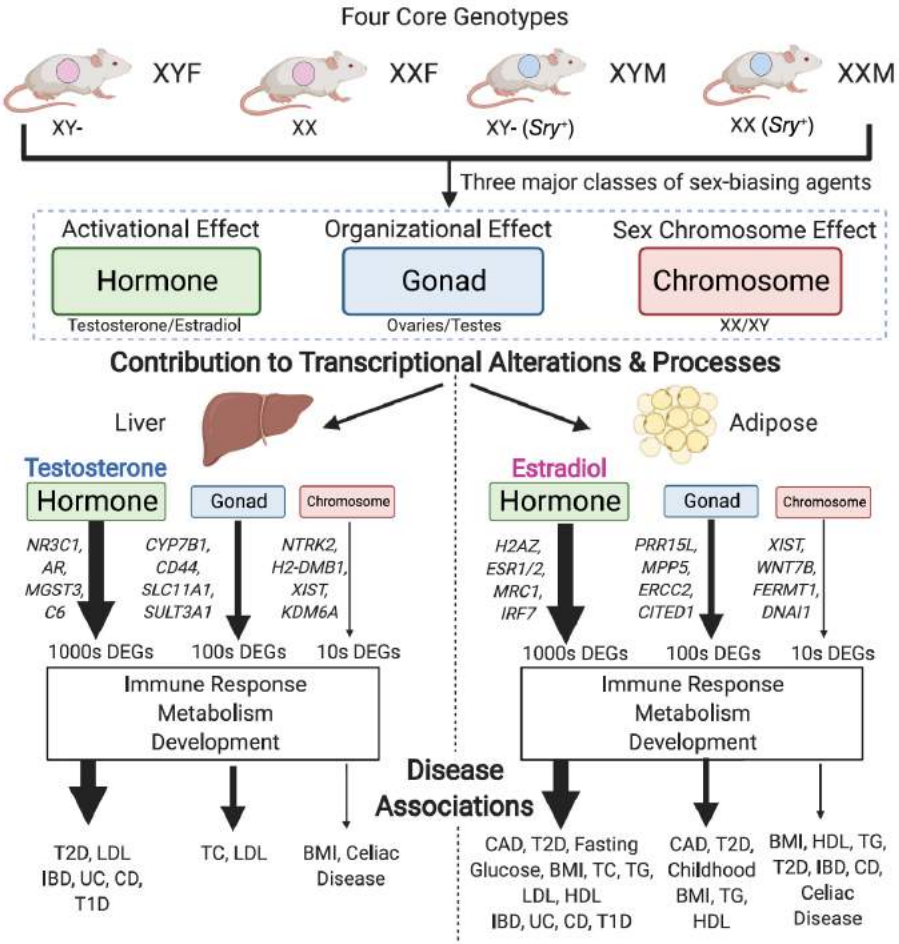


Figure 6. Study summary. Using the FCG model, we separated the effects of three major classes of sex-biasing agents and uncovered their relative contribution to transcriptional alterations in the liver and adipose tissue, the resulting biological processes enriched, and finally the diseases associated.

Blencowe et al, 2022, Genome Res. 32:807–824

COLLÈGE DE FRANCE 1530

Relative contributions of sex hormones, sex chromosomes, and gonads to sex differences in (non-gonadal) tissue gene regulation

- Sex differences in physiology and disease in mammals result from the effects of three classes of factors that are inherently unequal in males and females: reversible (activational) effects of gonadal hormones, permanent (organizational) effects of gonadal hormones, and cell-autonomous effects of sex chromosomes, as well as genes driven by these classes of factors.
- Often, these factors act together to cause sex differences in specific phenotypes, but the relative contribution of each and the interactions among them remain unclear.
- The four core genotypes (FCG) mouse model was used with or without hormone replacement to distinguish the effects of each class of sex-biasing factors on transcriptome regulation in liver and adipose tissues.
- Find that activational hormone levels have the strongest influence on gene expression, followed by the organizational gonadal sex effect, and last, sex chromosomal effect, along with interactions among the three factors.
- Tissue specificity was prominent, with a major impact of estradiol on adipose tissue gene regulation and of testosterone on the liver transcriptome.
- The networks affected by the three sex-biasing factors include development, immunity and metabolism, and tissue-specific regulators were identified for these networks.
- The genes affected by individual sex-biasing factors and interactions among factors are associated with human disease traits such as coronary artery disease, diabetes, and inflammatory bowel disease.
- Identify tissue effects by sex-biasing factors on gene regulation with a broad impact on systemic metabolic, endocrine, and immune functions.

Blencowe et al, 2022, Ge

E

The role of sex in human complex traits

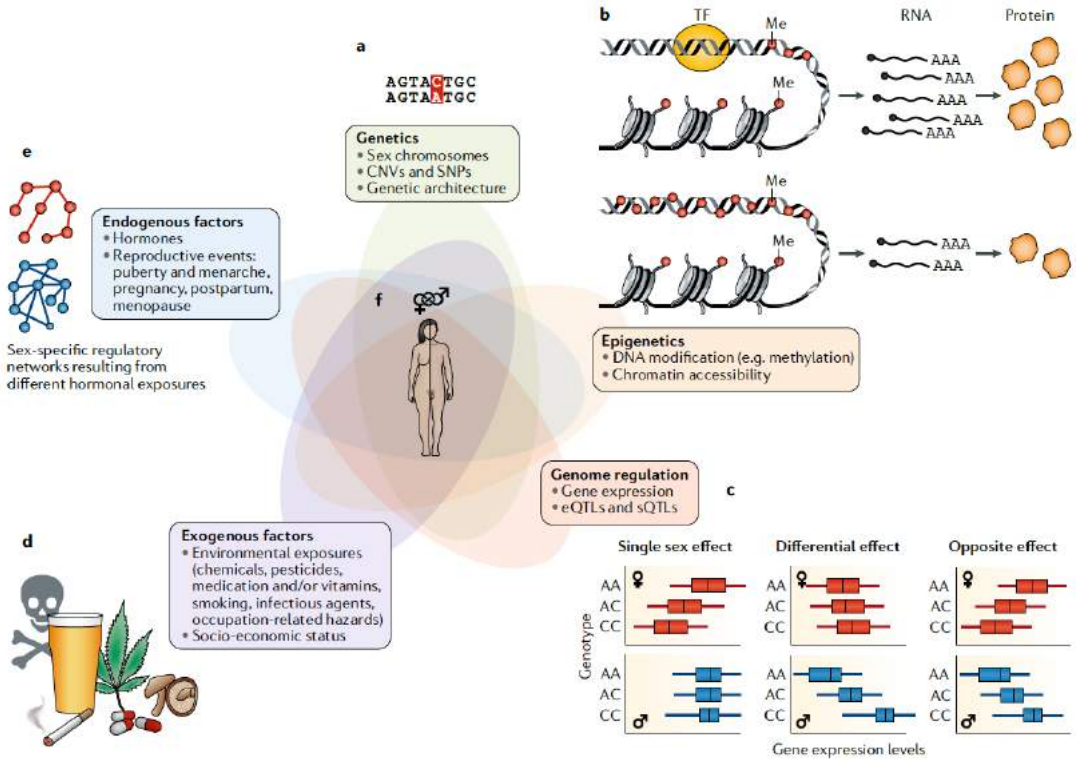
Throughout human life, sex is among the most important characteristics, but the role of sex in health and disease remains poorly understood.

Need to know effects of sex on disease risk, prognosis and treatment efficacy for true precision medicine.

Nearly all human complex traits and disease phenotypes exhibit some degree of sex differences, including in prevalence, age of onset, severity or disease progression.

Major challenges for studying sex differences include **adequate sample sizes** and **analytical tools**.

Advances in genomic technologies and analytical approaches are enabling a deeper investigation into the effect of sex on human health traits



Challenge to distinguish relative contributions of sex hormones, and sex chromosomal effects in humans compared to the FCG mouse and rat models

Khramtsova E et al, Nature Rev Genetic (2021)
https://doi.org/10.1038/s41576-018-0083-1

E. Heard, March 20th 2023

COLLÈGE DE FRANCE 1530

Evolutionary Conservation of Sex Differences in Gene Expression across Mammals

RESEARCH ARTICLE

COMPARATIVE GENETICS

RESULTS: Linear modeling revealed ~3000 genes with conserved (species-shared) sex bias in gene expression, most of which was tissue specific. The cumulative effects of conserved sex bias explain ~12% of the sex difference in mean human height, and cases such as that of *LCORL*, a TF with conservation of both female-biased expression and genetic association with height, suggest a contribution to sex differences in body size beyond humans. However, most sex-biased gene expression (~77%) was specific to single species or subsets of species, implying that it arose more recently during evolution. We identified 83 instances where TFs showed sex-biased expression in the same tissue, in which their motifs were associated with gain or loss of sex bias at other genes, accounting for a significant portion (~27%) of lineage-specific changes in sex bias.

Sex differences are widespread in humans and other mammals. Eg distribution of height or body size

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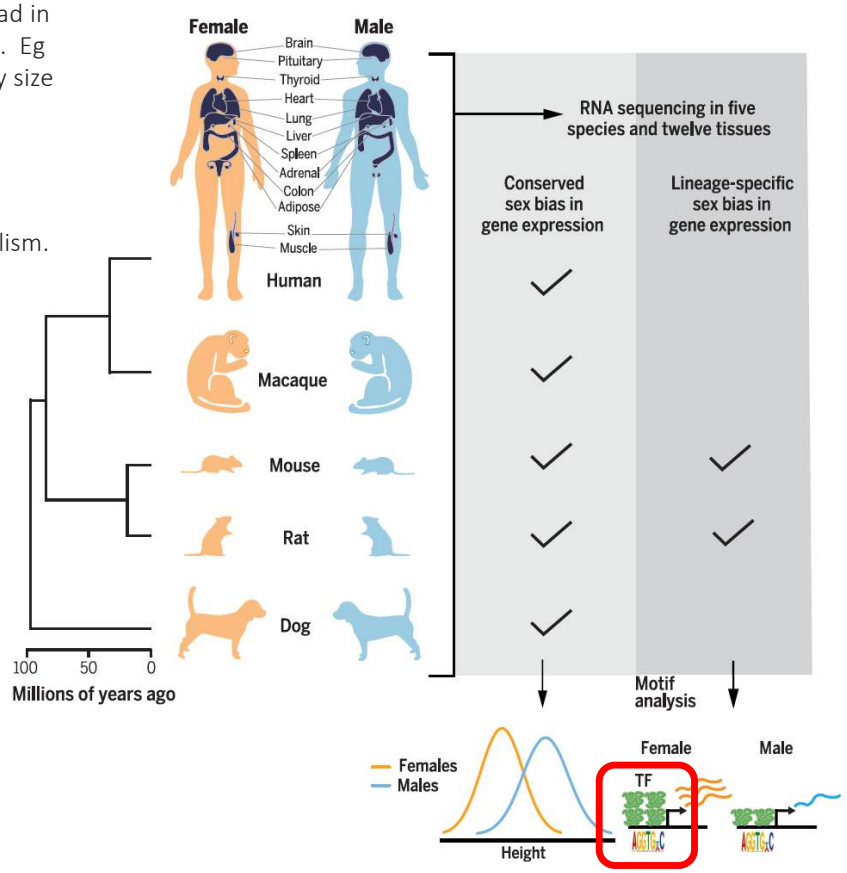


RNA sequencing of male and female samples in 12 tissues and five species reveals the functional impact and mechanistic underpinnings of sex-biased gene expression.

COLLÈGE DE FRANCE 1530

Evolutionary Conservation of Sex Differences in Gene Expression across Mammals

RESEARCH

RESEARCH ARTICLE

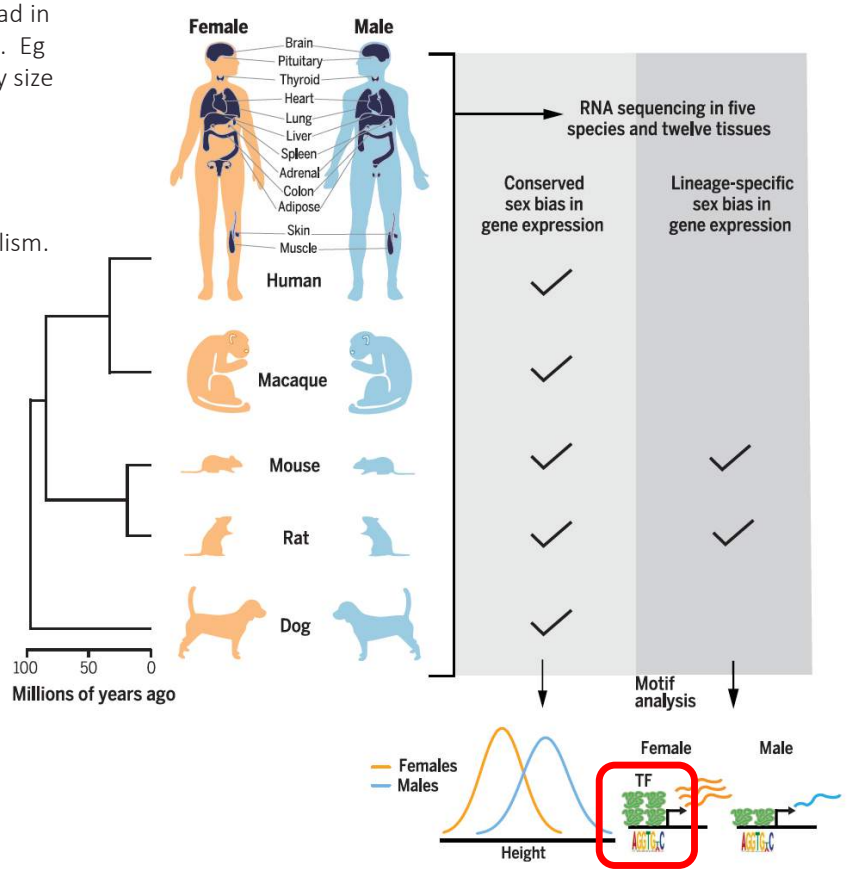
COMPARATIVE GENETICS

Conservation, acquisition, and functional impact of sex-biased gene expression in mammals

Sahin Naqvi[1,2], Alexander K. Godfrey[1,2], Jennifer F. Hughes[1], Mary L. Goodheart[1,3], Richard N. Mitchell[4], David C. Page[1,2,3]*

Sex differences abound in human health and disease, as they do in other mammals used as models. The extent to which sex differences are conserved at the molecular level across species and tissues is unknown. We surveyed sex differences in gene expression in human, macaque, mouse, rat, and dog, across 12 tissues. In each tissue, we identified hundreds of genes with conserved sex-biased expression—findings that, combined with genomic analyses of human height, explain ~12% of the difference in height between females and males. We surmise that conserved sex biases in expression of genes otherwise operating equivalently in females and males contribute to sex differences in traits. However, most sex-biased expression arose during the mammalian radiation, which suggests that careful attention to interspecies divergence is needed when modeling human sex differences.

Sex differences are widespread in humans and other mammals. Eg distribution of height or body size is shifted upwards in males relative to females, and sex differences are found in the immune and cardiovascular systems as well as in metabolism.



RNA sequencing of male and female samples in 12 tissues and five species reveals the functional impact and mechanistic underpinnings of sex-biased gene expression.

Most of the sex-biased TFs identified as contributing to lineage-specific evolution of sex bias are autosomal, suggesting that their sex bias is either due to trans-regulatory effects of sex chromosomes or sex hormones (_ie the levels of these TFs are influenced by X or X-linked gene products or by sex hormones_)

Also - changes in numbers of binding motifs for these TFs are associated with gains and losses of sex bias

E. Heard, March 20th 2023

COLLÈGE DE FRANCE 1530

The role of sex in human complex traits

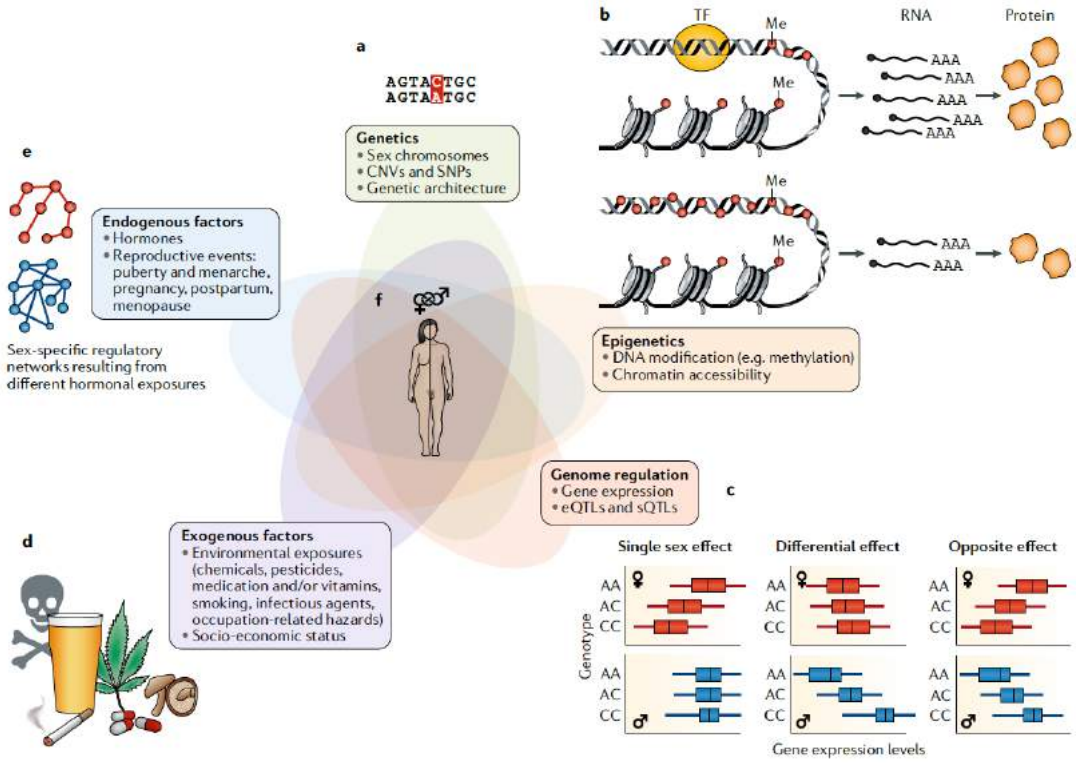
Throughout human life, sex is among the most important characteristics, but the role of sex in health and disease remains poorly understood.

Need to know effects of sex on disease risk, prognosis and treatment efficacy for true precision medicine.

Nearly all human complex traits and disease phenotypes exhibit some degree of sex differences, including in prevalence, age of onset, severity or disease progression.

Major challenges for studying sex differences include adequate sample sizes and analytical tools.
For example in GWAS studies, only recently have cohorts reached sufficient sample sizes to be well- powered for sex- stratified and interaction analysis.

