CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2022-2023

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

13 mars, 2023

Cours II

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

COLLÈGE DE FRANCE 1530

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**

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

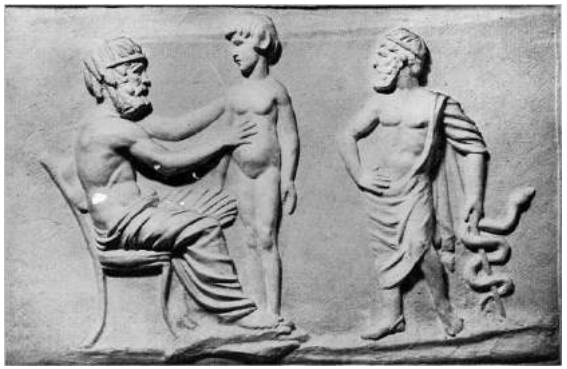
COLLÈGE DE FRANCE 1530

E. Heard, March 13th 2023

SUMMARY of LAST WEEK

- Perceptions and treatments of diseases of women from ancient history to modern times
- Early studies on the biology of Men and Women
- Sex determination strategies and Human Sex determination
- The Human Sex Chromosomes
- Bipotential tissues are directed to develop into male or female sex organs; hormonal changes bring about puberty, and the secondary sex characteristics of men and women
- Non-gonadal sex phenotypes
- Considerations on the definitions of Biological Sex (gametic, hormonal, genetic...) and Gender
- Sexual Dimorphism and Sexual Selection (what Darwin didn't see...)
- Human Health and towards Precision Medicine: assessing and addressing Sex Bias in Disease at last
- Sexual Dimorphism and Human Health – some examples

www.ucl.ac.uk/museums-static/digitalegypt/med/birthpapyrus.html



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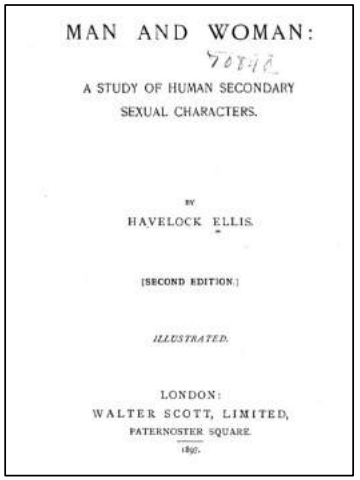

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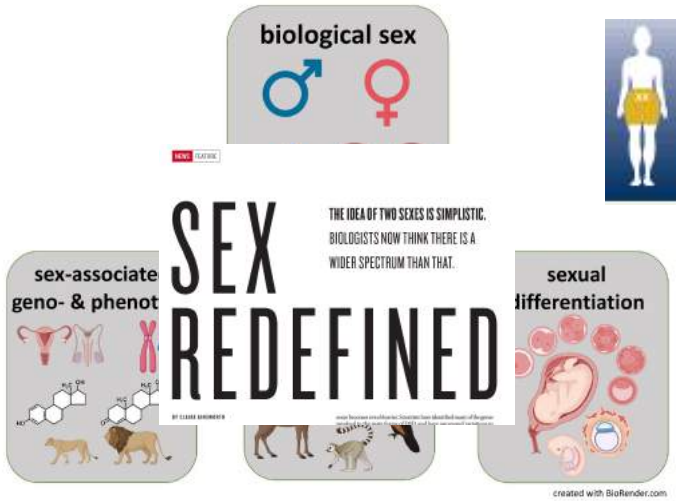
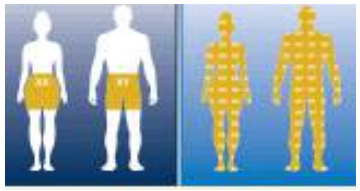
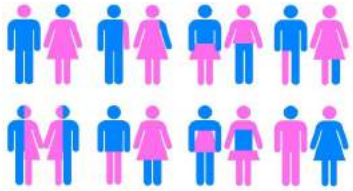
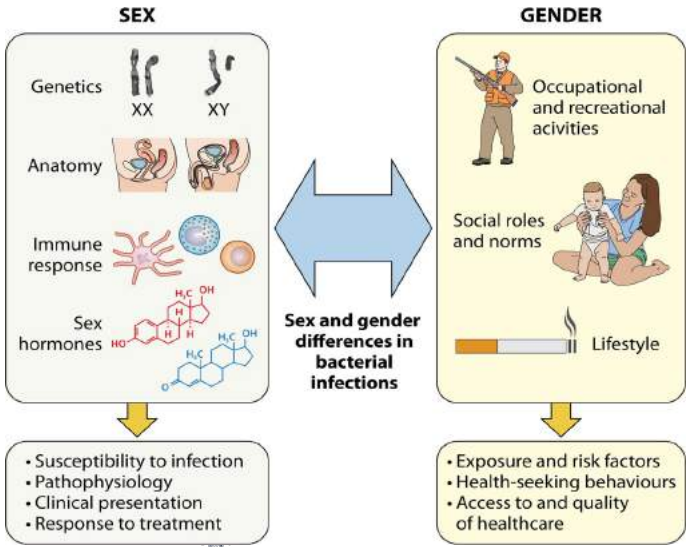
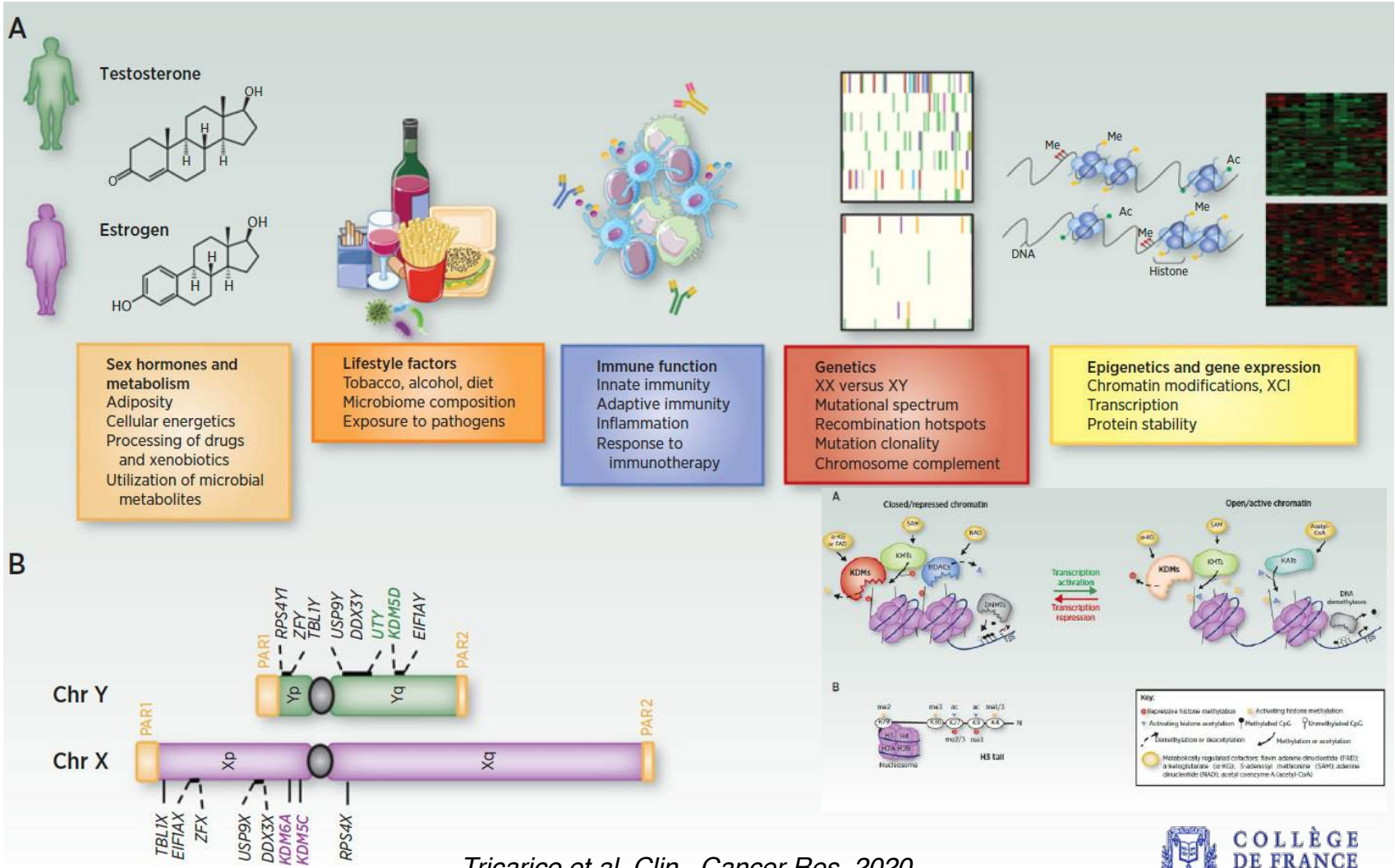