Advances in genomic technologies and analytical approaches are enabling a deeper investigation into the effect of sex on human health traits



Khramtsova E et al, Nature Rev Genetic (2021)
https://doi.org/10.1038/s41576-018-0083-1

COLLÈGE DE FRANCE 1530

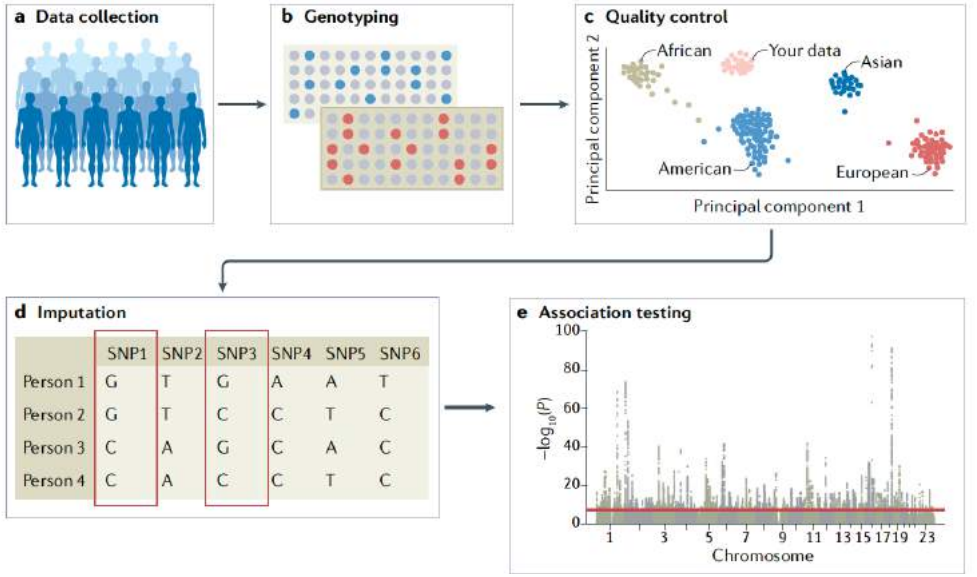
The role of sex in human complex traits

Genome-wide association studies

Emil Uffelmann[1], Qin Qin Huang[2], Nchangwi Syntia Munung[3], Jantina de Vries[3], Yukinori Okada[4,5], Alicia R. Martin[6,7,8], Hilary C. Martin[2], Tuuli Lappalainen[9,10,12] and Danielle Posthuma[1,11]

Abstract | Genome-wide association studies (GWAS) test hundreds of thousands of genetic variants across many genomes to find those statistically associated with a specific trait or disease. This methodology has generated a myriad of robust associations for a range of traits and diseases, and the number of associated variants is expected to grow steadily as GWAS sample sizes increase. GWAS results have a range of applications, such as gaining insight into a phenotype's underlying biology, estimating its heritability, calculating genetic correlations, making clinical risk predictions, informing drug development programmes and inferring potential causal relationships between risk factors and health outcomes. In this Primer, we provide the reader with an introduction to GWAS, explaining their statistical basis and how they are conducted, describe state-of-the art approaches and discuss limitations and challenges, concluding with an overview of the current and future applications for GWAS results.

The sex chromosomes contribute to the genetic basis of many sexually differentiated phenotypes but have historically been excluded from GWAS, mainly owing to the lack of statistical approaches to analyse the haploid Y chromosome (ChrY) and to account for dosage compensation and inactivation of the X chromosome (ChrX).



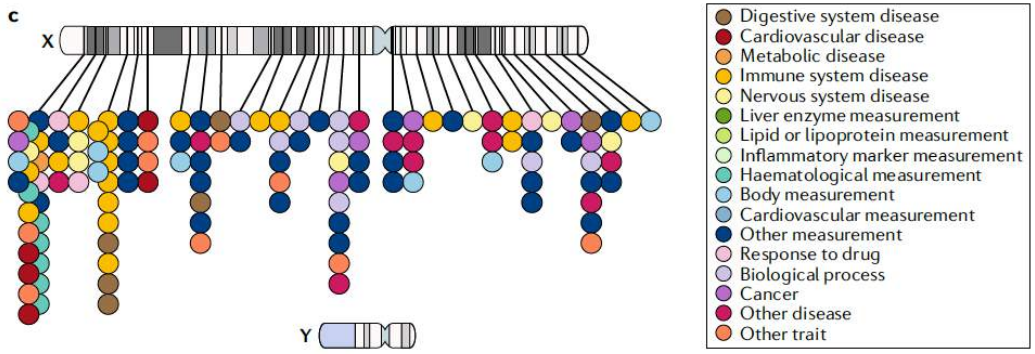


Fig. 3 | **GWAS loci identified on autosomes and the X chromosome. a** | Genome-wide association study (GWAS)

Khramtsova E et al, Nature Rev Genetic (2021)

Sex Differences in Gene Expression across Human Tissues

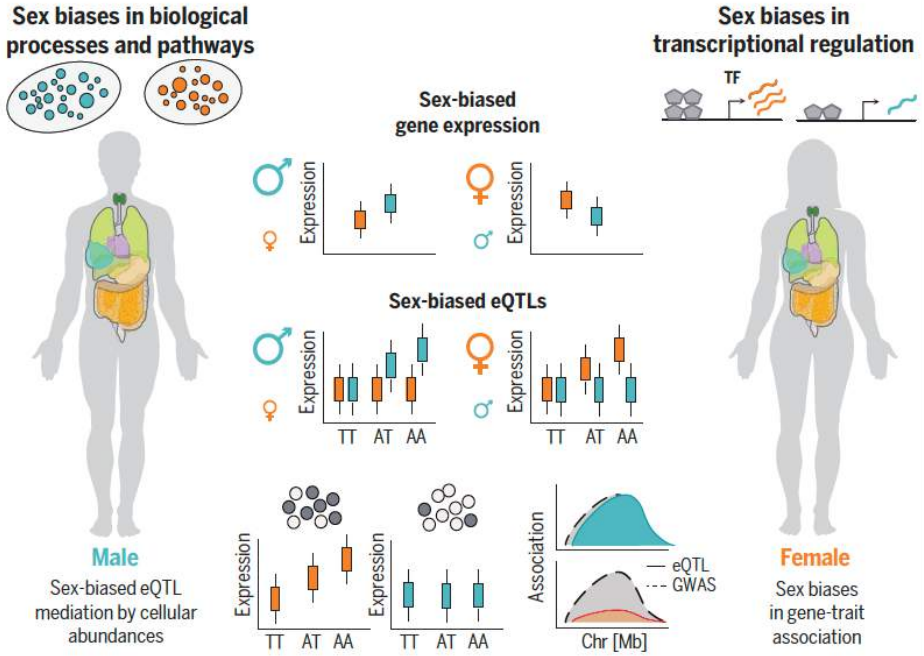
COMMENTARY

OPEN

The Genotype-Tissue Expression (GTEx) project

The GTEx Consortium*

Genome-wide association studies have identified thousands of loci for common diseases, but, for the majority of these, the mechanisms underlying disease susceptibility remain unknown. Most associated variants are not correlated with protein-coding changes, suggesting that polymorphisms in regulatory regions probably contribute to many disease phenotypes. Here we describe the Genotype-Tissue Expression (GTEx) project, which will establish a resource database and associated tissue bank for the scientific community to study the relationship between genetic variation and gene expression in human tissues.



- To create a data resource to enable the systematic study of genetic variation and the regulation of gene expression in multiple reference human tissues
- To provide the scientific community with a biospecimen resource including tissues, nucleic acids and cell lines upon which to determine other molecular phenotypes
- To support and disseminate the results of a study of the ethical, legal and social issues related to donor recruitment and consent
- To support the development of novel statistical methods for the analysis of human eQTLs, alone and in the context of other molecular phenotypes
- To make data available to the research community as rapidly as possible
- To support the dissemination of knowledge, standards and protocols related to biospecimen collection and analysis methods developed during the project

Sex Differences in Gene Expression across Human Tissues

RESEARCH ARTICLE SUMMARY

HUMAN GENOMICS

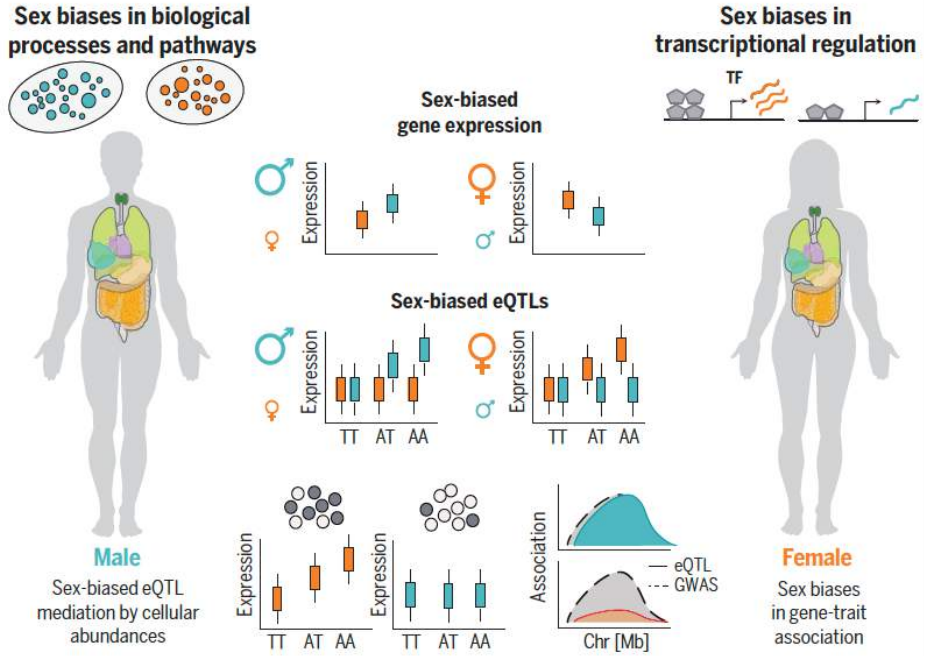
The impact of sex on gene expression across human tissues

Meritxell Oliva*†, Manuel Muñoz-Aguirre†, Sarah Kim-Hellmuth†, Valentin Wucher, Ariel D. H. Gewirtz, Daniel J. Cotter, Princy Parsana, Silva Kasela, Brunilda Balliu, Ana Viñuela, Stephane E. Castel, Pejman Mohammadi, François Aguet, Yuxin Zou, Ekaterina A. Khramtsova, Andrew D. Skol, Diego Garrido-Martín, Ferran Reverter, Andrew Brown, Patrick Evans, Eric R. Gamazon, Anthony Payne, Rodrigo Bonazzola, Alvaro N. Barbeira, Andrew R. Hamel, Angel Martinez-Perez, José Manuel Soria, GTEx Consortium, Brandon L. Pierce, Matthew Stephens, Eleazar Eskin, Emmanouil T. Dermitzakis, Ayellet V. Segrè, Hae Kyung Im, Barbara E. Engelhardt, Kristin G. Ardlie, Stephen B. Montgomery, Alexis J. Battle, Tuuli Lappalainen, Roderic Guigó, Barbara E. Stranger*

Many complex human phenotypes exhibit sex differentiated characteristics.

The molecular mechanisms underlying these differences remain largely unknown.

Not able to distinguish relative contributions of sex hormones, and sex chromosomal effects in humans unlike with the FCG mouse and rat models



Sex effects on gene expression (and genetic regulation) were measured in 44 GTEx human tissue sources and integrated with genotypes of 838 subjects

Oliva et al., Science 369, 1331 (2020)

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Sex Differences in Gene Expression across Human Tissues

RESEARCH ARTICLE SUMMARY

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The impact of sex on gene expression across human tissues

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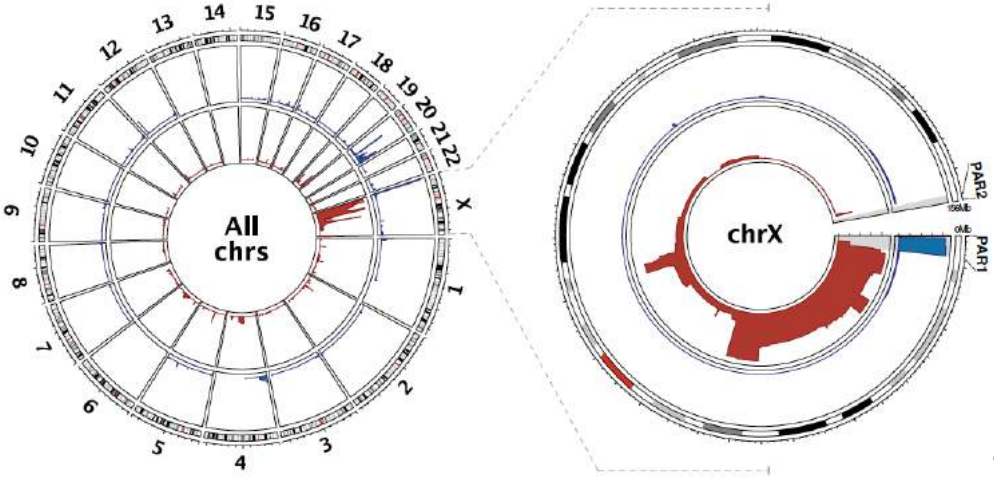
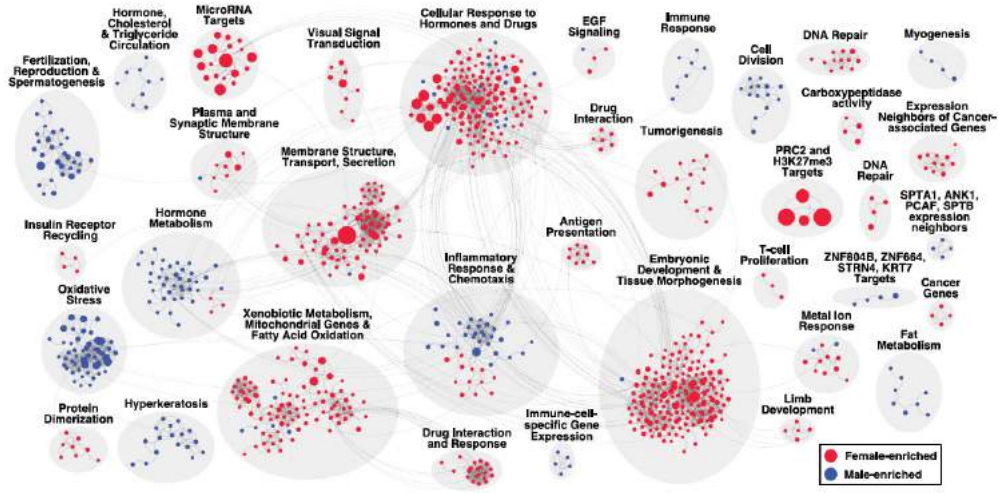
- Sex affects gene expression and its genetic regulation across tissues.
- Sex effects on gene expression were measured in 44 GTEx human tissue sources and integrated with genotypes of 838 subjects.
-

- Sex effects on gene expression were ubiquitous but small
- Sex-biased gene expression was largely tissue-specific *(only 30 genes (0.23%), 22 of which are known constitutive XCI escapees, exhibited consistent sex bias across all 44 tissues).*
- Sex and disease influence tissue cellular composition
- X-linked female-biased genes accurately predict sex and suggest tissue-specific candidates for escape from X-chromosome inactivation
- A total of 37% genes exhibit sex-biased expression in at least 1 tissue(4% were X-linked ie 531 (~50% X genes)
- Promoters of sex-biased genes are enriched for hormone-related and other TF binding sites (2 out of 92 are X-linked AR and ELK1)
- Sex differences in the genetic regulation of gene expression are highly tissue-specific and less common than sex effects on gene expression
- Sex-biased expression quantitative trait loci in cis (sex-biased eQTLs) are partially mediated by cellular abundances and reveal gene trait associations.
- Sex-biased expression is present in numerous biological pathways and is associated to sex-differentiated transcriptional regulation.





The forgotten sex chromosomes....

Just as females were excluded in scientific research cohorts etc similarly the sex chromosomes have been excluded from GWAS and other genomic approaches aimed at identifying heritability of different traits and diseases

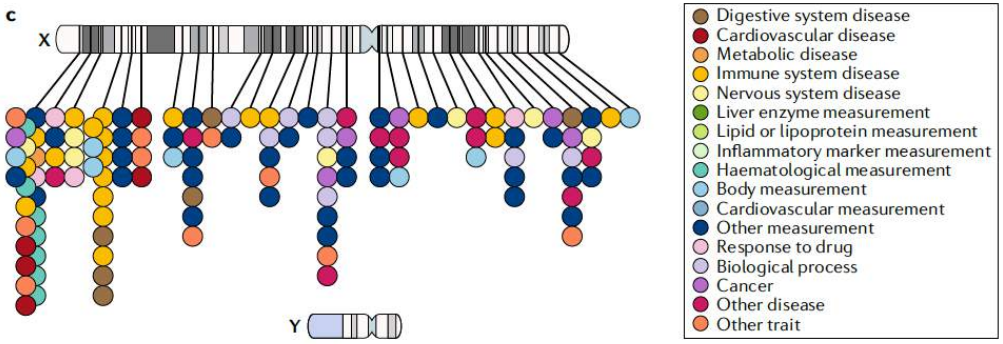


Fig. 3 | **GWAS loci identified on autosomes and the X chromosome. a** | Genome-wide association study (GWAS)

nature medicine

EDITORIAL

2017

Accounting for sex in the genome

Genetic association studies of the human genome often omit the X chromosome because of the unique analytical challenges it presents. A concerted effort to undo this exclusion could offer medically relevant insights into basic biology that might otherwise be missed.

The X chromosome makes up about 5% of the haploid human genome, and carries just around 800 protein-coding genes out of our total of 20,000 such genes. Even so, in some genetics research, the X chromosome has featured prominently: mutations within it contribute to almost 10% of Mendelian disorders. There is also a broad appreciation that certain illnesses, such as depression and most autoimmune diseases, occur more often in females than in males, suggesting an influence of the X chromosome (either directly or indirectly). Likewise, other diseases, such as autism, are more commonly diagnosed in males, underscoring that sex chromosomes might exert a significant influence on health. Despite these insights, the X chromosome is often less scrutinized in the era of population genetics analyses because of the unique statistical challenges it presents.

A literature search published several years ago found that just 242 among 743 genome-wide association studies (GWAS) in the review included the X chromosome in their analyses. It's not surprising then that, of the almost 300 traits explored using GWAS, only 15 of the 2,800 significant variants, or 0.5%, were reported on the X chromosome[1]. This disparity was highlighted earlier this year by Whitehead Institute Director David C. Page at the Keystone Symposia's meeting on Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity. Riffing on the observed shrinking of the Y chromosome over time, Page remarked that, whereas it may take ten million years for the Y chromosome to disappear, it has taken only ten years of GWAS for the X chromosome to do so.

In the past, genotyping chips contained very few X-chromosome markers, which created a bottleneck on data. This has since improved, but the significance of variants on the X chromosome still remains harder to assess than for variants on autosomal chromosomes. One reason is simply that there are two copies of X in women and one in men, so the signals for variants on this chromosome obtained with standard array genotyping platforms are comparatively lower for men. Another reason is the phenomenon of X inactivation—the process by which one of the two X chromosomes is randomly silenced in women's cells. It is not yet possible for standard sequencing technologies to discern which genetic variants are on the silenced version of the X chromosome. To make matters more compli-

more often in men than in women, another group showed that deleting *Cosmc* in mice contributes to gut inflammation in males but not in females. The study also reported gut microbiome changes in the rodents resulting from loss of *Cosmc*[4]. A follow-up published earlier this year characterized functional domains of the COSMC chaperone protein[5]. It's too early to say whether these initial insights will pave the way forward for new IBD therapeutics, but they offer an interesting starting point for exploration. This example underscores that there may be a lot to be gained in terms of discovering drug targets by including sex chromosomes in analyses.

The apparent exclusion of the X chromosome extends to association analyses conducted with more recent DNA sequencing tools as well. As of May 2016, of the 41 published genetic association studies for complex traits using whole-genome or whole-exome sequencing data, 25 completely omitted the X chromosome from their analyses. The majority of the remaining 16 did not apply any specialized computational and statistical methods to accurately scrutinize the X chromosome for genetic variants associated with disease (A. Keinan, personal communication).

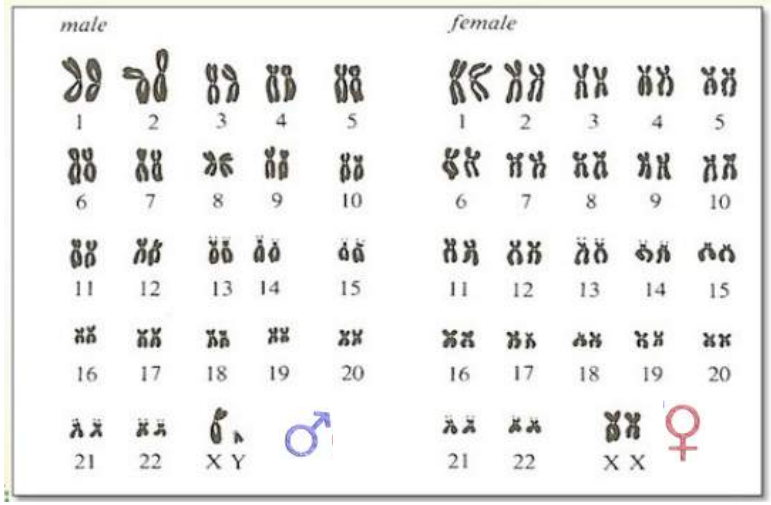
It is not just the X chromosome that has been neglected in genetic analyses. A review paper last year noted that the Y chromosome is "too often ignored by researchers but could potentially be the key to understanding the [coronary artery disease] prevalence differences between men and women" (ref. 6). And it's not only genetic association studies that need to take the influence of sex chromosomes into consideration. The agenda for a workshop facilitated by the US National Institutes of Health late last month on sex as a biological variable included a session on sex differences in gene expression. Studies in the last couple of years have, in fact, begun to explore the influence of the X chromosome and sex on gene expression[7,8]. Earlier this summer, a study appearing in this journal offered a characterization of male and female transcriptional profiles associated with major depressive disorder across six brain regions[9]. These findings form an important starting point for understanding such associations, but there are relatively few publications as of yet and plenty of room for far more study in this area.

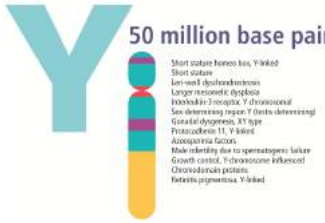
The failure to assess the influence of sex chromosomes in studies of the genome doesn't necessarily boil down to a lack of tools; there is also a

All of the myriad differences between human males and females—from anatomy to disease susceptibility—arise from differences in the genes of the X and Y chromosomes that appeared as these chromosomes diverged in gene content from their autosomal ancestors (D. Page, Nature 2014)

Khramtsova E et al, Nature Rev Genetic (2021)
https://doi.org/10.1038/s41576-018-0083-1

Human Sex Chromosomes



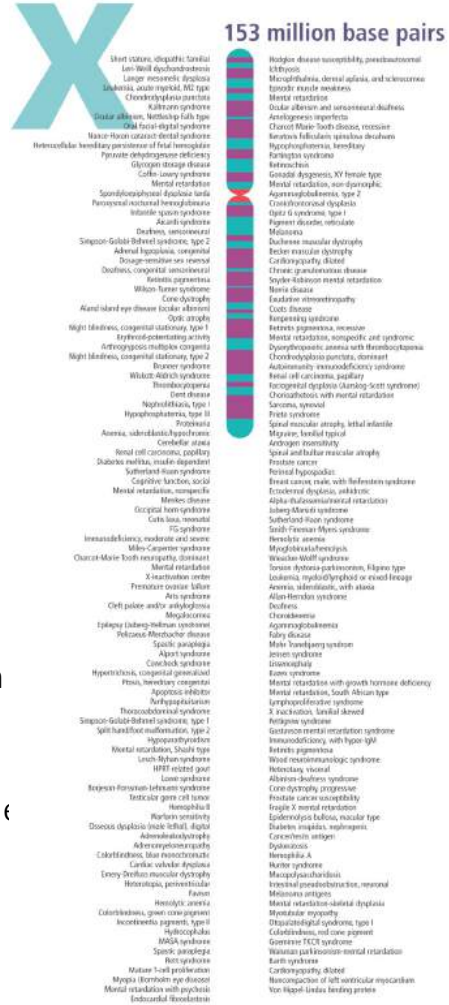


X: >1000 genes

Y: ~100 genes

Sry (testis determinant factor)

Eif2s3y (spermatogenesis)

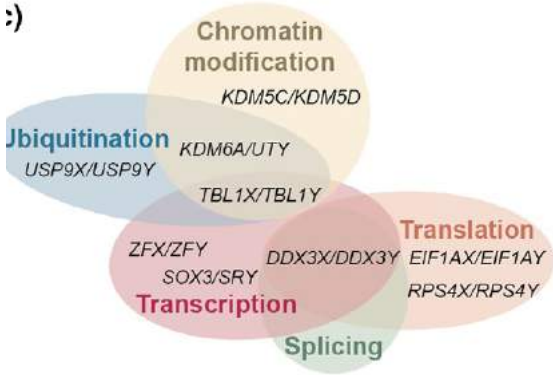


- Humans normally have 46 chromosomes: 23 pairs, one set from each parent
- Includes 1 pair of sex chromosomes, the X and the Y or the inactive X and the active X
- The sex chromosomes lead to dramatic differences in gene content and expression
- To accommodate this imbalance, dosage compensation mechanisms have evolved
- X-chromosome inactivation is the process that leads to silencing of almost all genes on one X in individuals with more than one X
- Sex chromosomes also evolved functional specialization of their gene content
- Accumulation of male-beneficial genes on the Y chromosome is probably due to its sole transmission in males
- Intriguingly, the X chromosome also has a special complement of genes
- Sex chromosomes evolve as a consequence of sexual reproduction (enabling sex determination and gonad differentiation)
- But their genes also influences non-gonadal tissues with implications for diseases

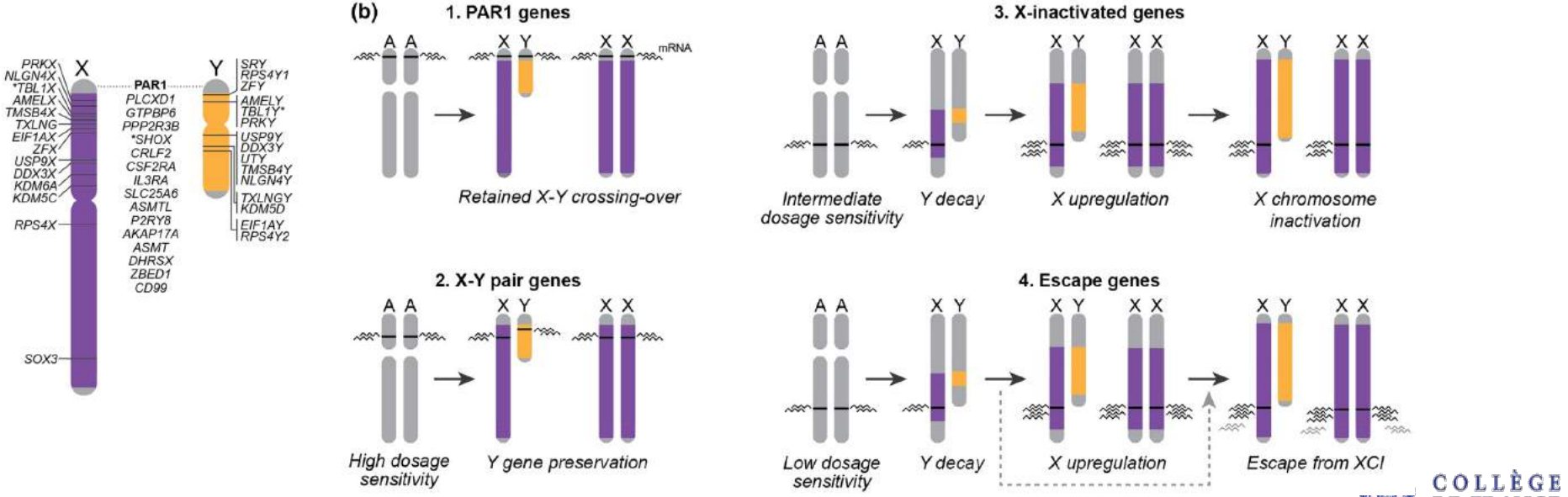
Evolution of the Human Sex Chromosomes

- Of the 17 surviving ancestral genes on the human Y chromosome, four (SRY, RBMY, TSPY, and HSFY) have clearly diverged in function from their X homologues (SOX3, RBMX, TSPX and HSFX) to play male-specific roles in reproductive development or gametogenesis.
- All genes on the Y chromosome were exposed to selection only in males, even widely expressed ancestral genes may exhibit subtle functional differences from their X-linked homologues.
- Particularly interesting are eight global regulators of gene activity that exist as X-encoded and Y-encoded (male-specific) protein isoforms in diverse human tissues.
- These exemplify a fundamental sexual dimorphism, at a biochemical level, throughout the human body, that derives directly from genetic differences between the X and Y chromosomes –

:)



UTX/UTY, EIF1AX/EIF1AY, ZFX/ZFY, RPS4X/RPS4Y1, KDM5C/ KDM5D, DDX3X/DDX3Y, USP9X/USP9Y and TBL1X/TBL1Y



E. Heard, March 20th 2023

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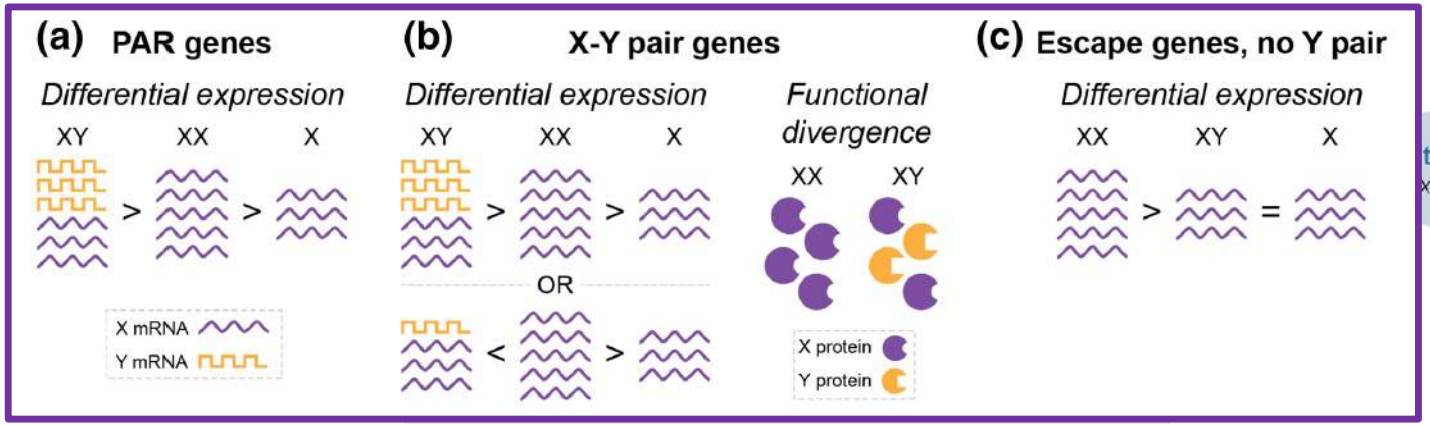
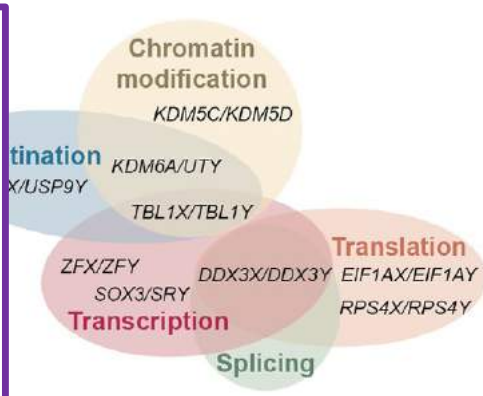
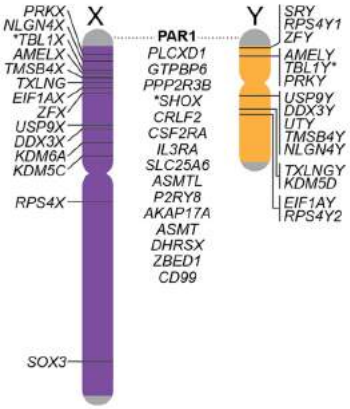
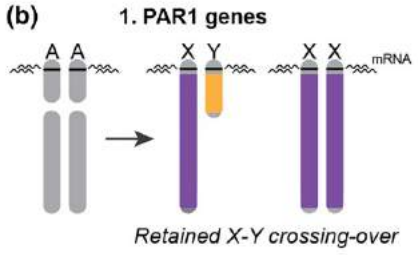
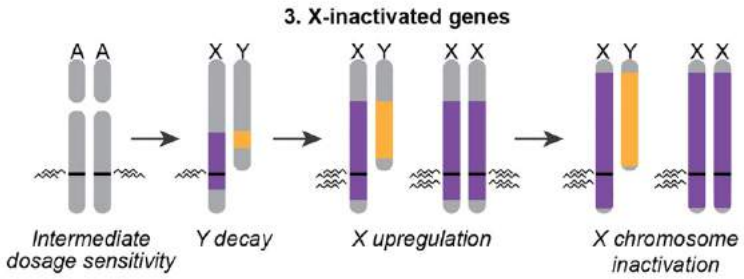
Human Sex Chromosomes lead to Sex Differences in Gene Expression

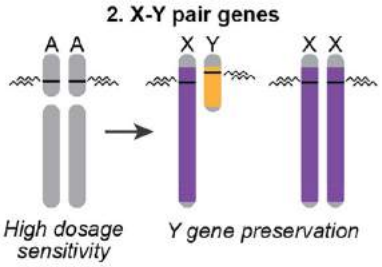


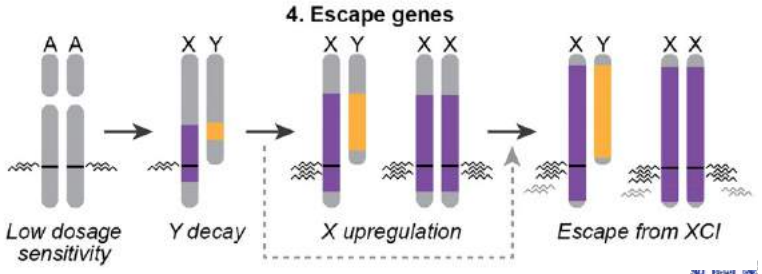












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Turner's Syndrome (XO)

Genes or hormones?

Women with Turner's syndrome have reduced estrogen levels compared to 46,XX females (Turner, 1938).

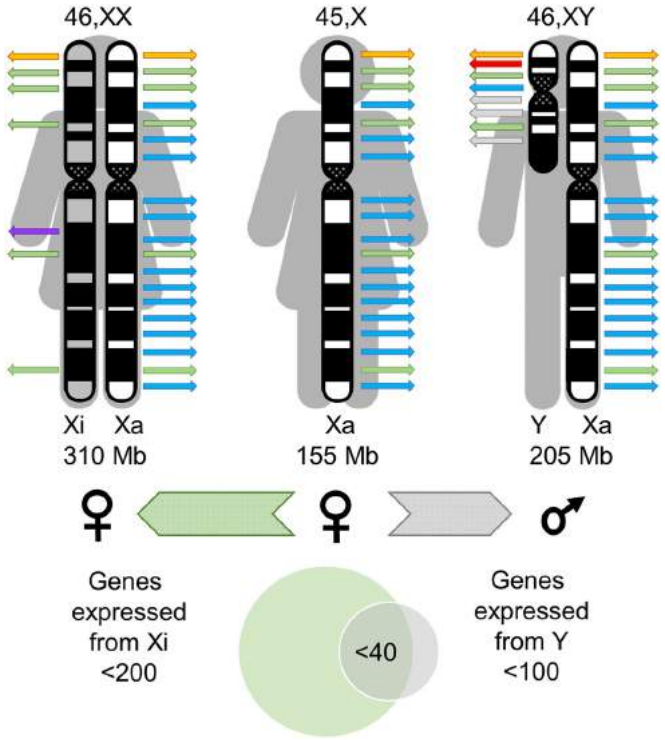
Mosaicism is common in TS

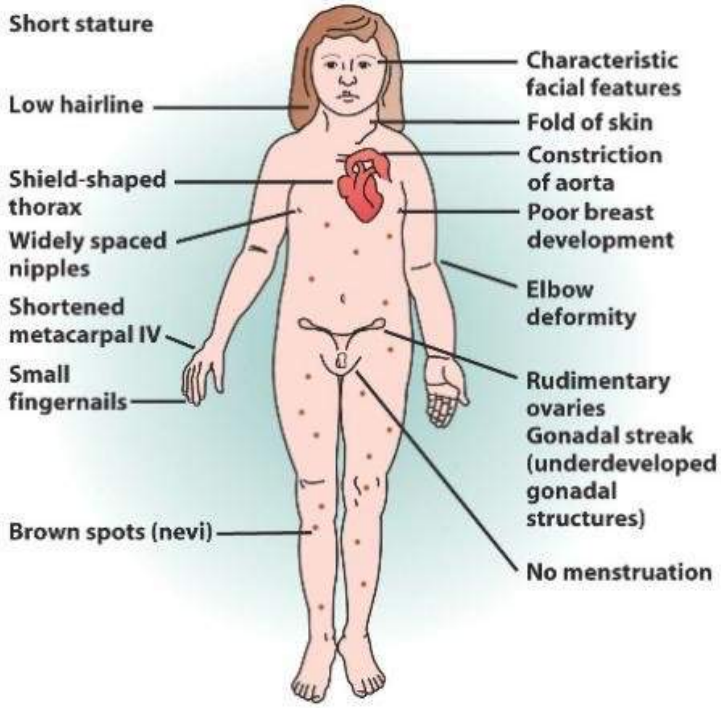
In fact it is thought that all women with TS must be mosaic for cells of another karyotype, such as 46,XX, because 99% of fetuses with a complete 45,X karyotype do not survive fetal development (Hook & Warburton, 1983, 2014)

Mouse model for TS?

XO mice do not recapitulate infertility, embryo lethality or the phenotypes present in TS.

7 of the 14 X-Y pair genes are not present on mouse Y. Almost all of the human PAR genes are on mouse autosomes or missing in mouse genome.





Turner syndrome, a condition that affects only females, **results when one of the X chromosomes (sex chromosomes) is missing or partially missing**.

Turner syndrome can cause a variety of medical and developmental problems, including short height, failure of the ovaries to develop and heart defects.

Peeters et al, 2018

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Differences in XX, XO and XY Development

The genetic basis of XX-XY differences present before gonadal sex differentiation in the mouse

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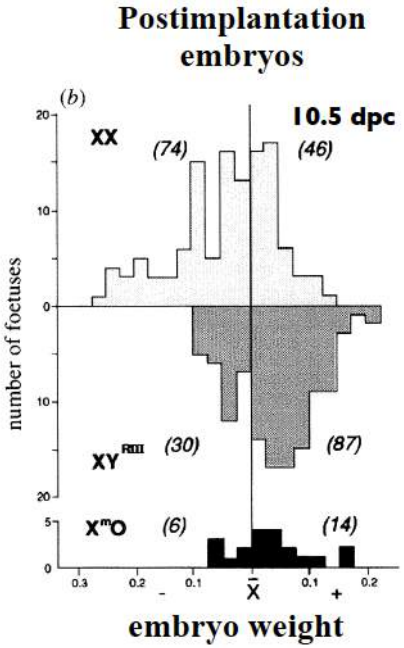
[2] *MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, U.K.*
[3] *Institute of Child Health, 30 Guilford Street, London WC1N 1EH, U.K.*
[4] *Department of Anatomy & Developmental Biology, University College London, Gower Street, London WC1E 6BT, U.K.*
[5] *INSERM U242, Faculté de Médecine, 27 Boulevard Jean Moulin, 13385 Marseilles Cedex 5, France*
[6] *MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.*

SUMMARY

There is now a substantial body of data showing that in eutherian mammals (mouse, rat, cow and man) XY conceptuses are developmentally more advanced (and consequently larger) than XX conceptuses of equivalent gestational age. This developmental difference is already discernible in the preimplantation period and it has been suggested that the more advanced development of XY embryos may be a consequence of the preimplantation expression of Y chromosomal genes such as *Sry* or *Zfy*. In the present paper sex-chromosomally variant mice were used to analyse the genetic basis of XX-XY differences as manifest at 10.5 days *post coitum*. The results show that the XX-XY difference is due to a combination of a Y chromosome effect and an effect of the difference in X chromosome constitution (2X v 1X). The Y effect is not dependent on the presence of *Sry*. In the light of this and other studies, it is concluded that the Y chromosome of most mouse strains carries a factor which accelerates preimplantation development and that the resulting developmental advantage is carried over into the postimplantation period. The retarding effect of two X chromosomes is then superimposed on this Y effect subsequent to the blastocyst stage but prior to 9.5 days *post coitum*.