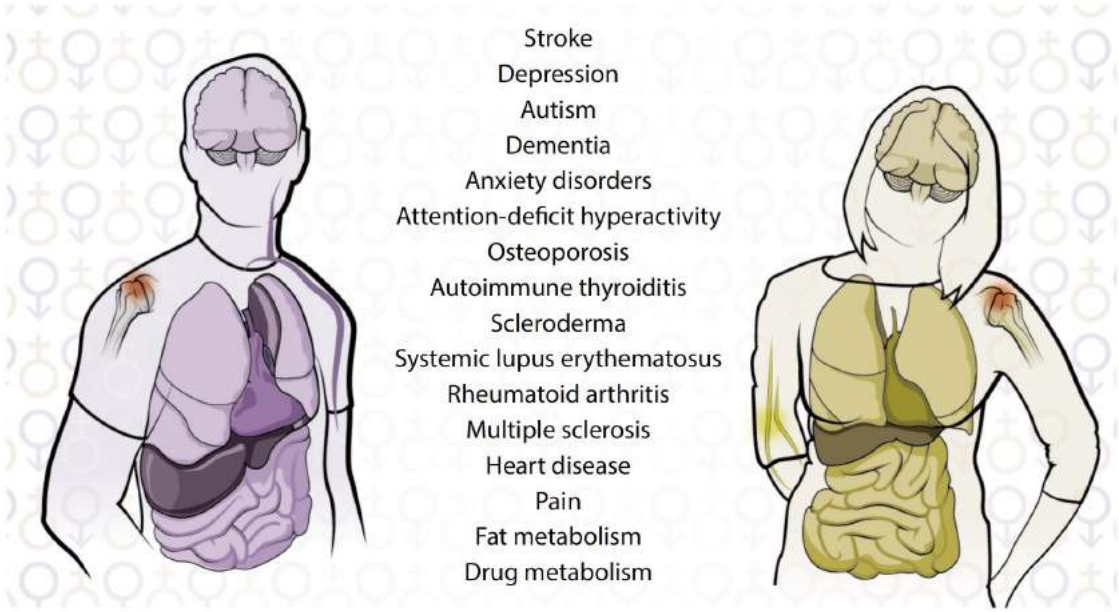
Sexual Dimorphism and Human Health



Tricarico et al, Clin.. Cancer Res. 2020

Sex and gender exert wide-ranging effects on human health

- Sex is the now generally considered as the constellation of biological attributes of sexually reproducing organisms, including physical characteristics.
- Gender refers to cultural and social attitudes that influence a continuum of traits considered to be feminine or masculine, social interactions, issues of gender identity.
- It is crucial to design studies that can capture variation within and between sexes.
- Sex and gender must be considered in preclinical study design and analysis...



Sex equity. Men and women display differences in presentation and risk for certain human diseases, but the underlying mechanisms remain elusive.

Biomedical science has shown a strong bias and blindness to sex and gender

After many centuries of misconceptions and misleading information, today, the medical field understands that there are marked sex-based differences in the epidemiology, clinical manifestations, course, and therapy of disease.

Still, it is only in the last decade that biomedical research and clinical trials are actually including both sexes, not just males.

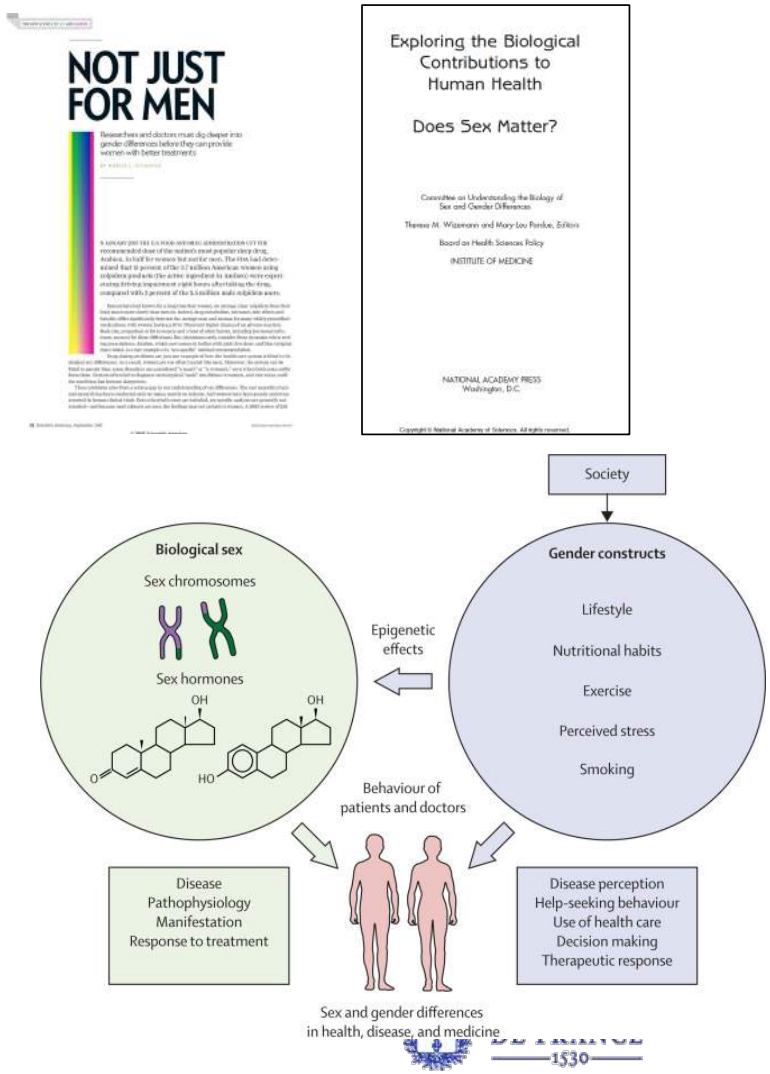
Very few sex-based differences are understood in molecular or cellular terms, yet many of the explanations will derive from the fundamental biological differences between the sexes.

These are due to both **hormonal** and **genetic** differences, that impact both gonadal and non-gonadal tissues.

Certain differences also derive from **environmental** factors and **life style** differences.

2023 COURS III and IV *for the role of sex chromosomes and Xi chromosome in particular.*

E. Heard, March 13th 2023



Sex bias in disease: overcoming bias in *practices*

NIH Policy on the Inclusion of Women in Clinical Research

Women now account for roughly half of all participants in NIH-supported clinical research. More often than not, basic and preclinical biomedical research has focused on male animals and cells.

An over-reliance on **male animals** and **cells** obscures understanding of key sex influences on health processes and outcomes.

- Accounting for sex as a biological variable begins with the development of research questions and study design.
- It also includes data collection and analysis of results, as well as reporting of findings. Consideration of sex may be critical to the interpretation, validation, and generalizability of research findings.
- Adequate consideration of both sexes in experiments and disaggregation of data by sex allows for sex-based comparisons and may inform clinical interventions.
- Appropriate analysis and transparent reporting of data by sex may therefore enhance the rigor and applicability of preclinical biomedical research.

NIH Policy on Sex as a Biological Variable

The 4 Cs of Studying Sex to Strengthen Science:-

In June 2020, the Journal of Women's Health published "Sex as a Biological Variable: A 5-Year Progress Report and Call to Action," an article commenting on the development and implementation of NIH's SABV policy, which went into effect in January 2016.

https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable

Sex bias in disease: overcoming bias in *practices*

Sex bias in disease: overcoming bias in *practices*

SYNTHÈSE

HCE HAUT CONSEIL à l'EGALITE ENTRE LES FEMMES ET LES HOMMES

Prendre en compte le sexe et le genre pour mieux soigner : un enjeu de santé publique

Les différences de santé entre les femmes et les hommes résultent d'interactions complexes entre des facteurs biologiques, socioculturels et économiques. Si des spécificités anatomiques et physiologiques liées au **sexe** biologique participent de ces différences, elles ne sont pas exclusives. L'influence du **genre** - qui réfère à la construction sociale des identités et des rapports sociaux entre les sexes - est un facteur d'inégalité entre les femmes et les hommes dans la santé et dans la prise en charge médicale.

Chez les malades tout d'abord, les codes sociaux liés au genre féminin et masculin influencent l'expression des symptômes, le rapport au corps, le recours aux soins. Chez les personnels soignants, les préjugés liés au genre sont susceptibles d'influencer l'interprétation des signes cliniques et la prise en charge des pathologies. Dans les recherches cliniques et biomédicales enfin, ils peuvent induire des biais dans les expérimentations et les applications médicales. A cela, s'ajoutent les conditions de vie, sociales et économiques, qui exposent différemment les femmes et les hommes à des risques de santé. La combinaison de tous ces facteurs conduit à des situations d'inégalité de santé et de discrimination entre les sexes dans l'accès aux soins et dans la prise en charge médicale.

Ces questions sont au cœur du présent rapport dont le fil directeur est de montrer comment la dimension du genre alliée à celle du sexe permet de mieux comprendre comment se forgent les différences et les inégalités de santé entre les femmes et les hommes.