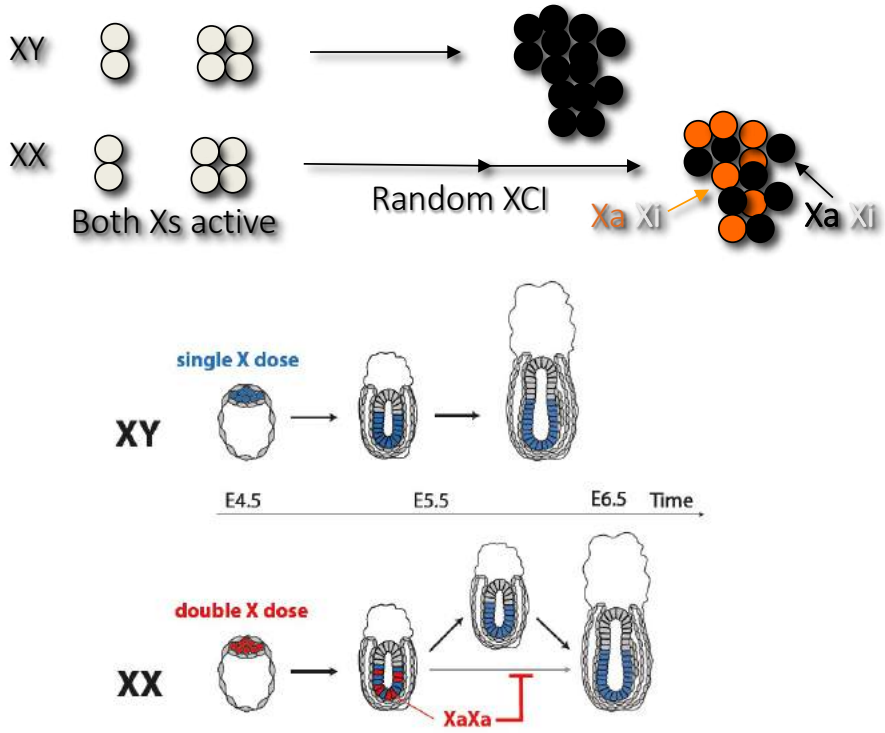


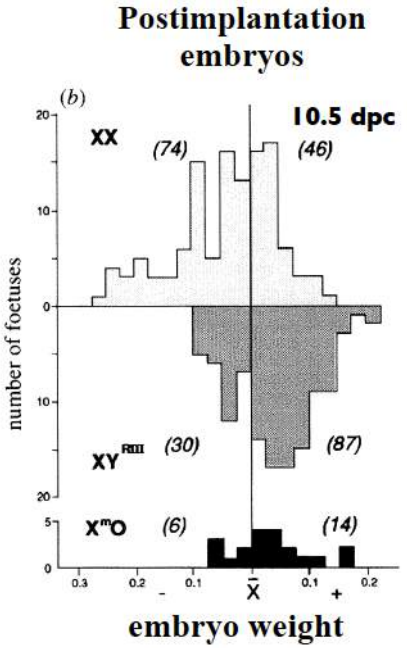
Burgoyne et al, *The genetic basis of XX-XY differences present before gonadal sex differentiation in the mouse*, Philosophical transactions: Biological Sciences, 1995

Delayed post-implantation development of XX embryos compared to XY or XO
(Scott and Holson, 1977; Burgoyne et al, 1995)

E. Heard, March 20th 2023

Differences in XX, XO and XY Development





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Slower differentiation of XX ESCs compared to XO or XY ESCs

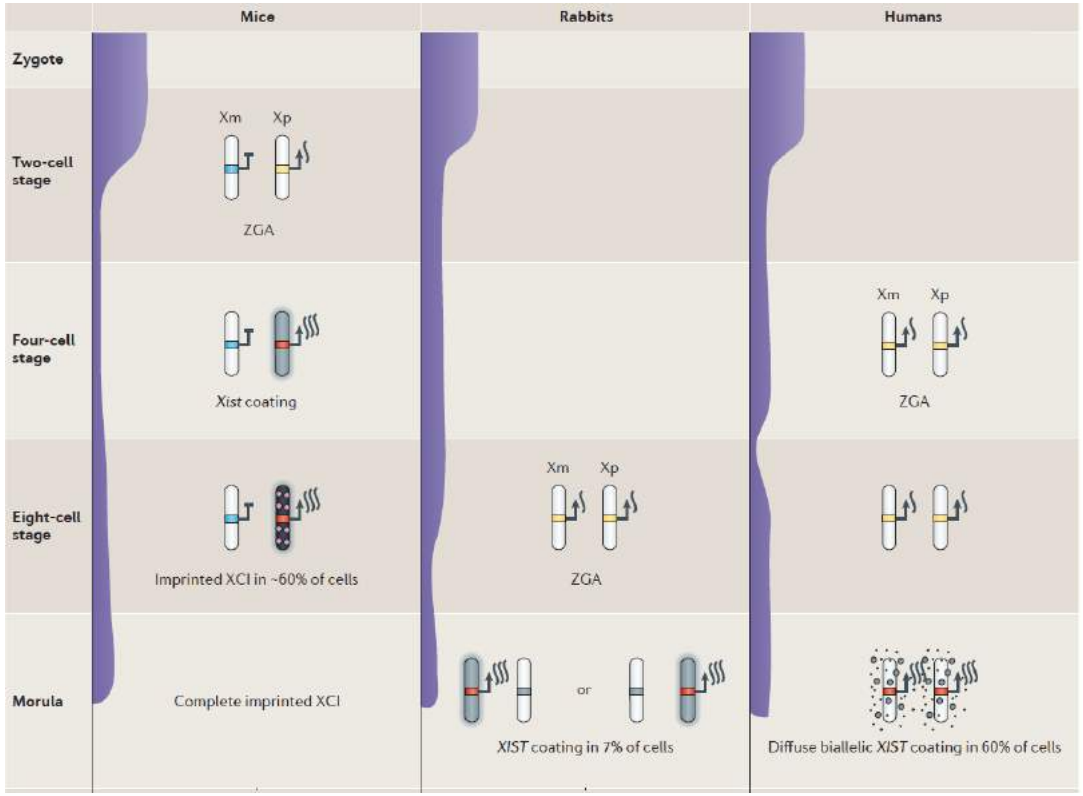
Two active X chromosomes inhibit Fgf/MAPK signaling
=> slow down exit from pluripotency (Schulz et al, 2014)

Delayed post-implantation development of XX embryos compared to XY or XO

(Scott and Holson, 1977; Burgoyne et al, 1995)

E. Heard, March 20th 2023

Sex differences in early (pre-gonadal) development

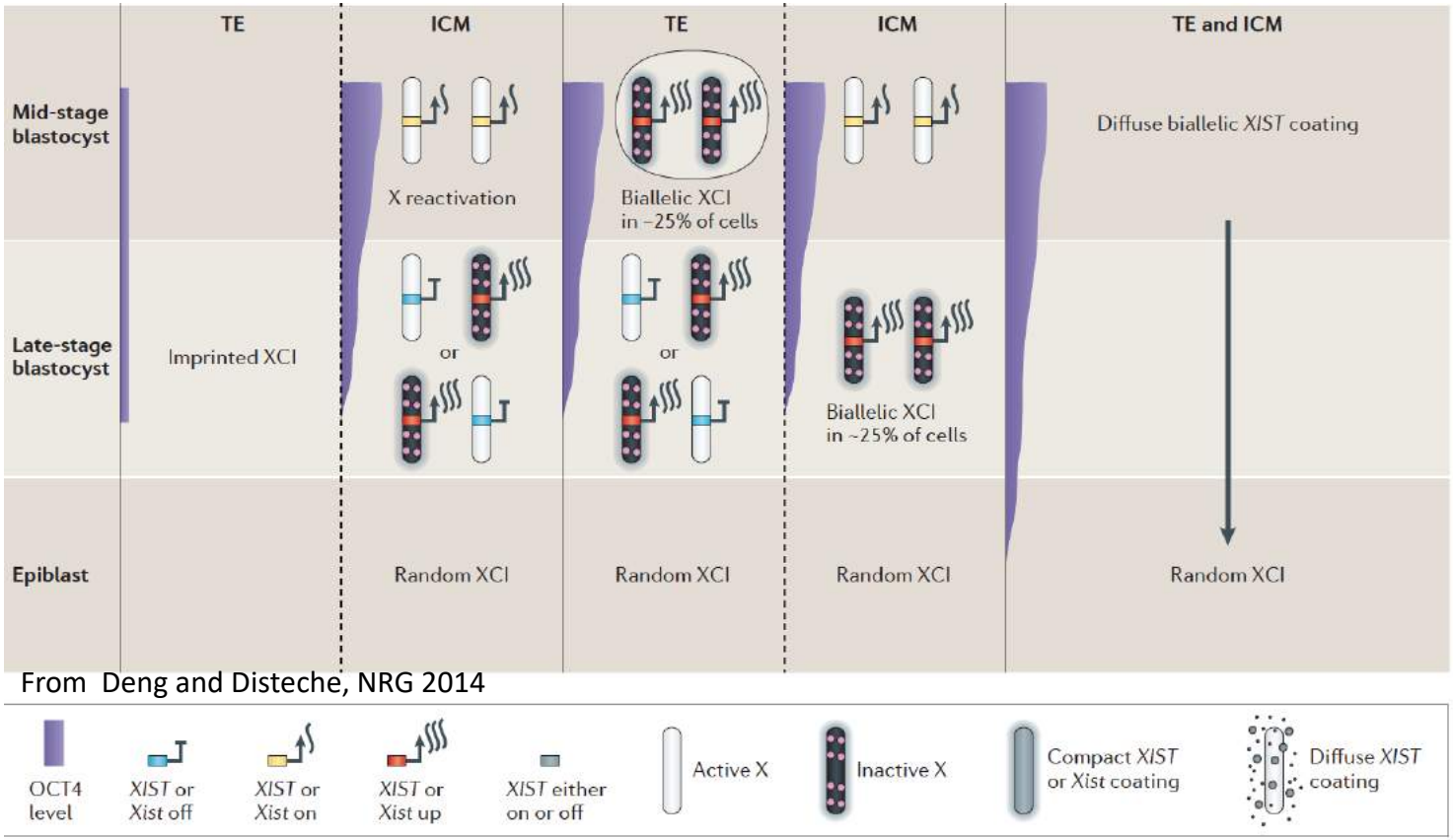


From Deng and Disteche, NRG 2014

Very different timing of X-linked dosage compensation between different mammaliam species! (more next week)

See Okamoto et al, 2011; Okamoto et al, 2021 and Rougeulle and Heard for review

Sex differences in early development



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Different categories of X and Y genes can cause phenotypic differences between XX and XY cells and tissues

Sex differences in non-gonadal tissues and diseases that are currently known or suspected in XX vs. XY cells due to inherent imbalance of representation of sex chromosome genes:

(1) The male-specific expression of Y genes causes XY cells to differ from XX cells

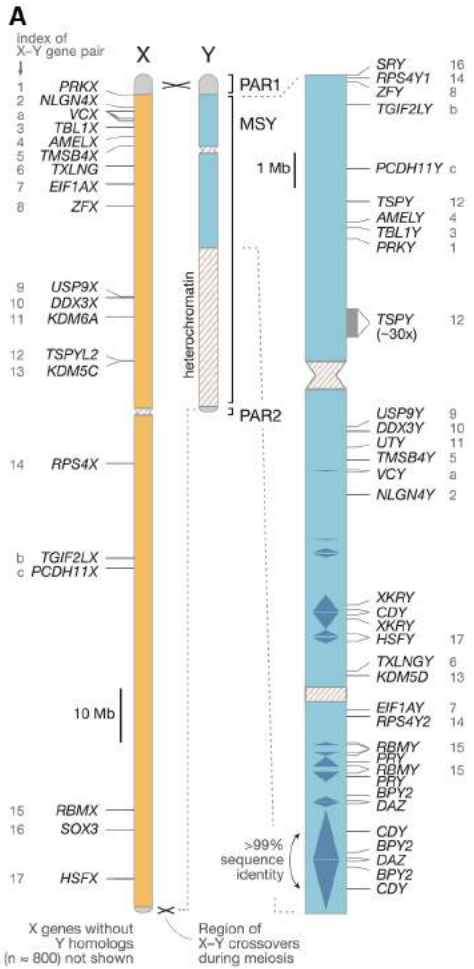
(2) The constitutive difference in number of X chromosomes leads to four classes of X genes that underlie sex differences in phenotype:

- Genes on the X that escape XCI are expressed higher in XX than XY cells
- Genes in the pseudoautosomal regions (PAR) of the sex chromosomes are often expressed at higher levels in XY cells, relative to XX cells, because some of them are subject to X inactivation in XX cells
- X:Y homologs whose tissue/developmental regulation may have diverged

Table 1. Published evidence for functional equivalence or difference of proteins encoded by widely expressed, ancestral X–Y gene pairs

X–Y Pair	a.a. % id.	Evidence supporting at least partial equivalence	Evidence supporting difference[a]
KDM6A/UTY	86%	*Uty* rescues inviability of *Kdm6a*-knockout mice (Lee et al. 2012; Shpargel et al. 2012; Welstead et al. 2012). Concomitant loss of *KDM6A* and *UTY* in cancer (van Haaften et al. 2009; Gozdecka et al. 2018). KDM6A and UTY demethylate trimethylated histone 3 lysine 27 in vitro (Walport et al. 2014).	Compared with KDM6A, UTY shows substantially reduced or absent demethylase activity in vitro and in cellular assays (Hong et al. 2007; Lan et al. 2007; Shpargel et al. 2012; Walport et al. 2014).
KDM5C/KDM5D	87%	KDM5C and KDM5D demethylate di- and trimethylated histone 3 lysine 4 in vitro (Iwase et al. 2007). *Kdm5d* rescues inviability of *Kdm5c*-knockout mice (Kosugi et al. 2020).	Compared with KDM5C, KDM5D shows reduced demethylase activity in vitro (Iwase et al. 2007).
USP9X/USP9Y	91%	—	—
DDX3X/DDX3Y	92%	Human *DDX3X* and *DDX3Y* rescue cell proliferation defect conferred by *Ddx3x* mutation in hamster cell line (Sekiguchi et al. 2004). *DDX3Y* is essential for cell proliferation in a lymphoma cell line with a truncating mutation in *DDX3X* (Wang et al. 2015).	—
PRKX/PRKY	92%	—	—
RPS4X/RPS4Y1	93%	Human *RPS4X* and *RPS4Y1* rescue cell proliferation defect conferred by *Rps4x* mutation in hamster cell line (Watanabe et al. 1993).	—
ZFX/ZFY	93%	—	—
EIF1AX/EIF1AY	99%	—	—
NLGN4X/NLGN4Y	99%	—	—

(a.a. % id.) Percentage amino-acid sequence identity (Skaletsky et al. 2003); dashes indicate an absence of published evidence, to our knowledge.
[a]A functional "difference" could include quantitative differences in the same protein function (e.g., differences in enzymatic activity) or qualitatively distinct protein functions.



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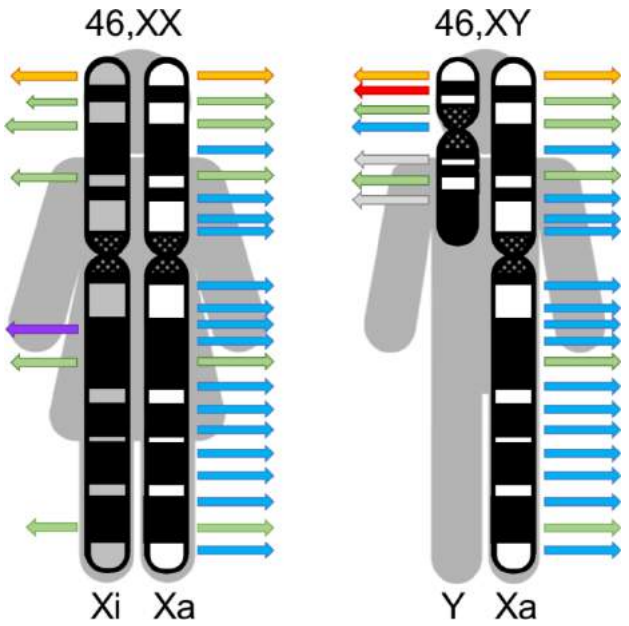
- X:Y homologs whose tissue/developmental regulation may have diverged
- X genes that have a parental imprint can be expressed at a higher or lower level in XX vs. XY cells
- The difference in epigenetic regulation of the X and Y chromosomes could impact the epigenetic status of the autosomes (heterochromatic Xi present only in XX cells might sequester heterochromatizing factors and reduce their availability for autosomal heterochromatin, affecting autosomal gene expression.

(3) XY males have **hemizygous exposure of X alleles**, so that they express X allelic variations more prominently than XX females. XY individuals with X-linked lethal alleles are removed from the population, and shift the mean phenotype of males vs. females.

(4) XX individuals are mosaic for X alleles and X gene imprints, because the parental origin of the active X varies among XX cells but not among XY cells. The mosaicism itself can have a protective effect for XX but not XY tissues.



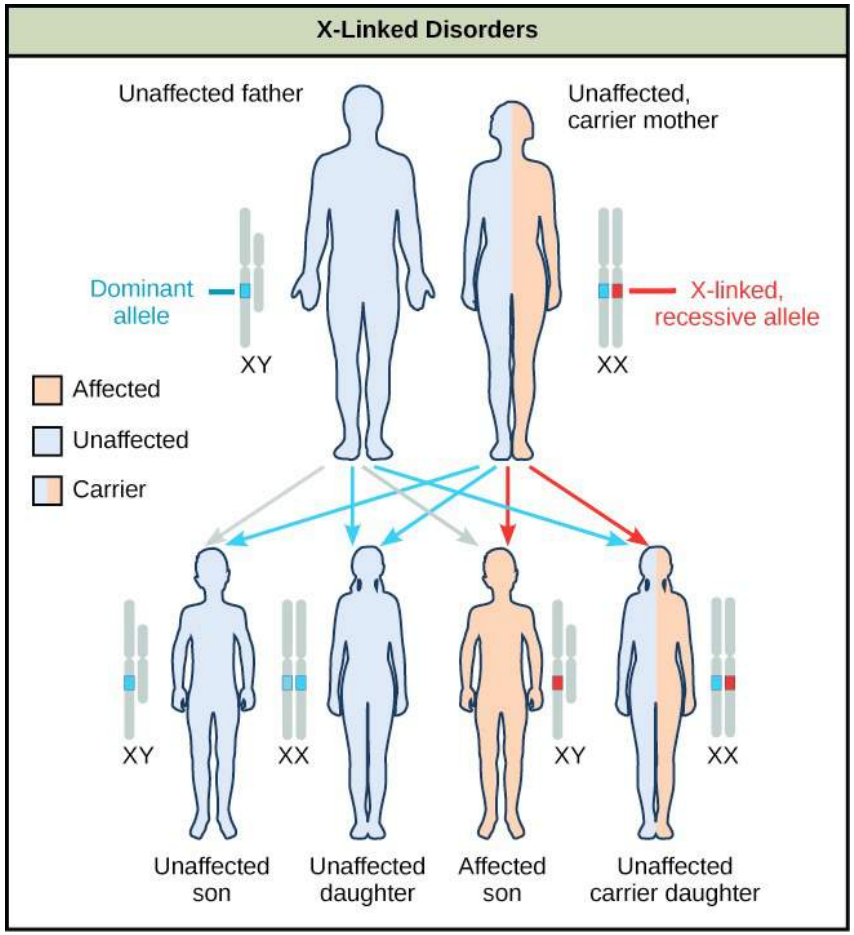
Adapted from Peeters et al, 2018

Adapted from: Rethinking sex determination of non-gonadal tissues (Arthur P. Arnold)

Sex Differences due to X-linked Mutations

Severe phenotypes or lethality in males due to haploid state (no second copy)
Variable and sometimes no phenotypes in females

Eg Fragile X syndrome, Haemophilia, muscular dystrophy, Incontinentia pigmenti



COLLÈGE DE FRANCE 1530

X-linked genes implicated in Human Diseases

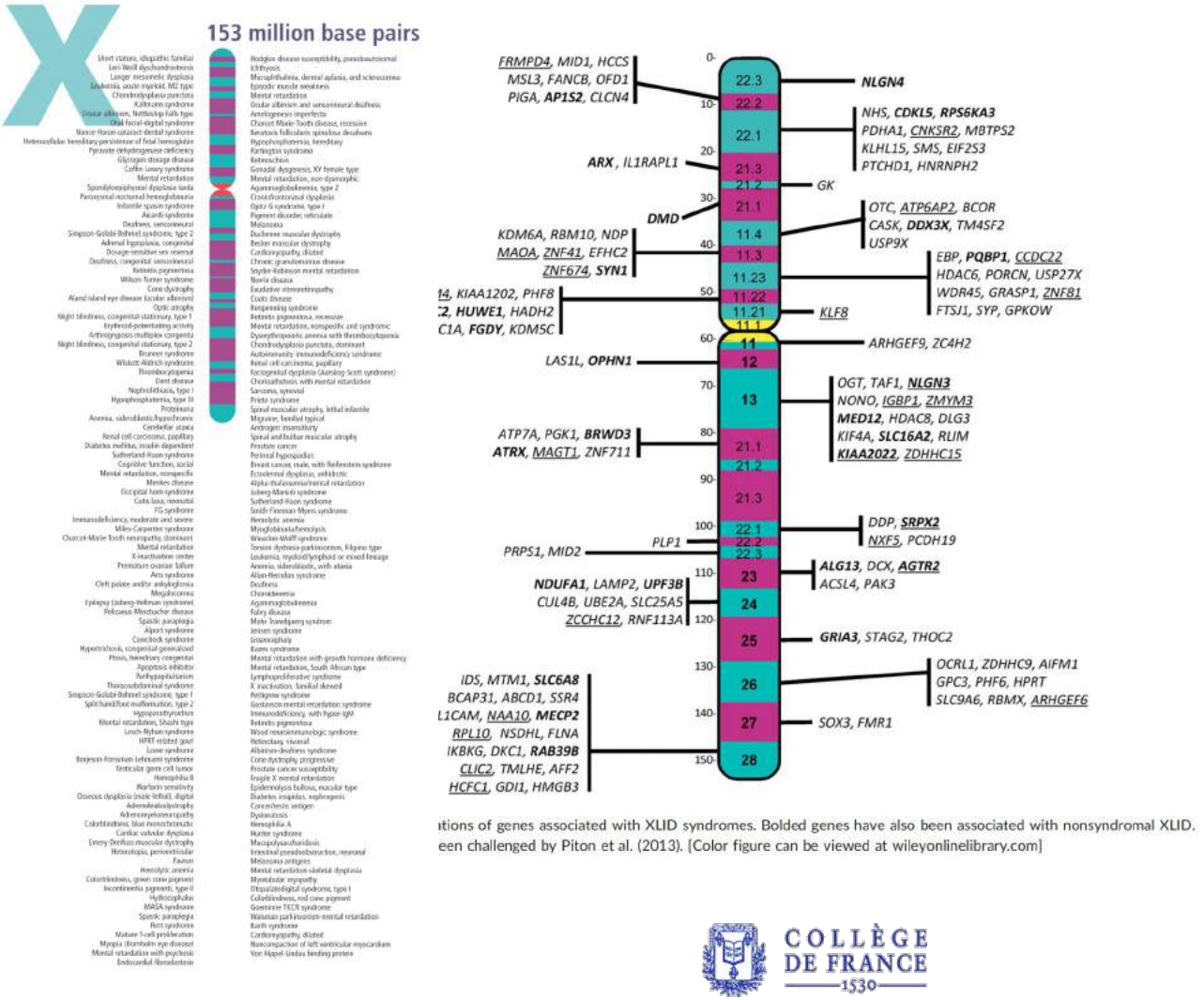
Genes expressed in the brain especially in the cortex and the hypothalamus are enriched on the X chromosome, a phenomenon probably driven by sexual selection. (COURS 2018)

The X-chromosome comprises only about 5% of the human genome

It accounts for about 15% of the genes currently known to be associated with intellectual disability

3.5-fold higher incidence of X-linked versus autosomal mutations that cause intellectual disability

Genetic disability that is X-linked is more readily detectable in males due to haploid state (no second copy)



itions of genes associated with XLID syndromes. Bolded genes have also been associated with nonsyndromal XLID. een challenged by Piton et al. (2013). [Color figure can be viewed at wileyonlinelibrary.com]

COLLÈGE DE FRANCE 1530

X-linked genes implicated in Human Diseases

nature communications

Article

https://doi.org/10.1038/s41467-022-34264-y

Systematic analysis and prediction of genes associated with monogenic disorders on human chromosome X

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Check for updates

Elsa Leitão[1,29], Christopher Schröder[1,29], Ilaria Parenti[1], Carine Dalle[2], Agnès Rastetter[2], Theresa Kühnel[1], Alma Kuechler[1], Sabine Kaya[1], Bénédicte Gérard[3], Elise Schaefer[4], Caroline Nava[2], Nathalie Drouot[5,6,7,8], Camille Engel[5,6,7,8], Juliette Piard[9,10], Bénédicte Duban-Bedu[11], Laurent Villard[12,13], Alexander P. A. Stegmann[14,15], Els K. Vanhoutte[15], Job A. J. Verdonschot[15,16], Frank J. Kaiser[1], Frédéric Tran Mau-Them[10,17], Marcello Scala[18,19], Pasquale Striano[18,19], Suzanna G. M. Frints[15,20], Emanuela Argilli[21,22], Elliott H. Sherr[21,22], Fikret Elder[23], Julien Buratti[23], Boris Keren[23], Cyril Mignot[2,24], Delphine Héron[24], Jean-Louis Mandel[3,5,6,7,8], Jozef Gecz[25,26,27], Vera M. Kalscheuer[28], Bernhard Horsthemke[1], Amélie Piton[3,5,6,7,8] & Christel Depienne[1]

Disease gene discovery on chromosome (chr) X is challenging owing to its unique modes of inheritance. We undertook a systematic analysis of human chrX genes. We observe a higher proportion of disorder-associated genes and an enrichment of genes involved in cognition, language, and seizures on chrX compared to autosomes. We analyze gene constraints, exon and promoter conservation, expression, and paralogues, and report 127 genes sharing one or more attributes with known chrX disorder genes. Using machine learning classifiers trained to distinguish disease-associated from dispensable genes, we classify 247 genes, including 115 of the 127, as having high probability of being disease-associated. We provide evidence of an excess of variants in predicted genes in existing databases. Finally, we report damaging variants in *CDK16* and *TRPC5* in patients with intellectual disability or autism spectrum disorders. This study predicts large-scale gene-disease associations that could be used for prioritization of X-linked pathogenic variants.

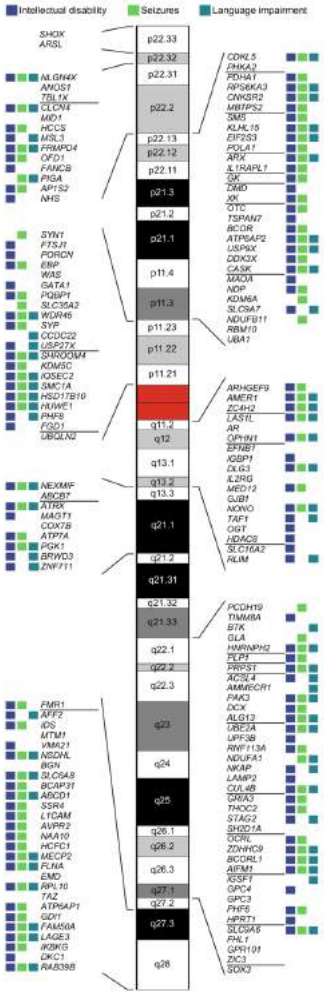

Fig. 2 | Scheme of genes associated with neurological features on chrX. Squares next to the genes represent association with intellectual disability (blue), seizures (green) or language impairment (cyan). A horizontal line separates genes present in different chromosome bands. Data underlying this scheme can be found in Supplementary Data 2. The banded chrX was generated using karyoploteR[85].

Chromosome X is enriched in disorder genes and in genes relevant to brain function

Chromosome X comprises 829 protein-coding genes annotated in HUGO Gene Nomenclature Committee (HGNC), including 205 associated with at least one monogenic disorder (referred to as 'disorder genes') in OMIM

Used the clinical synopsis to compare the proportion of disorder genes and their associated clinical features on chrX (available for 202 genes) and autosomes.

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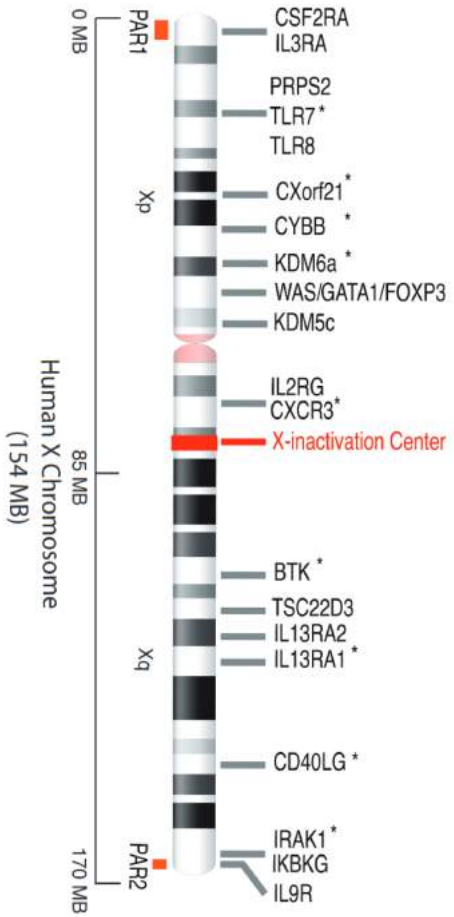
X-linked genes implicated in Human Diseases

Other types of genes enriched on the X chromosome are genes involved in muscle function, metabolic functions, immune response

And many X-linked genes have pleiotropic and tissue specific functions eg chromatin modifiers and chromatin remodelling complexes
Eg UTX, ATRX, SMCX, MED12 and MED14 (NEXT WEEK COURS IV)



X-linked genes implicated in Human Diseases

And many X-linked genes have pleiotropic and tissue specific functions eg chromatin modifiers and chromatin remodelling complexes

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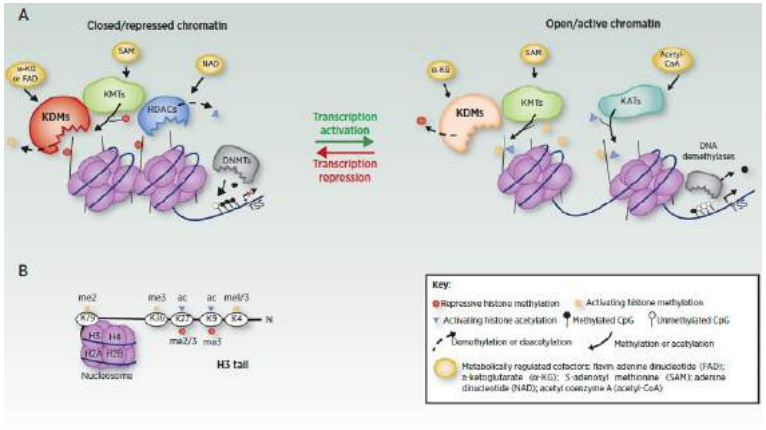
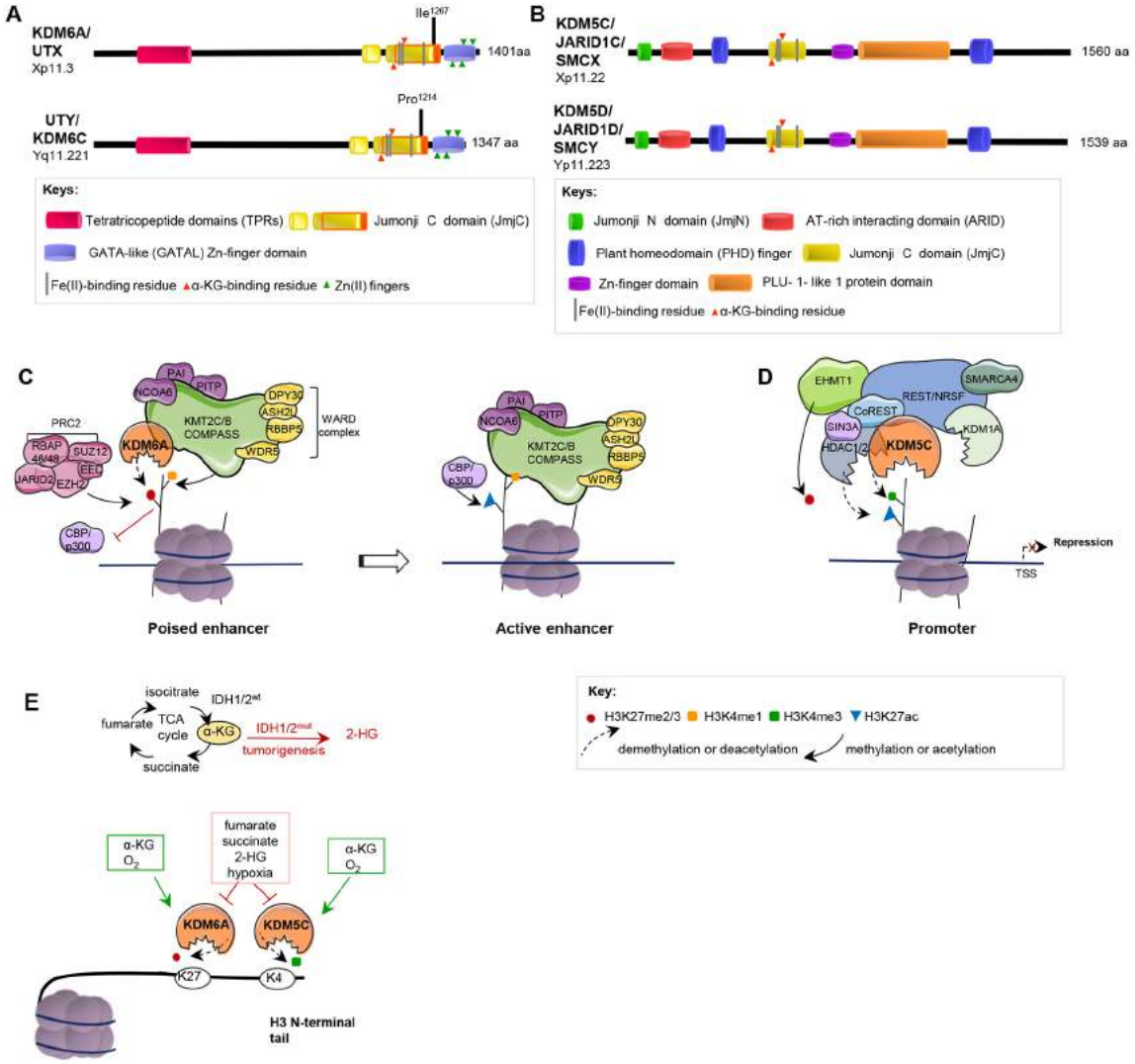
MOST CAN ESCAPE XCI and are DOSAGE SENSITIVE FACTORS WITH MAJOR IMPLICATIONS FOR DISEASE





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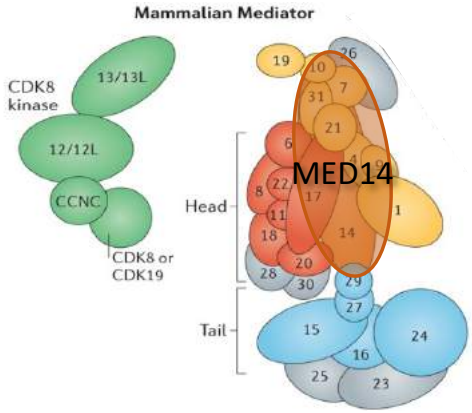
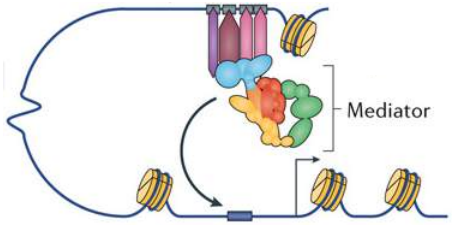




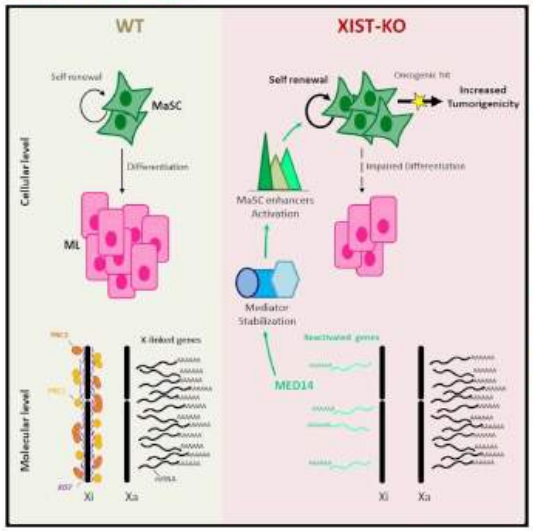
E. Heard, March 20th 2023

Cell

Article

XIST loss impairs mammary stem cell differentiation and increases tumorigenicity through Mediator hyperactivation

Graphical abstract



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Correspondence

raphael.margueron@curie.fr (R.M.), christophe.ginestier@inserm.fr (C.G.)

In brief

Outside the context of initiating X chromosome inactivation, XIST contributes to human mammary stem cell homeostasis, and loss of XIST and Xi transcriptional instabilities enhances tumorigenesis and is a common feature among human breast tumors with poor prognosis.

Highlights

- *XIST*-null cells display reactivation of a few X-linked genes, including *MED14*
- *MED14* overdosage impacts stem cell homeostasis through Mediator stabilization
- Loss of *XIST* enhances the tumorigenic potential of cells upon transformation
- Xi transcriptional reactivation is common among aggressive breast tumors

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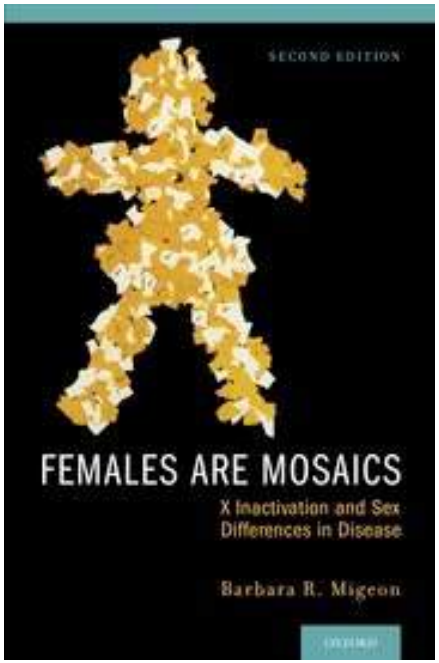
The Inactive X Chromosome contributes to Male-Female Differences in Disease

The role of X-inactivation is often ignored as a prime cause of sex differences in disease. Yet, the way males and females express their X-linked genes has a major role in the dissimilar phenotypes that underlie many rare and common disorders, such as intellectual deficiency, epilepsy, congenital abnormalities, and diseases of the heart, blood, skin, muscle, and bones. Summarized here are many examples of the different presentations in males and females. Other data include reasons why women are often protected from the deleterious variants carried on their X chromosome, and the factors that render women susceptible in some instances.

Genetics in Medicine (2020) 22:1156–1174;
https://doi.org/10.1038/s41436- 020-0779-4

Barbara Migeon
(1931-2023)
Human Geneticist
Specialist of X-linked diseases and X inactivation in human females.