L'enjeu est de démontrer que la prise en compte du genre permet d'analyser plus précisément les pathologies, de formuler de nouvelles hypothèses de recherche et de construire des stratégies de prévention et de traitement. Il est aussi de montrer que cette approche constitue une innovation dans la médecine et la recherche pour le plus grand bénéfice de la santé des femmes comme de celle des hommes.

Notre investigation aborde de nombreuses disciplines de recherche - médecine, biologie, sociologie, épidémiologie - qui sont questionnées dans leurs rapports avec les modes de vie, le monde du travail, les nuisances de l'environnement, y compris la Covid-19. Quatre axes ont été retenus dans cette analyse.

Prendre en compte le sexe et le genre pour mieux soigner : un enjeu de santé publique

Rapport n°2020-11-04 Santé 45 voté le 04 11 2020

E. Heard, March 13th 2023

Axe 1. Les maladies dites féminines ou masculines : une réalité à nuancer

Axe 2. Les recherches pluridisciplinaires sur le sexe et le genre dans la santé : des clarifications nécessaires

Axe 3. Inégalités de santé : conditions de vie et environnement

Axe 4. Formation sur genre et santé et l'accès à la gouvernance : des lacunes et des résistances

Quatre objectifs au coeur des politiques publiques :

1. Mieux soigner en sensibilisant les soignant.es à prendre en compte les interactions entre sexe et genre dans les pathologies ;
2. Mieux rechercher en soutenant les recherches pluridisciplinaires sur le sexe et le genre dans la santé ;
3. Mieux maîtriser les risques d'inégalité de santé en prenant en compte les conditions de vie et l'environnement (polluants physiques, chimiques et microbiologiques - Covid) ;
4. Mieux former les étudiant.es, soignant.es et chercheur.euses, et instaurer une dynamique paritaire pour l'accès aux responsabilités.

Quarante préconisations

Sex bias in disease: overcoming bias in *practices*

HCE — HAUT CONSEIL à l'EGALITE ENTRE LES FEMMES ET LES HOMMES

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HCE - *Prendre en compte le sexe et le genre pour mieux soigner : un enjeu de santé publique*

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E. H

Why were females excluded from scientific studies?

Beery and Zucker, Neuroscience and Biobehavioral Reviews, 2011

"79% of articles dealing with non-human mammals in the early 20th century failed to report subject sex in the Journal of Physiology (London) and the Journal of Pharmacology and Experimental Therapeutics (Fig. 4A). The percentage of articles reporting subject sex increased steadily through 1969, without substantially affecting the relative abundance of male and female single-sex studies. A marked increase in male-only reports after 1969 stabilized at around 50% (Fig. 4A).

Single-sex studies of males still predominate in the biological literature, and neglect of females is widespread in many disciplines, including neuroscience, pharmacology, endocrinology, zoology, and physiology. One cannot assume that beyond the reproductive system, sex differences either do not exist or are irrelevant; despite this, a high proportion of studies failed to specify sex, and in experiments performed on both sexes data often were not analyzed by sex."

Just as the absence of statistical analysis from biological research prior to the 1940s did not prevent major advances in biology, the neglect of females has not prevented progress in non-human animal research. Several arguments nevertheless can be made for abandoning the status quo ante for biomedical research.

To understand the biology of women or develop safe treatments for diseases of women one must do more than study men.

Why were females excluded from scientific studies?

Reasons that have led to medical research being mainly focused on males:

(i) Historical assumption that biological mechanisms unrelated to sex organs and reproduction do *not* differ appreciably between sexes.

(ii) Female hormonal fluctuations were believed to confound biological measurements and so studies of males are favored.

(iii) Considering sex as a variable requires more animals/participants and thus demands greater resources.

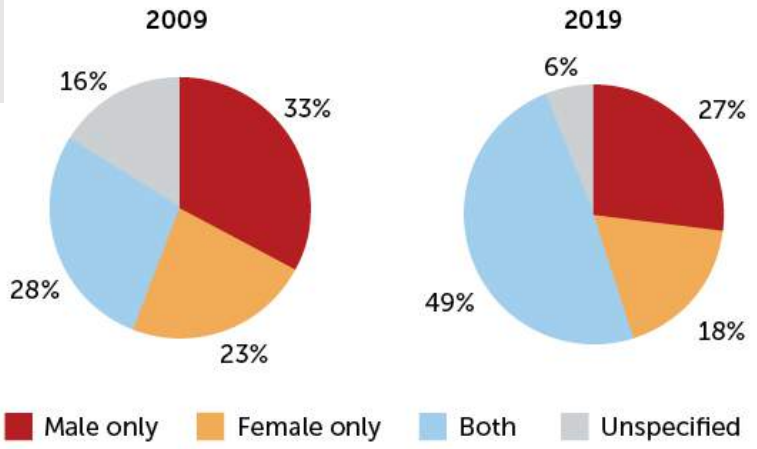
(iv) Clinical trials designed to avoid women in reproductive age groups due to potential risk of pregnancy

Meta-analysis of 293 publications reported that trait variability was no greater in female than in male mice despite not considering estrous cycle stage in the primary studies.

SN: *Why weren't people including females in their scientific studies a decade ago?*

Beery: "*Researchers were making an active choice to exclude females from their studies. One rationale for this is that a lot of people assume that females are more variable than males [due to their hormone cycles]. There have now been several papers that have looked explicitly at that question and shown that no, females aren't more variable than males.*

Male bias also gets historically entrenched. If everyone in your field has studied males, and the body of knowledge that's been built up has always used male-only subjects, then you might be inclined to continue studying male-only subjects…. I think that's been part of perpetuating the male bias for a long time."



Meta-Research: A 10-year follow-up study of sex inclusion in the biological sciences (Elife, 2019)
In 2019, 49 percent of articles surveyed in biomedical science used both male and female subjects, almost twice as many as a decade earlier

https://www.sciencenews.org/article/biomedical-research-sex-male-female-animal-human-studies

COLLÈGE DE FRANCE 1530

Are Females really more “variable” than Males?