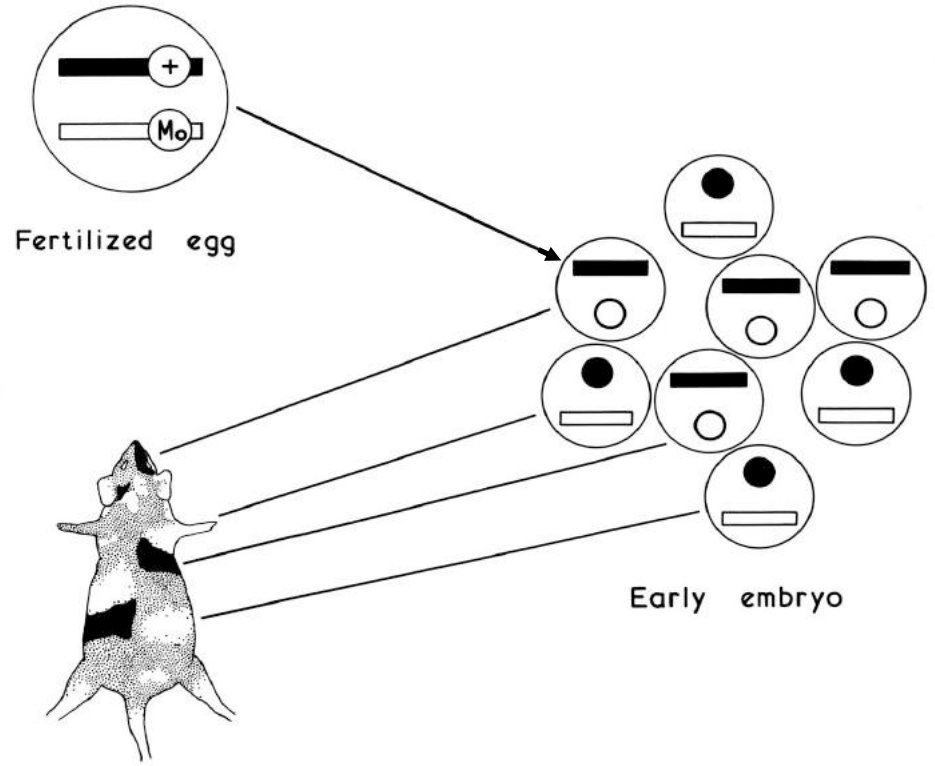
COLLÈGE DE FRANCE 1530

X-Chromosome Inactivation

Establishment of one inactive X chromosome during early development and its subsequent clonal propagation

A classic model of Epigenetics

Mary Lyon
(1929-2014)
Mouse Geneticist
Discovered X inactivation



Adapted from Mary Lyon, Henry Stewart Talks

Lyon, Nature 1961

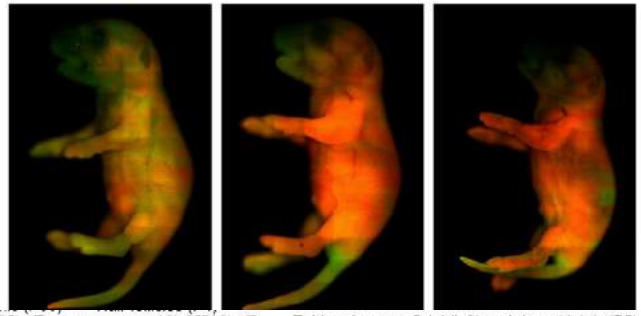
COLLÈGE DE FRANCE 1530

X-Chromosome Inactivation

XCI mosaicism creates diversity between and within individuals

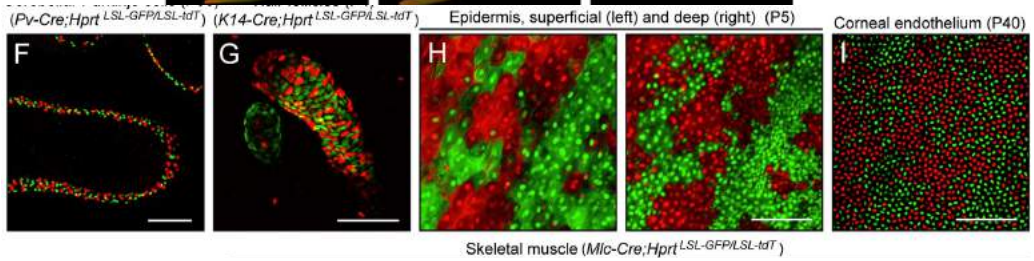
Genotype: X^{gfp} X^{tomato}

Phenotype: green *or* red



Phenotypic variation within the **same** individual…

Mosaicism varies **between** individuals



XCI mosaicism creates diversity between and within individuals.
Within an individual, inhomogeneities in the XCI mosaic in any particular tissue reflects the interplay of :
(1) the ratio of founder cells to adult cells
(2) the timing and extent of cell migration during development
(3) alleles that may be counter-selected in specific cell types

A diversity-generating mechanism such as XCI, which operates on all cells within a tissue and creates diversity on a spatial scale has the potential to add functionally relevant capabilities.

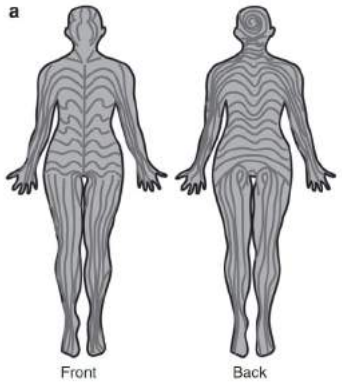
Jeremy Nathans lab
Wu et al (2014)
"Cellular Resolution Maps of X -Chromosome inactivation: Implication for Neural development, function, and disease."
Neuron 81, 103–119

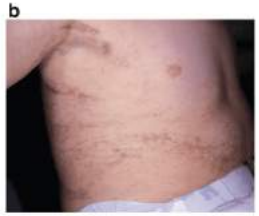
COLLÈGE DE FRANCE 1530

Impact of the X chromosome on human disease

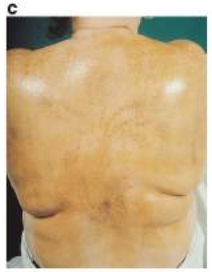
X-Chromosome Inactivation

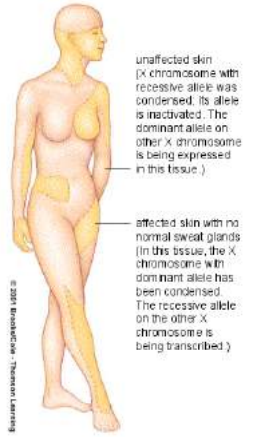
- Random XCI leads to cellular mosaicism within and between individuals.
- Every female is a unique mosaic for X-linked gene expression: outcome will depend on interplay of all alleles between the two (active) Xs, as well as the inherent variation in primary “random” XCI, also in cell mixing and migration.
- As XCI is complete after the blastocyst stage, the manifestation of X-linked phenotypes depends largely on the way in which these cells subsequently divide, mingle, and migrate to form the organs of the body.





Incontinentia pigmenti. (lethal in males)
The hyperpigmentation stage of incontinentia pigmenti shows the characteristic whorled pattern that follow Blaschko’s lines.



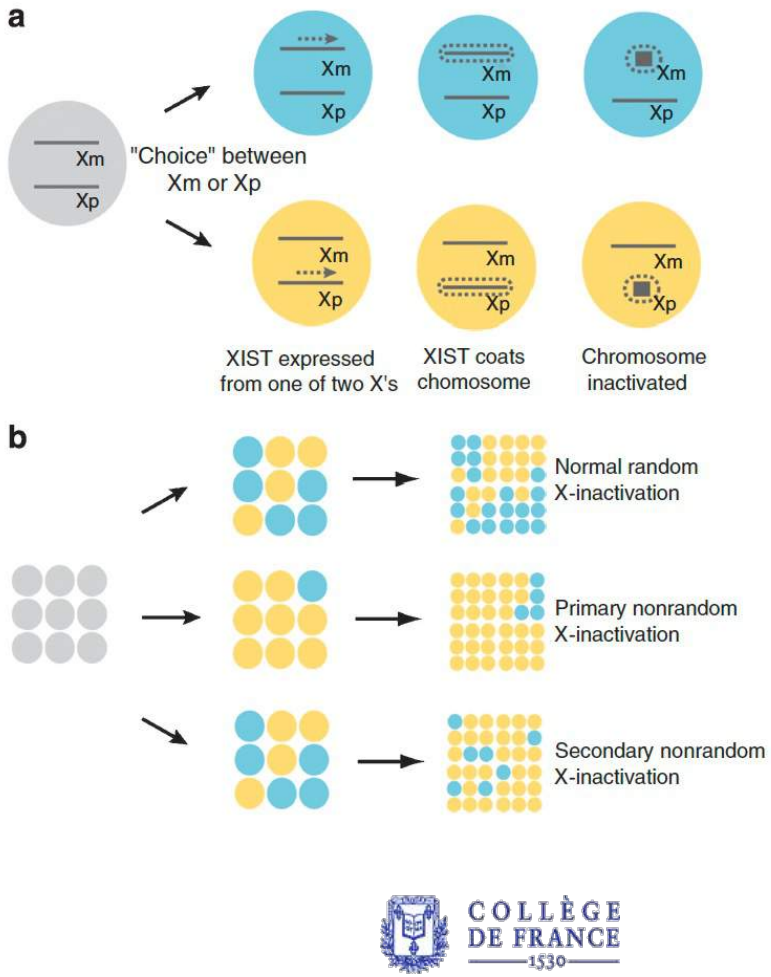


Iodine starch test in female carrier of anhidrotic ectodermal dysplasia. Application of starch in this female carrier shows a characteristic ‘fountain pattern’’ of dark areas with normal sweat glands as well as light areas that have no sweat glands.

Impact of the X chromosome on human disease

X-Chromosome Inactivation

- Random XCI leads to cellular mosaicism within and between individuals.
- Every female is a unique mosaic for X-linked gene expression: outcome will depend on interplay of all alleles between the two (active) Xs, as well as the inherent variation in primary “random” XCI, also in cell mixing and migration.
- As XCI is complete after the blastocyst stage, the manifestation of X-linked phenotypes depends largely on the way in which these cells subsequently divide, mingle, and migrate to form the organs of the body.
- In the context of a mutation in the initiation of XCI primary skewing can occur (most mutations are lethal)
- In the context of a mutation, or variant X-linked allele that is disadvantageous to growth or function, secondary skewing can occur



COLLÈGE DE FRANCE 1530

Timing of XCI determines lineage-specific XCI ratio probability

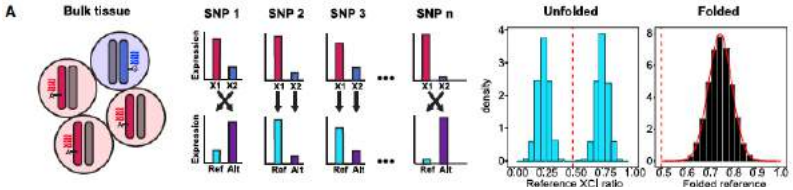
Developmental Cell

Article

Variability of cross-tissue X-chromosome inactivation characterizes timing of human embryonic lineage specification events

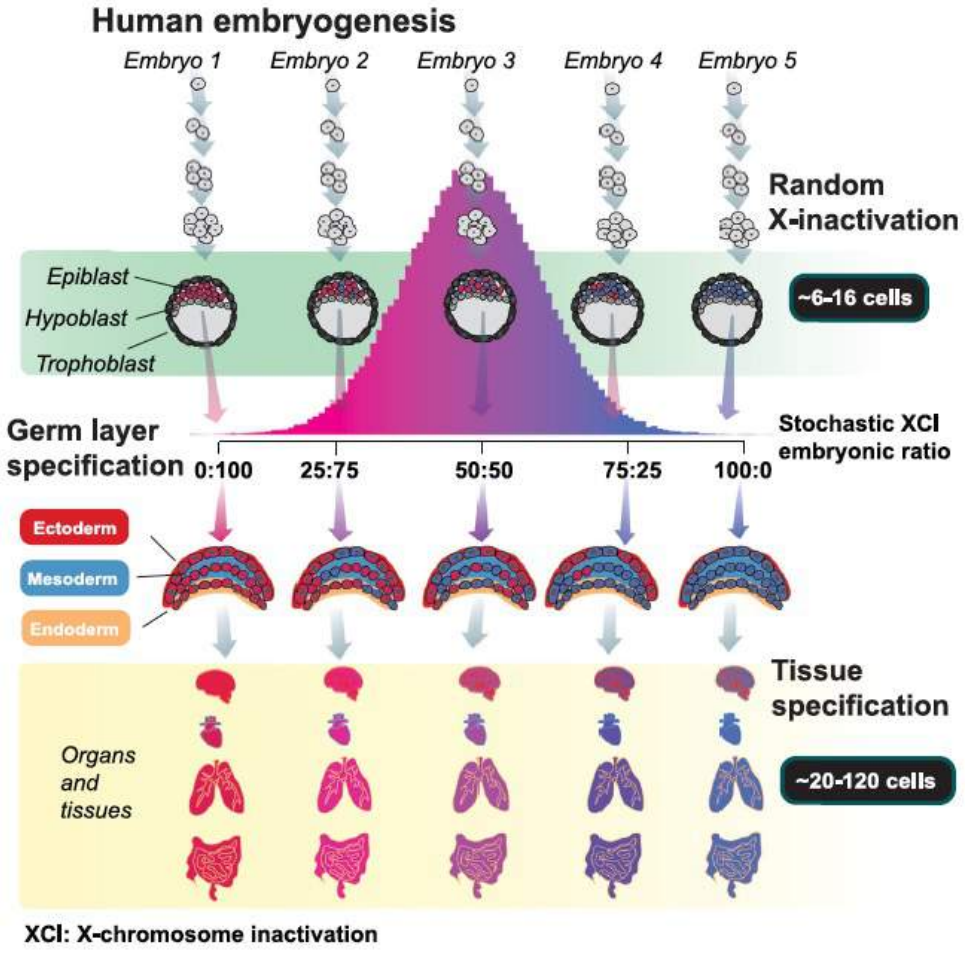
Jonathan M. Werner,[1] Sara Ballouz,[1,2] John Hover,[1] and Jesse Gillis[1,3,4,*]

- Tissue XCI ratios can be determined using reference-aligned
- bulk RNA-seq data
- XCI ratios are shared across all human tissues
- XCI variance in adult populations is explained by the inherent stochasticity of XCI
- Human XCI occurs when the embryonic epiblast is composed of at least 6–16 cells



- determine the tissue XCI ratio from unphased bulk RNA-sequencing data
- assess XCI ratios from any publicly available RNA-sequencing datasets
- tissue sampling scheme of the Genotype- Tissue Expression (GTEx v8)
- XCI ratios for 49 tissues both within and across individuals for 311 female donors
- XCI ratios are shared for tissues both within and across germ layers

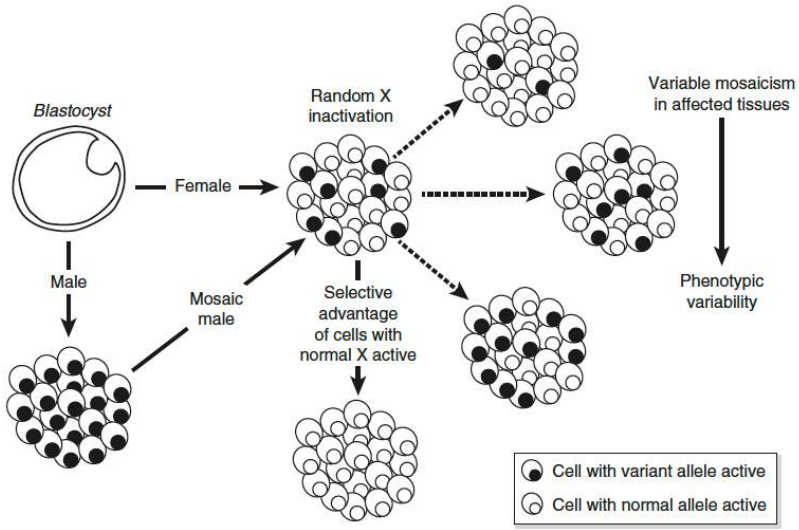
=> XCI is completed before any significant embryonic lineage decisions are made



COLLÈGE DE FRANCE 1530

Are Sex Differences in Disease manifestation due to X-inactivation?

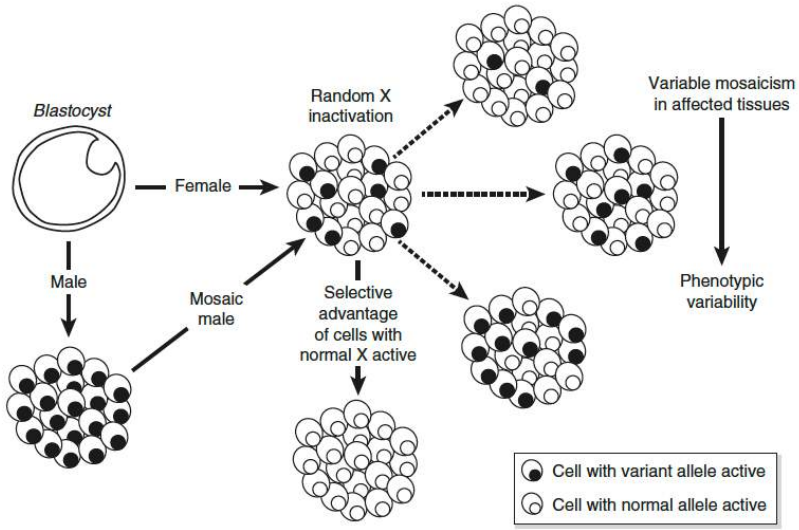
- There is usually a greater severity of X-linked diseases in males than females
- If the X-linked mutation or variant is so functionless that it is lethal to fetal or newborn males, then females with a single mutant allele are the only ones that can have the disease.
- The sex differences in the effect of X-linked pathologic variants is due to the process of X chromosome dosage compensation, X-inactivation
- A woman is less susceptible to the pathogenic variants in genes on her active X chromosome because the variant is not expressed in all her cells
- The mix of normal and abnormal cells moderates females' disease.
- In some disorders the variant is so lethal that most males with severe deficiency of the gene product die in utero.
- The only survivors are females or mosaic males, who also have a mix of variar and normal cells, they are the ones with nonlethal manifestation of the disorder. X-linked diseases, such as incontinentia pigmenti, or orofacial digital syndrome type 1, occur only in *females* or *mosaic males*
- Some, but not all, females with the same X-linked deleterious allele are protected from its effects
- When so many women are protected from manifesting severe X-linked diseases, why are some of them susceptible?
- What is the role of X inactivation and escape from X inactivation on the manifestation and potential alleviation of X-linked mutations?
- What is the role of X-linked genes in specific disorders eg immune disorders
- Or in cancer : XX individuals can be protected by escapees (EXIT hypothesis) or Xi epigenetic instability can lead to aberrant gene dosage and participate in tumorigenesis
- What are the potential therapeutic approaches?



From Migeon, Genetics in Medicine, 2020

Are Sex Differences in Disease manifestation due to X-inactivation?

- Les maladies liées au chromosome X sont généralement plus graves chez les hommes que chez les femmes.
- Si la mutation ou la variante liée au chromosome X est si peu fonctionnelle qu'elle est létale pour les fœtus ou les nouveau-nés de sexe masculin, les femmes porteuses d'un seul allèle mutant sont les seules à pouvoir être atteintes de la maladie.
- Les différences entre les sexes dans l'effet des variantes pathologiques liées au chromosome X sont dues à l'inactivation du chromosome X. Une femme est moins sensible aux mutations liées a l'X qu'un homme.
- Les femmes sont moins sensibles aux mutations dans les gènes sur le chromosome X actif parce que la variante n'est pas exprimée dans toutes leurs cellules. Le mélange de cellules normales et anormales modère les maladies féminines.
- Dans certaines maladies, la variante est si délétère que la plupart des hommes présentant une déficience grave du produit du gène meurent in utero.
- Les seuls survivants sont les femelles - ou les mâles mosaïques qui ont également un mélange de cellules normales et de variantes, et qui présentent une manifestation non létale de la maladie. Les maladies liées à l'X, telles que l'incontinentia pigmenti ou le syndrome digital orofacial de type 1, ne se manifestent que chez les femmes ou les hommes en mosaïque
- Certaines femmes, mais pas toutes, porteuses du même allèle délétère lié à l'X sont protégées de ses effets.
- Si tant de femmes sont protégées contre les maladies graves liées à l'X, pourquoi certaines d'entre elles y sont-elles sensibles ?
- Quel est le rôle de l'inactivation de l'X et de l'échappement à l'inactivation de l'X dans la manifestation et l'atténuation potentielle des mutations liées à l'X ?
- Quel est le rôle des gènes liés à l'X dans des troubles spécifiques, par exemple les troubles immunitaires ?
- Quel est le role sans le cancer : les individus XX peuvent être protégés par les genes qui ecappent a l'inactivation du X (hypothèse EXIT)
- Mais l'instabilité épigénétique due Xi peut conduire à un dosage aberrant des gènes et participer à la tumorigenèse
- Quelles sont les approches thérapeutiques potentielles ?