Current Biology

CellPress
OPEN ACCESS

Report

Mouse spontaneous behavior reflects individual variation rather than estrous state

Dana Rubi Levy,[1] Nigel Hunter,[1] Sherry Lin,[1] Emma Marie Robinson,[1] Winthrop Gillis,[1] Eli Benjamin Conlin,[1] Rockwell Anyoha,[1] Rebecca M. Shansky,[2,*] and Sandeep Robert Datta[1,3,*]
[1]Department of Neurobiology, Harvard Medical School, Boston, MA, USA
[2]Department of Psychology, Northeastern University, Boston, MA, USA

Tracking open-field behaviour of female and male mice over weeks revealed that behaviour reflects *individual identity*, far more than estrous state in females. Males are much more variable than females, however, arguing for the inclusion of both sexes in studies of spontaneous behaviours.

- Spontaneous behaviour of female mice is only negligibly affected by estrous state
- Females and males exhibit strongly individualized patterns of exploration
- Female spontaneous behaviour is less variable than male behaviour.

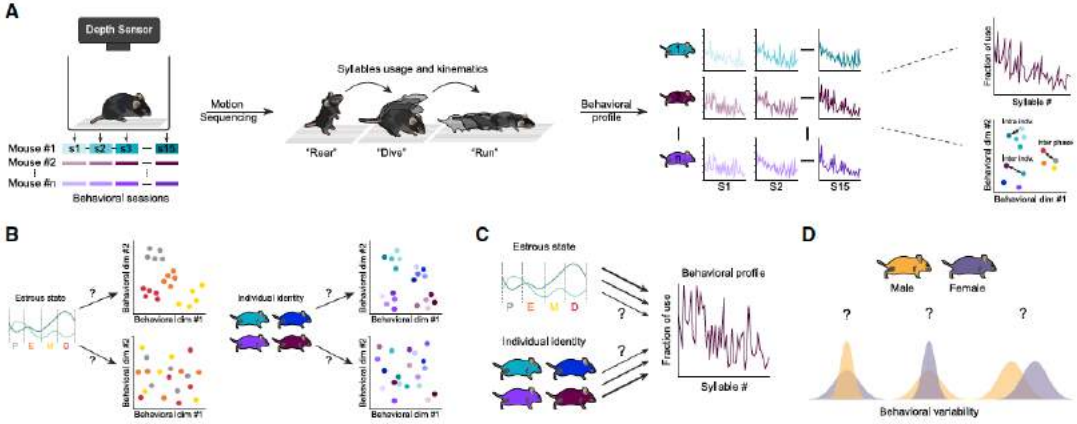
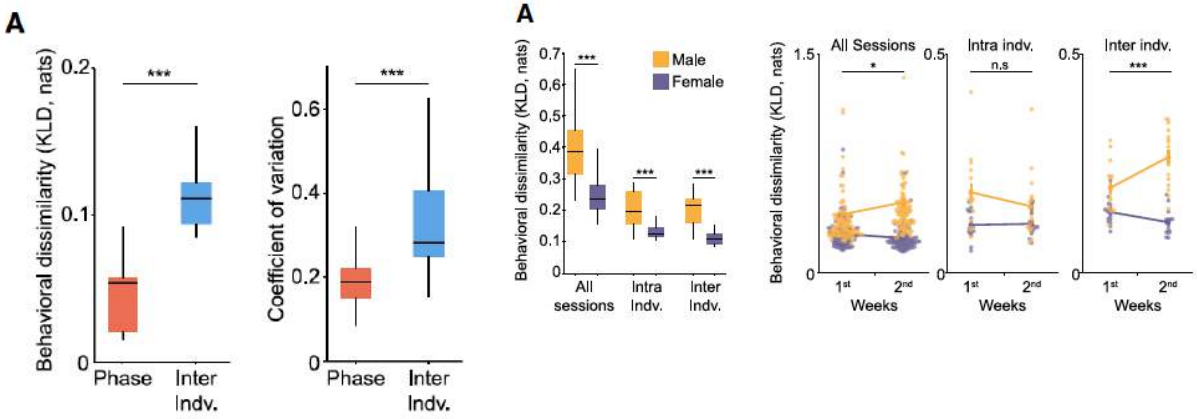


Figure 1. Weighing the relative influence of estrous state and individual variation on spontaneous behavior
(A) Individual mice (n = 16 females, n = 16 males) were recorded in an open-field arena using a depth camera for 15 consecutive sessions, and the estrous phase of



COLLÈGE DE FRANCE 1530

Sex bias in disease: what are the causes?

After many centuries of misconceptions, misleading information or lack of information, today, the medical field understands that there are marked sex-based differences in the epidemiology, clinical manifestations, course, and therapy of disease.

Still, it is only in the last decade that biomedical research and clinical trials are actually including both sexes, not just males.

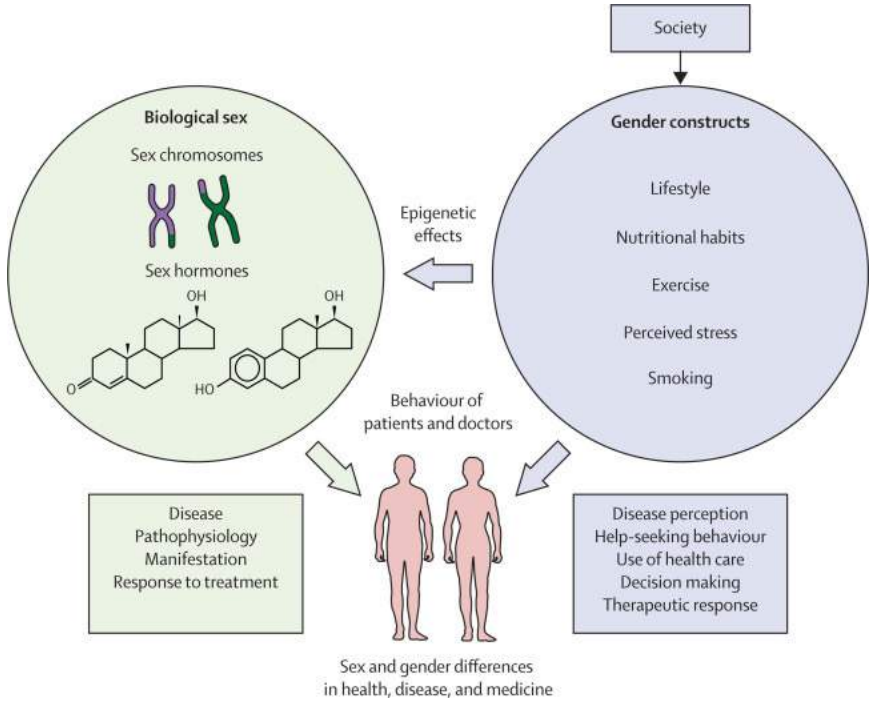
Very few sex-based differences are understood in molecular or cellular terms, yet many of the explanations will derive from the fundamental biological differences between the sexes.