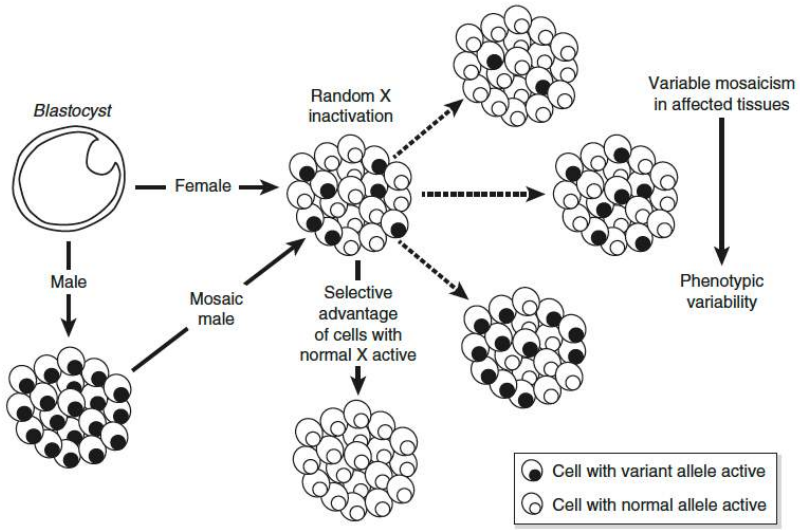


From Migeon, Genetics in Medicine, 2020

Are Sex Differences in Disease manifestation due to X-inactivation?

Table 1. Examples of inherited X-linked diseases affected by XCI patterns

X-linked disease	Gene	XCI status in carriers	Mechanism	Phenotyp
Duchenne muscular dystrophy	*DMD*	Random XCI	Sufficient number of cells expressing cell autonomous protein	N
Duchenne muscular dystrophy	*DMD*	Skewed XCI toward mutated allele	X:autosome translocation causes skewing	A
Hunter syndrome	*IDS*	Random XCI	Sufficient amount of secreted protein	N
Fabry disease	*GLA*	Random XCI	Normal protein product not taken by mutant cells	A
Lesh-Nyhan	*HPRT*	Random XCI in fibroblasts/skewed XCI toward normal in blood	Gap junctions between fibroblasts/cell selection in blood	N
Adrenoleukodystrophy	*ABCD1*	Skewed XCI toward mutated allele	Growth advantage of cells expressing mutation	A
Craniofrontonasal syndrome	*EFNB1*	Random XCI	No substitution for EFNB1 deficiency	A
Rett syndrome	*MECP2*	Variable XCI skewing	Critical protein; mutation lethal in males	A
ICF syndrome	*DNMT3B*	Aberrant XCI	Hypomethylation of various sequences	A
XLID due to escape genes	e.g. *KDM5C*, *KDM6A*	Escape XCI; partial XCI skewing	Haploinsufficiency	A
X aneuploidy	Escape genes; e.g. *KDM6A*	Random XCI	Dosage imbalance; genome-wide expression and DNA methylation effects	A
SLE	*TLR7*, *TLR8*, *IRAK1*, *CXORF21*	Eroded XCI	Higher gene expression in B- and T-cells	A



From Migeon, Genetics in Medicine, 2020

Are Sex Differences in Disease manifestation due to X-inactivation?

Females who express an X-linked pathogenic variant, even though most carriers of pathogenic variants in similar genes do not?

Fabry Disease Symptoms

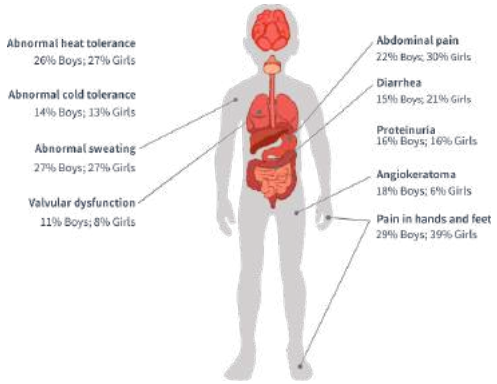
•Burning pain in the arms and legs, which worsens in hot weather or following exercise.

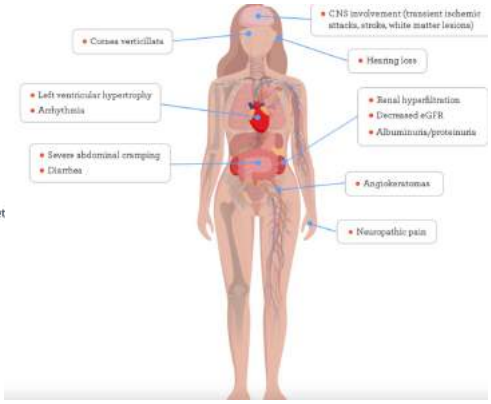
•The buildup of excess material in the clear layers of the cornea (resulting in clouding but no change in vision)

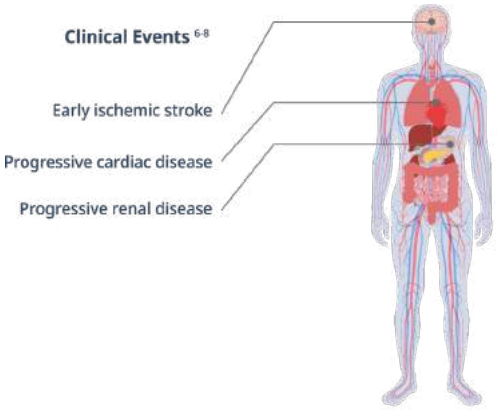
•Impaired circulation that may lead to stroke or heart attack due to fatty storage in blood vessel walls.

Heterozygous women are not just carriers

It is a common misconception that females are just carriers of a defective *GLA* gene. Heterozygous women with Fabry disease can experience a variable presentation, ranging from asymptomatic or mild symptoms to symptoms that are just as severe and multisystemic as those experienced by male patients, such as cardiac, renal, and cerebrovascular complications. On average, many women are not diagnosed until about 16 years after symptoms first appear. Symptoms in women tend to occur at a later age than in men and typically have a more drawn-out or lengthened course of disease compared with men.







Signs and symptoms that present at any age[6-8]

- Arrhythmias
- Corneal and lenticular opacities
- Heat, cold and exercise intolerance
- Peripheral neuropathy of extremities / episodic pain crises
- Hearing loss, tinnitus
- Hypohidrosis
- Gastrointestinal manifestations
- Angiokeratomas

Patients may experience a subset of these signs and symptoms

From Migeon, Genetics in Medicine, 2020

COLLÈGE DE FRANCE 1530

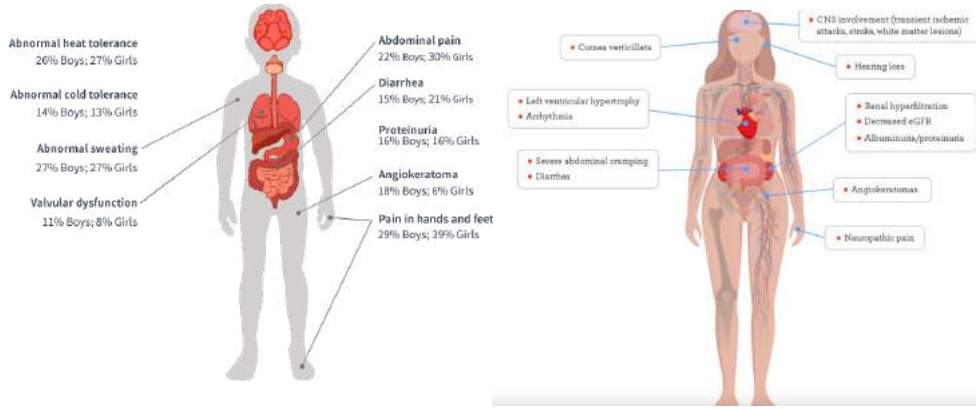
Are Sex Differences in Disease manifestation due to X-inactivation?

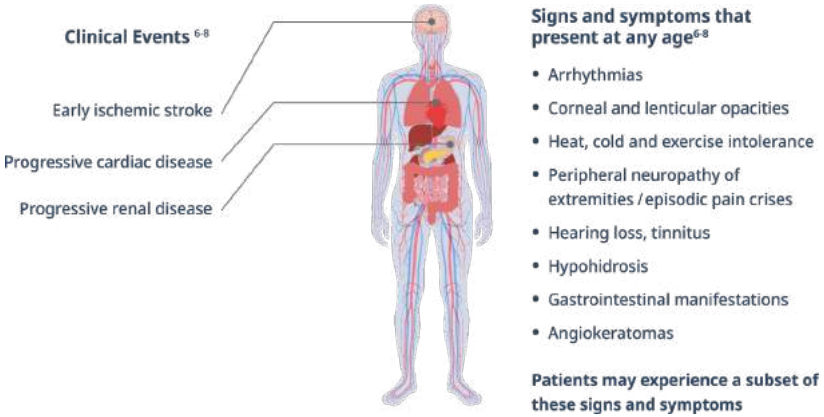
Females who express an X-linked pathogenic variant, even though most carriers of pathogenic variants in similar genes do not?

Females with Fabry disease, caused by lack of the lysosomal enzyme α-galactosidase may have some of the clinical symptoms seen in affected males, whereas carriers of a variant in a gene encoding another lysosomal enzyme, iduronic sulfatase, rarely have any clinical symptoms associated with Hunter syndrome.

Both enzymes are made in the lysosomes, and can be transported from the lysosomes of one cell to those of another cell. The two lysosomal disorders differ because iduronate sulfatase is taken up by cells better than the low uptake enzyme, α-galactosidase.

This difference in the ability of the lysosome to take up a product is responsible for normal Hunter heterozygotes and manifesting Fabry heterozygotes.





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Are Sex Differences in Disease manifestation due to X-inactivation?

EFFECT OF CELL SELECTION IN MOSAIC FEMALES

ARTICLE

Defining the Cause of Skewed X-Chromosome Inactivation in X-Linked Mental Retardation by Use of a Mouse Model

Mary R. Muers, Jacqueline A. Sharpe, David Garrick, Jacqueline Sloane-Stanley, Patrick M. Nolan, Terry Hacker, William G. Wood, Douglas R. Higgs, and Richard J. Gibbons

Extreme skewing of X-chromosome inactivation (XCI) is rare in the normal female population but is observed frequently in carriers of some X-linked mutations. Recently, it has been shown that various forms of X-linked mental retardation (XLMR) have a strong association with skewed XCI in female carriers, but the mechanisms underlying this skewing are unknown. ATR-X syndrome, caused by mutations in a ubiquitously expressed, chromatin-associated protein, provides a clear example of XLMR in which phenotypically normal female carriers virtually all have highly skewed XCI biased against the X chromosome that harbors the mutant allele. Here, we have used a mouse model to understand the processes causing skewed XCI. In female mice heterozygous for a null *Atrx* allele, we found that XCI is balanced early in embryogenesis but becomes skewed over the course of development, because of selection favoring cells expressing the wild-type *Atrx* allele. Unexpectedly, selection does not appear to be the result of general cellular-viability defects in Atrx-deficient cells, since it is restricted to specific stages of development and is not ongoing throughout the life of the animal. Instead, there is evidence that selection results from independent tissue-specific effects. This illustrates an important mechanism by which skewed XCI may occur in carriers of XLMR and provides insight into the normal role of ATRX in regulating cell fate.

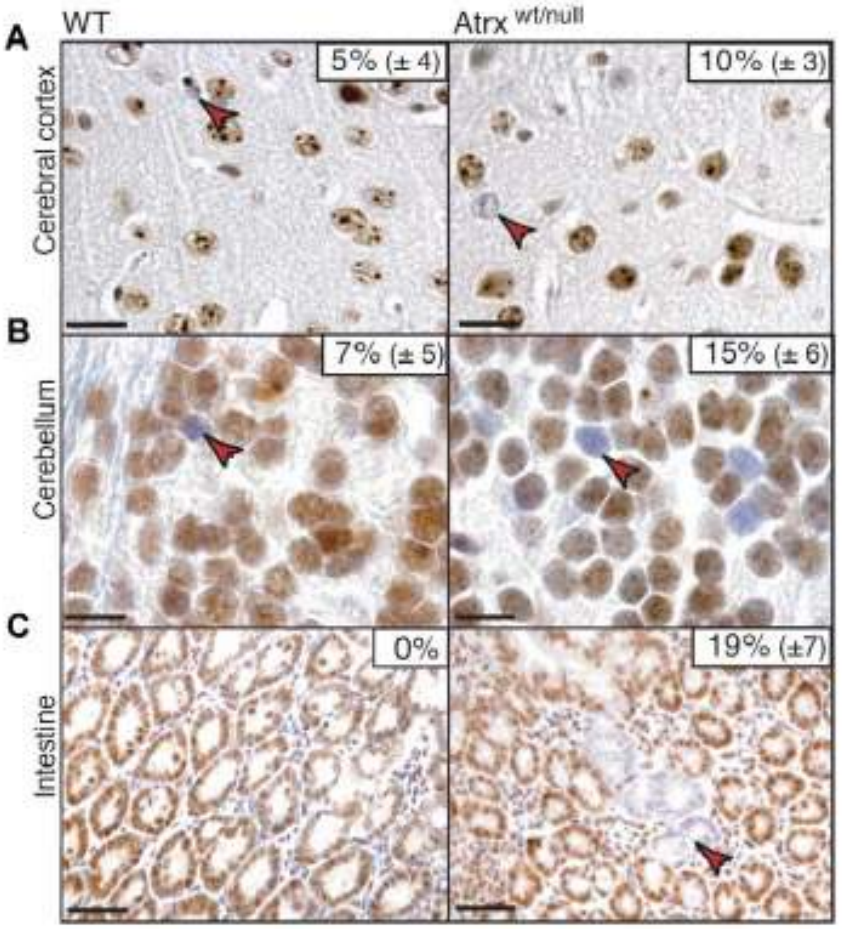

Figure 1. XCI in adult $Atrx^{wt/null}$ mice. Paraffin sections of tissues from wild-type (WT) and $Atrx^{wt/null}$ female mice, stained with the anti-Atrx antibody (H300) detected by horseradish peroxidase (*brown*) with hematoxylin counterstain (*blue*). Sections stained under the same conditions with an IgG control antibody gave a weak background signal, as shown by Garrick et al.[25] Red arrowheads indicate examples of Atrx-negative cells. The mean percentage (±1 SD) of Atrx-negative cells in the cerebral cortex and the granular layer of the cerebellum and the percentage of Atrx-negative intestinal crypts is given (three animals, with >2,000 cells or crypts per genotype). Scale bars are 25 μm (*A*), 10 μm (*B*), and 50 μm (*C*).

E. Heard, March 6th 2023

Are Sex Differences in Disease manifestation due to X-inactivation?

ARTICLE

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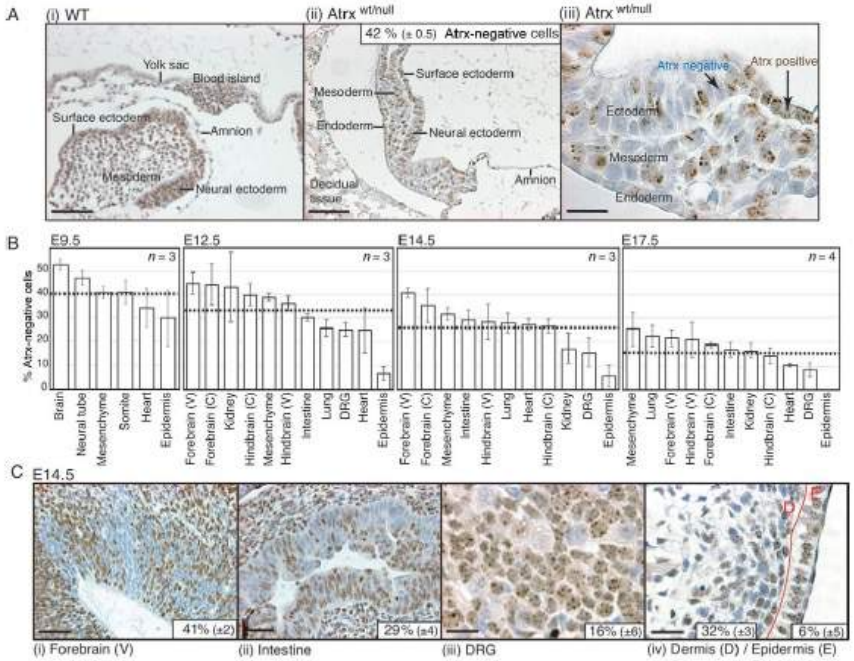
Systematic, detailed analysis of the XCI pattern in a range of tissues at different stages of development. Atrx can be detected in virtually all cells of wild-type embryos throughout gestation , but skewed XCI (ratio 180:20) was apparent in most tissues by late gestation (E17.5) in Atrxwt/null mice (fig. 2B).

However, although the overall impression for the embryo as a whole is of a gradual decline in Atrx-null cells during development, at the level of individual tissues, the degree of cell selection varied significantly. For example, at E14.5 (fig. 2B and 2C), the percentage of negative cells remained 30%–40% in the forebrain and dermis (mesenchyme) but had declined to !20% in the epidermis and dorsal root ganglia



Pattern of XCI during embryonic development. *A*, Paraffin sections of ~E8 wild-type (WT) and Atrx$^{wt/null}$ littermate embryos, ith the anti-Atrx antibody (H300) and hematoxylin counterstain (as in fig. 1). Examples of Atrx-positive and -negative cells

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Are Sex Differences in Disease manifestation due to X-inactivation?

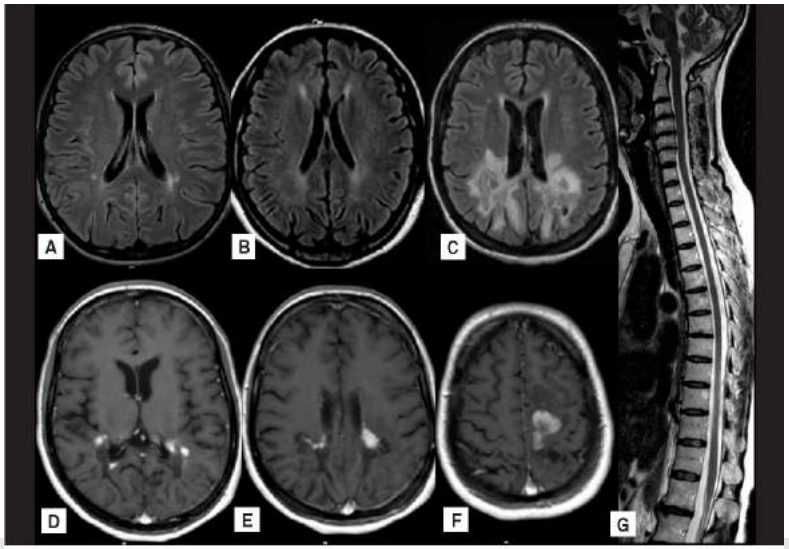
Women are not just carriers. In some cases it is the *mutant expressing cell* that has a selective advantage.

Adrenoleukodystrophy is the only known X-linked disease where the variant allele has a selective advantage.
ALD affects approximately 1 in 17,000 people worldwide

For reasons not yet understood, heterozygotes with the variant causing adrenoleukodystrophy slowly lose their population of wild type cells.

Therefore, as they age, they usually manifest some symptoms of the disease. Although both men and women develop spinal cord disease, there are differences: in women the onset of spinal cord disease is usually later in life, and progression is considered to be slower

ALD is caused by mutations in *ABCD1*, a gene located on the X chromosome that codes for ALD, a peroxisomal membrane transporter protein. Saturated fatty acids build up in the brain, nervous system, and adrenal gland and eventually destroy the myelin sheath that surrounds the nerves.