These are due to both **hormonal** and **genetic** differences, that impact both gonadal and non-gonadal tissues.

Certain differences also derive from **environmental** factors and **life style** differences.

Inclusion of **both sexes** in **studies.**

Where possible, use of models that **allow hormonal, genetic and environmental differences** to be distinguished.



COLLÈGE DE FRANCE 1530

Sex bias in disease: what are the causes?

The example of known sex bias in Autoimmune Diseases

Prevalence and genetic basis of autoimmune diseases.

Disease	Sex (F/M)	Prevalence (rate per 100,000)	Monozygotic disease concordance rates	Dizygotic disease concordance rates
Thyroiditis/Hypothyroidism	18:1	792	55% TPO Ab (64–65%)	0% TPO Ab (13–35%)
Sjogren's syndrome	(9–15):1	14–1600	N/A	N/A
Systemic sclerosis (Schleroderma)	12:1	4	4–5%	4–5%
Addison's Disease	12:1	5	N/A	N/A
Systemic lupus erythematosus (SLE)	9:1	2–7.6	24–57%	2–5%
Type 2 autoimmune hepatitis	9:1	10–20	N/A	N/A
Primary biliary cirrhosis	8:1	3	N/A	N/A
Graves' Disease	7:1	1151	17–36%	0–4%
Chronic active hepatitis	7:1	0.4	N/A	N/A
Multiple sclerosis (MS)	3:1	58	25%, 30–35%	0–5%
Rheumatoid arthritis (RA)	3:1	860	12–15%	3–4%
Type 1 autoimmune hepatitis	3:1	10–20	N/A	N/A
Myasthenia gravis	3:1	5	35%	4–5%
Glomerulonephritis, IgA	2:1	23	N/A	N/A
Pernicious anemia	2:1	151	N/A	N/A
Polymyositis/dermatomyositis	2:1	5	N/A	N/A
Celiac disease	2:1	39	75–83%	11%
Psoriasis	1:1	79	70%	20%
Inflammatory bowl disease (IBD)	1:1	2	17%	8%
Type 1 diabetes mellitus (IDDM)	1:1	192	32–50%	5–6%
Uveitis	1:1	2	N/A	N/A
Vitiligo	1:1	400	23%	0%
Guillain–Barré syndrome	1:2	2	N/A	N/A
Glomerulonephritis, primary	1:2	40	N/A	N/A
Ankylosing spondylitis (AS)	1:2	129	N/A	N/A
Goodpasture's syndrome	1:(2–9)	0.5	N/A	N/A

Autoimmune diseases comprise a range of diseases in which the immune response to self-antigens results in damage or dysfunction of tissues (Mackay and Burnet, 1963).

Criteria:

- the identification of a target antigen
- the presence of antibodies and/or T cells in the target organ,
- the transfer of disease to animals by cells or antibodies (Rose and Bona, 1993; Witebsky et al., 1957).

COLLÈGE DE FRANCE 1530

Sex bias in disease: what are the causes?

The example of known sex bias in Autoimmune Diseases

The mother's role in directly nurturing the fetus during gestation may have important implications for autoimmune disorders. Where there is disparity between the sexes.

Graves' disease, systemic lupus erythematosus, scleroderma, and multiple sclerosis share few clinical features, but these diseases affect women 3 to 10 times as often as men.

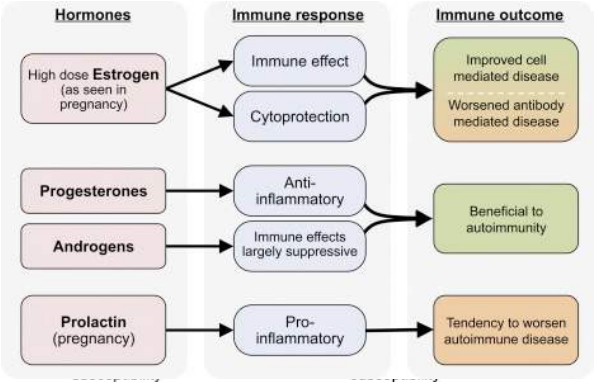
The assumption was that gonadal steroids play a major role in this disparity, but recent work suggests that **genetic** and **epigenetic** differences could also have a role gene expression from the inactive X
(COURS V, 2018 and more in next 2 weeks: COURS III and IV 2023)

The discovery that fetal cells can persist in the mother's circulation for decades after delivery has led to hypotheses that the presence of such foreign cells provides antigenic exposure that may be the source of heightened immune reactions in women.

The challenge: distinguishing the effects of hormones, sex chromosome and environmental variables in order to better predict and treat such diseases.

E. Heard, March 13th 2023

Hormones



X chromosomes

X Chromosome Dose and Sex Bias in Autoimmune Diseases

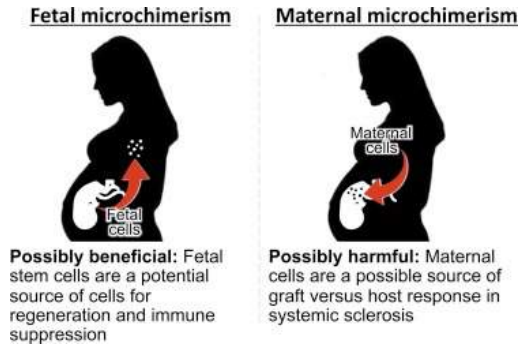
Increased Prevalence of 47,XXX in Systemic Lupus Erythematosus and Sjögren's Syndrome

Table 2. Relative risk of SLE and primary SS with X chromosome numbers*

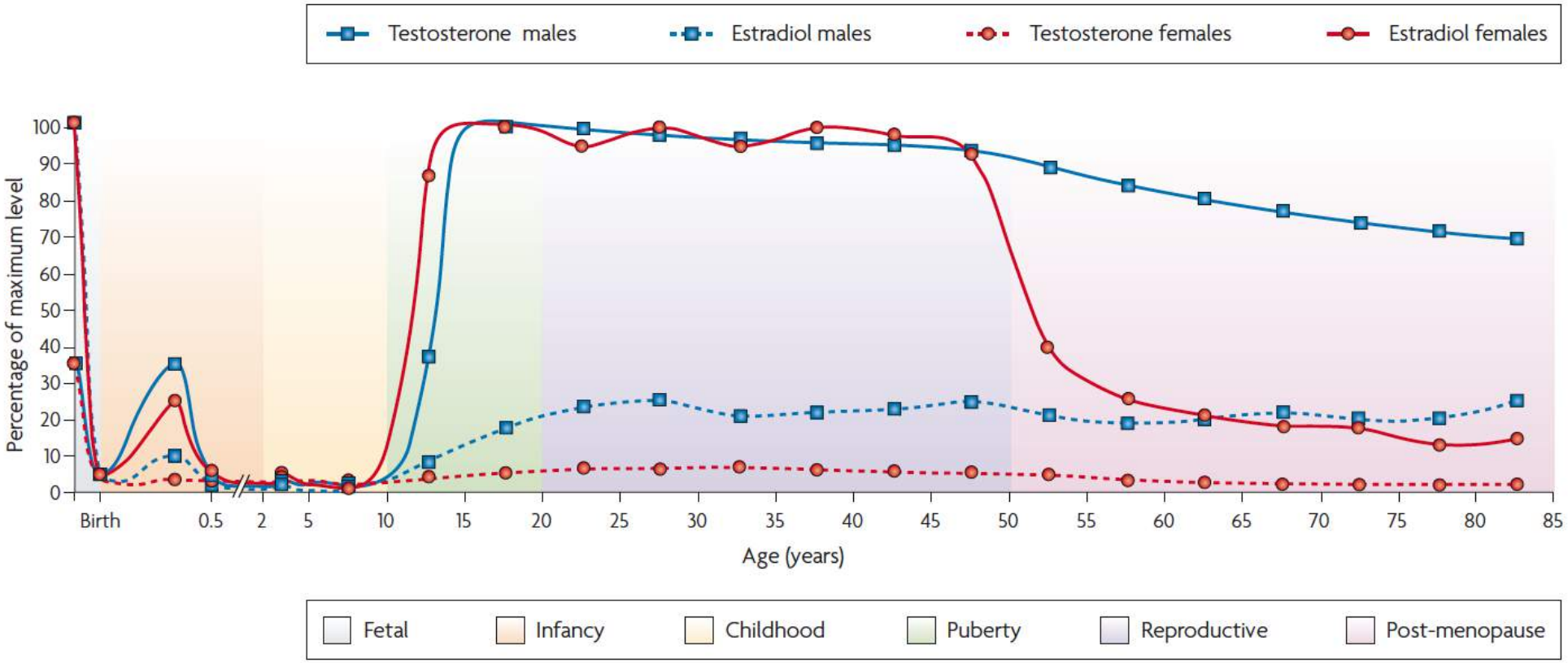
X chromosome number	Karyotype	SLE relative risk	SS relative risk
1	45,X; 46,XY	1	1
2	46,XX; 47,XXY†	~10	~14
3	47,XXX	~25	~41

* SLE = systemic lupus erythematosus.
† Data for 47,XXY with Sjögren's syndrome (SS) are not available.

Fetal cells



Sex Hormones changes during Life



E. Heard, March 13th 2023

COLLÈGE DE FRANCE 1530

Discovery of the hormonal regulation of sexual differentiation

Much of our understanding of how sex determination works in vertebrates comes from a paradigm established by the heroic experiments of Alfred Jost at the end of World War II.

Working with rabbits, Jost developed a surgical method of removing the gonads from developing embryos and returning operated embryos to the uterus to complete development. Jost discovered that removal of the gonads from all embryos at mid-gestation led to the exclusive development of rabbits with female morphological sex characteristics.

These experiments proved that (at least in rabbits) development of a phenotypic female does not require a gonad, but development of a phenotypic male does. From these experiments, Jost concluded that primary sex determination involves the **decision to initiate testis or ovary development**, which in turn leads to the **production of substances** that control the development of the **sex ducts** and **genitalia**.

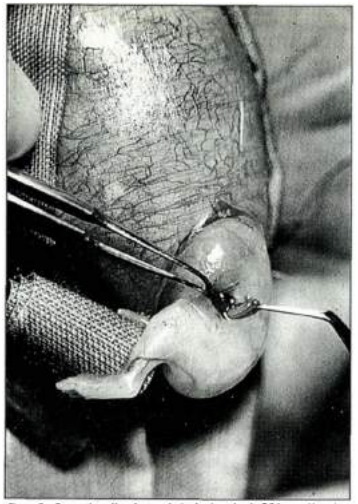


Figure 7. **Castration d'un fœtus de lapin de près de 23 jours.** Une chambre utérine a été ouverte, la partie postérieure du fœtus est extériorisée, son flanc est ouvert et le testicule, inséré sur le mésonéphros est exposé avant d'être réséqué. Après ablation bilatérale des testicules, le fœtus est replacé dans l'utérus jusque vers le terme. Pour des raisons de commodité, on a photographié l'opération sur un fœtus déjà trop âgé et trop grand pour donner des résultats figurant dans les figures 8 et 9.