Adrenoleukodystrophy (ALD) is a result of fatty acid buildup caused by failure of peroxisomal fatty acid beta oxidation which results in the accumulation of very long chain fatty acids in tissues throughout the body. The most severely affected tissues are the myelin in the central nervous system, the adrenal cortex, and the Leydig cells in the testes. The long chain fatty acid buildup causes damage to the myelin sheath of the neurons of the brain, resulting in seizures and hyperactivity. Other symptoms include problems in speaking, listening, and understanding verbal instructions. Clinically, ALD presents as a heterogeneous disorder, showing several distinct phenotypes, and no clear pattern of genotype–phenotype correlation. As an X-linked disorder, ALD presents most commonly in males; however, approximately 50% of heterozygote females show some symptoms later in life.

From Migeon, Genetics in M

Are Sex Differences in Disease manifestation due to X-inactivation?

WHY ARE SOME FEMALE HETEROZYGOTES AFFECTED BY DISEASE?

Cellular interference: females specifically manifest a disease (not males) because the variant allele interacts with the normal one.

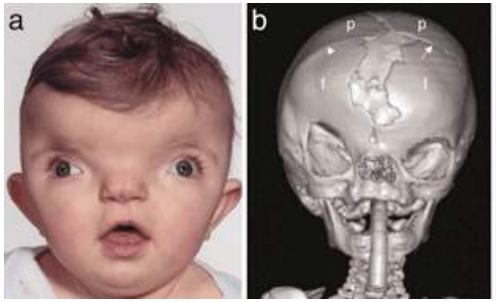
Examples include **Craniofrontonasal dysplasia**, **primary open angle closure glaucoma** and **epilepsy** and **mental retardation** limited to females (EMFR).

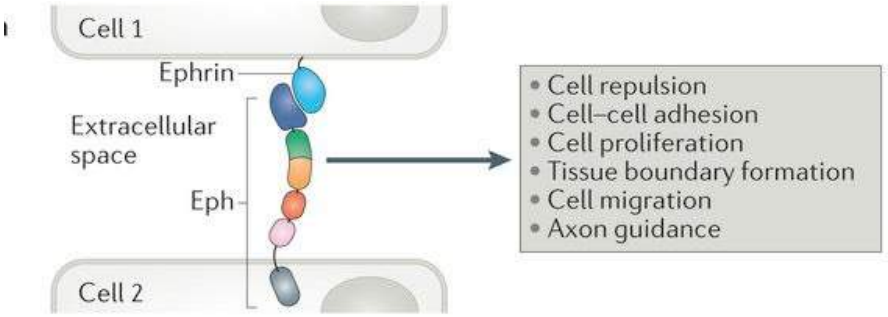
Craniofrontonasal dysplasia syndrome, caused by a deficiency of Ephrin-B1 (EFNB1). Other members of the ephrin family of proteins can substitute for a deficiency of EFNB1 in males with the deleterious variant >> minimal clinical symptoms.

Heterozygotes, have a mixture of mutant and wild type cells; such mixtures do not permit ephrin substitutes, and consequently, females have a deficiency more severe than that in males, who can substitute one ephrin for another.

It is heterozygosity, not the complete loss of function, that produces the severe disorder. It is the mosaic loss of EFNB1 that disturbs tissue boundary formation at the developing coronal suture. Several forms of infantile epilepsy also show similar cellular interference, but fortunately no other examples are known.

With so many mechanisms available to protect females with an X-linked pathologic variant, why do some heterozygotes manifest any symptoms of a disease?





E. Heard, March 6th 2023

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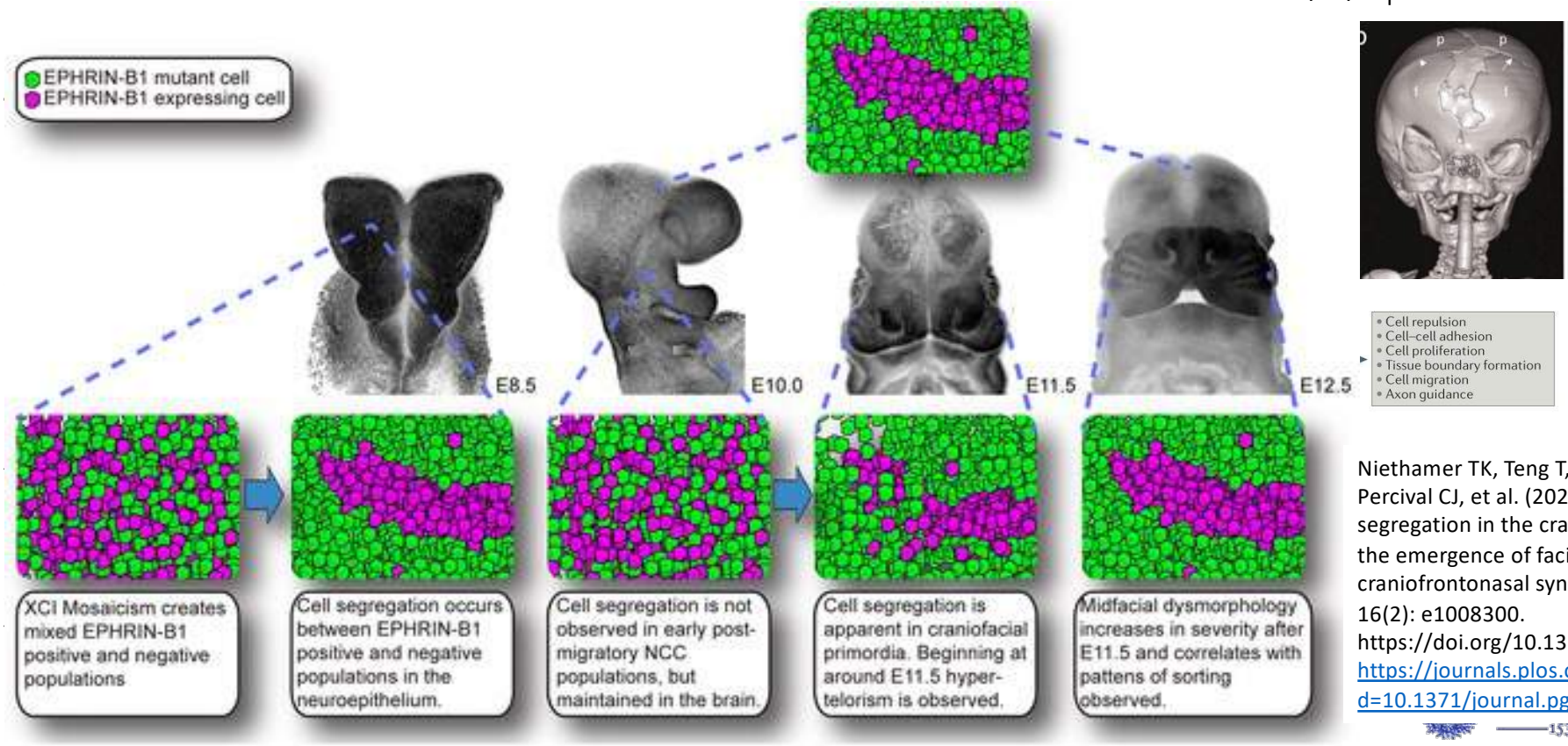


- Cell repulsion
- Cell–cell adhesion
- Cell proliferation
- Tissue boundary formation
- Cell migration
- Axon guidance

Niethamer TK, Teng T, Franco M, Du YX, Percival CJ, et al. (2020) Aberrant cell segregation in the craniofacial primordium and the emergence of facial dysmorphology in craniofrontonasal syndrome. PLOS Genetics 16(2): e1008300. https://doi.org/10.1371/journal.pgen.1008300 https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1008300

Are Sex Differences in Disease manifestation due to X-inactivation?

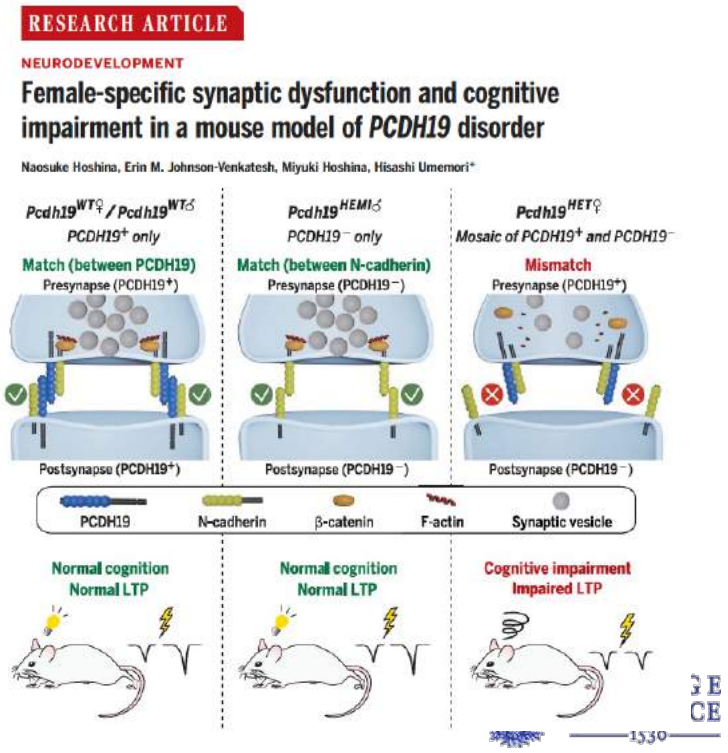
WHY ARE SOME FEMALE HETEROZYGOTES AFFECTED BY DISEASE?

Unique X-Linked Mutation Associated with Female-Specific Epilepsy and Intellectual Disability

Juberg RC, Hellman DC. A new familial form of convulsive disorder and mental retardation limited to females. J Pediatr 1971;79:726-3.

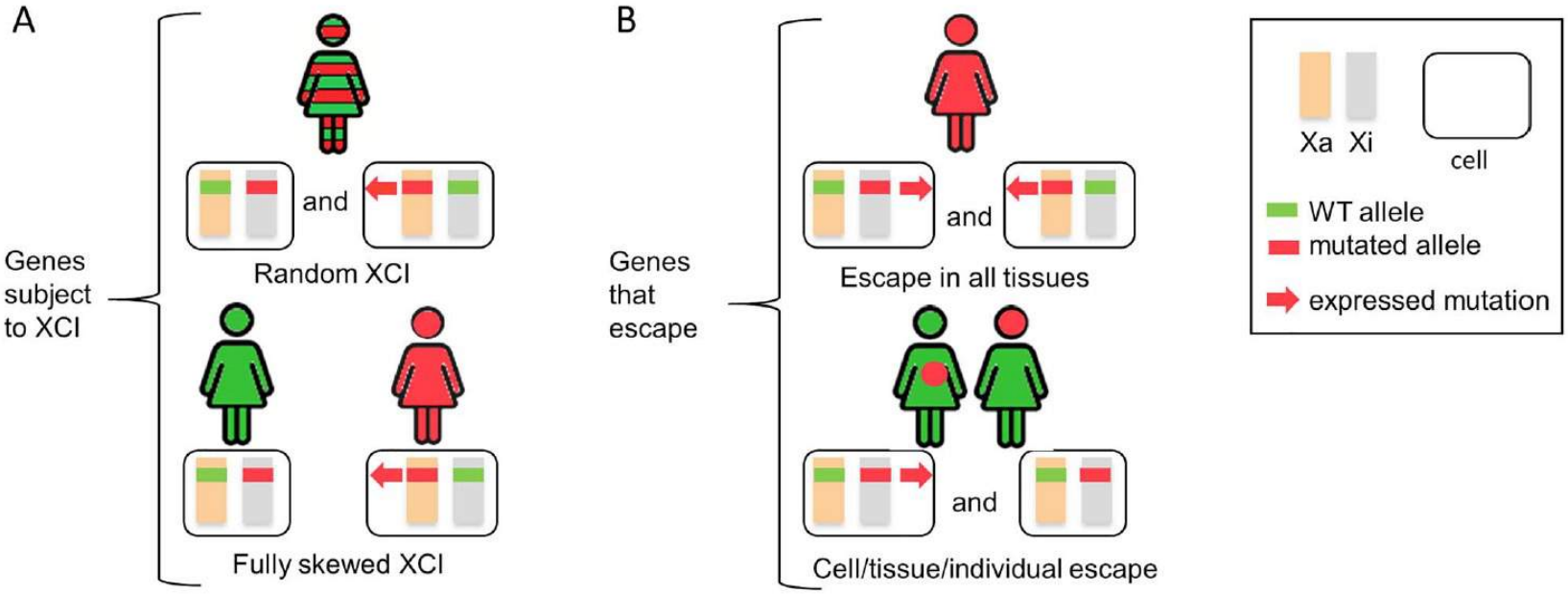
Cellular interference: females specifically manifest a disease (not males) because the variant allele interacts with the normal one.

- Juberg and Hellman reported the occurrence of a new form of epilepsy, sometimes concomitant with intellectual disability, which only occurred in female patients.
- 15 female patients, related as either sisters or first cousins through their fathers, were documented to have seizures. It was postulated that this was the result of an inherited genetic mutation within a single gene with sex-limited expression. This clinical presentation was later called epilepsy and mental retardation limited to females (EMFR).
- In 2008, Dibbens et al identified the X-linked protocadherin 19 (PCDH19) gene mutations in patients with EMFR. PCDH19, located on chromosome Xq22.1, is thought to mediate cell–cell adhesion and movement contributing to neural development in utero.
- > 200 documented mutations of PCDH19, with clinical symptoms varying from early-onset epilepsy to intellectual disabilities, to behavioral disturbances.
- Overall, estimated 15 000 - 30 000 female patients with EMFR in USA.
- Somatic tissue mosaicism in which mosaic female patients have PCDH19-positive and PCDH19-negative cells is postulated to adversely alter brain neuronal cell communication in females resulting in EMFR. Conversely, male patients with the PCDH19 mutation are hemizygous and thus have a homogeneous population of cells that are PCDH19 negative. It is hypothesized that the role of PCDH19 in phenotypically normal transmitting male patients is compensated by a gene on the Y chromosome, PCDH11Y. Both PCDH19 and PCDH11Y are expressed in the amygdala and developing cortical plate.



Impact of the inactive X chromosome on human disease

X-Chromosome Inactivation and Escape



Mutations in escape genes are an especially common cause of XLID (and is often lethal in males eg MECP2)
Autoimmune diseases, common in women, are likely caused by abnormal expression of escape genes.
Abnormal escape gene dosage due to X aneuploidy contributes to a milieu of deleterious phenotypes including infertility, intellectual disability, immune diseases and cancer.

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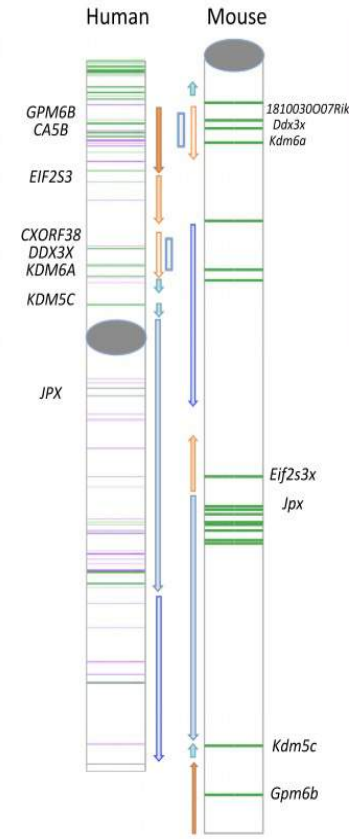
Escape from XCI in females: an added layer of cellular mosaicism in X-linked gene expression

In humans, up to 25% of X-linked genes can escape from X inactivation!

10% of these escape constitutively
15% of these genes show variability between individuals – and tissue specificity

X-inactivation profile reveals extensive variability in X-linked gene expression in females

Carrel and Willard (2005) *Nature* 434, 400-404



Balaton et al., *Trends in Genetics* 2016.

Everyone is unique: females even more so…

Huntington Willard – 2005:
"Genetically speaking, if you've met one man, you've met them all.
We are, I hate to say it, predictable. You can't say that about women.
Men and women are farther apart than we ever knew. It's not Mars or Venus.
It's Mars or Venus, Pluto, Jupiter and who knows what other planets."

Xi Escapees in Twin Studies: Genetic and Stochastic/Environmental

Escape from X-inactivation in twins exhibits intra- and inter-individual variability across tissues and is heritable

Antonino Zito[1¤a¤b]*, Amy L. Roberts[1☯], Alessia Visconti[1☯], Niccolo' Rossi[1], Rosa Andres-Ejarque[2], Stefano Nardone[3], Julia S. El-Sayed Moustafa[1], Mario Falchi[1], Kerrin S. Small[1]*

Escape from X-inactivation exhibits both constitutive and tissue-specific patterns

Escape from X-inactivation exhibits intra- and inter-individual variability

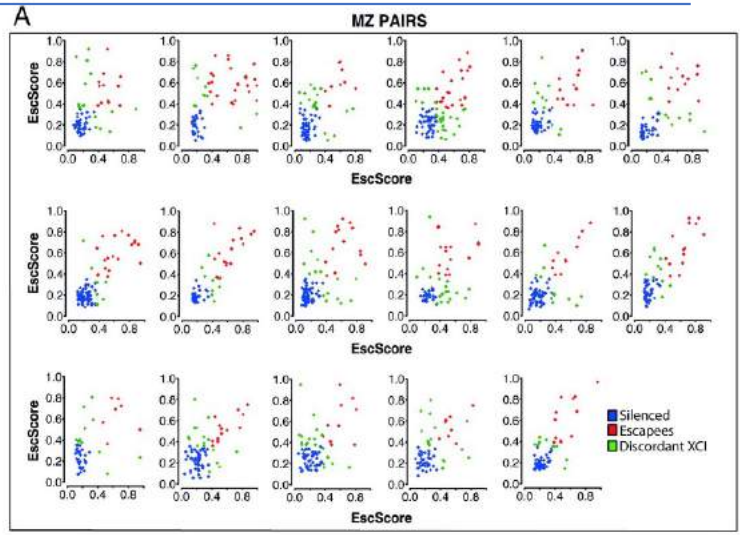
Escape from X-inactivation exhibits immune cell type-specificity
Females have

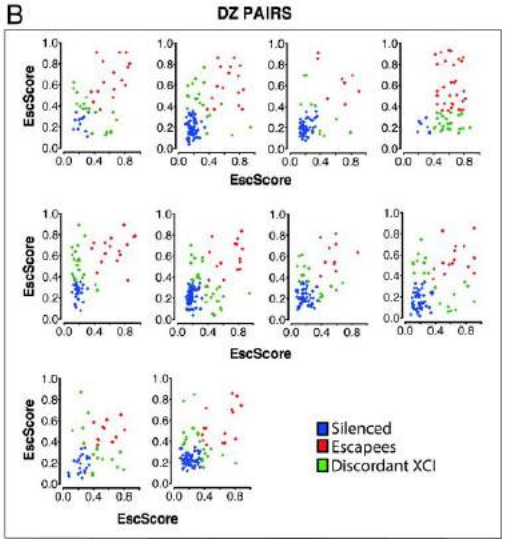
Escape from X-inactivation is influenced by heritable and environmental factors: Twin studies can assess the contribution of genetic factors to complex traits

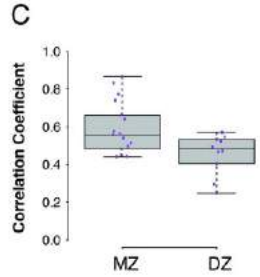
Using 27 complete twin pairs (17 monozygotic (MZ or identical); 10 dizygotic (DZ or fraternal)), we quantified the concordance in the escape in LCLs between co-twins and compared such concordance between MZ and DZ twins.

MZ share significantly more similar escape genes than DZ twins
⇒significant genetic component of escape
MZ twin discordance suggests environmental influences.
=> both genetic and environmental factors – or stochastic variation - interplay to regulate XCI escape.

Variability between immune cell types may also suggest an immune cell type-specific response to environmental influences.







CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2022-2023

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

27 mars, 2023

Cours IV

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

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