Alfred Jost (1916 -1991)
Endocrinologist, Professeur
Collège de France 1974 – 19
Chaire Physiologie du développe

From B. Capel, Nature Reviews Genetics, 2017

E. Heard, March 13th 2023

COLLÈGE DE FRANCE 1530

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He proceeded to show that the developing testis produces two critical substances that control sex determination. The first is testosterone and its derivatives, which support the development of the **male reproductive ducts** (the epididymis and the vas deferens) and the **male genitalia**. The second substance, identified later as anti-Müllerian hormone (AMH), controls the degeneration of the **female duct** primordia (which would otherwise give rise to the oviduct and uterus).

Similarly, the primordium for the **external genitalia** is identical in all embryos but differentiates as male genitalia in the presence of dihydrotestosterone or as female genitalia in its absence.

From B. Capel, Nature Reviews Genetics, 2017

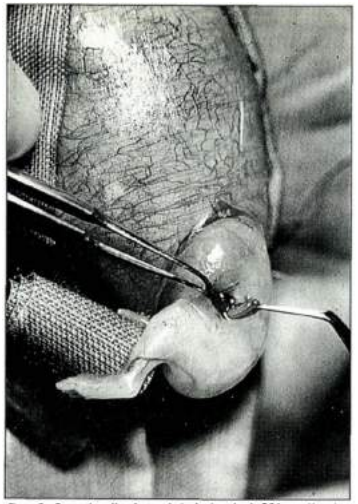

Figure 7. **Castration d'un foetus de lapin de près de 23 jours.** Une chambre utérine a été ouverte, la partie postérieure du foetus est extériorisée, son flanc est ouvert et le testicule, inséré sur le mésonéphros est exposé avant d'être réséqué. Après ablation bilatérale des testicules, le foetus est replacé dans l'utérus jusque vers le terme. Pour des raisons de commodité, on a photographié l'opération sur un foetus déjà trop âgé et trop grand pour donner des résultats figurant dans les figures 8 et 9.

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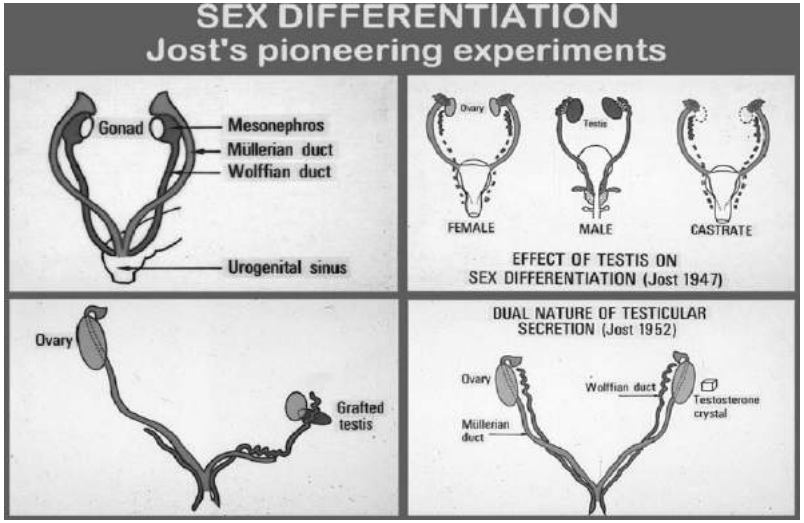


E. Heard, March 13th 2023

Discovery of the hormonal regulation of sexual differentiation

Alfred Jost's work in the 1940s laid the foundation of the current **paradigm of sexual differentiation of reproductive tracts**, which contends that testicular hormones drive the male patterning of reproductive tract system whereas the female phenotype arises by default.

Once established, the sex-specific reproductive tracts undergo morphogenesis, giving rise to anatomically and functionally distinct tubular organs along the rostral–caudal axis.

Impairment of sexual differentiation of reproductive tracts by genetic alteration and environmental exposure are the main causes of disorders of sex development, and infertility at adulthood.

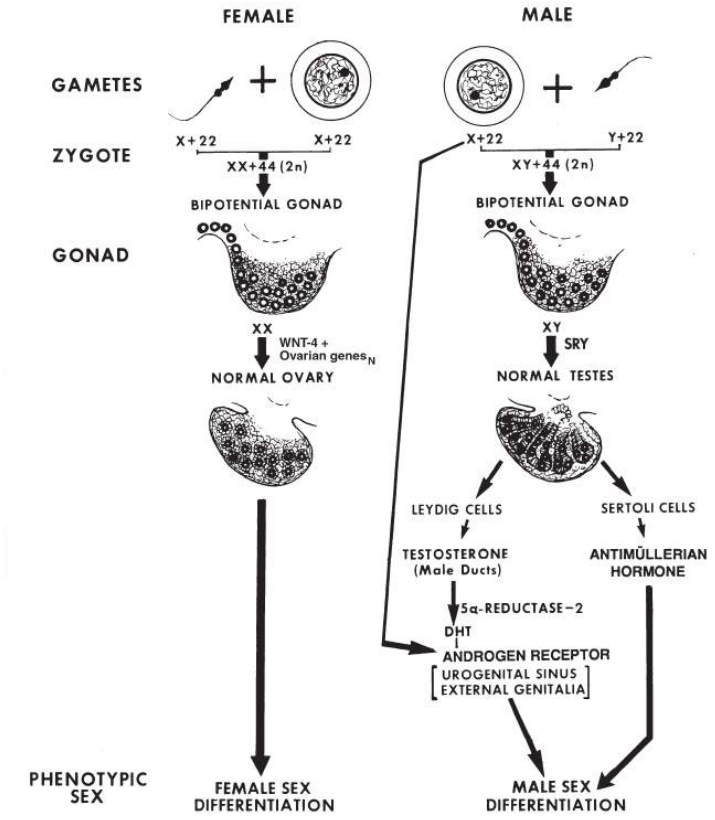


FIGURE 3-1 From genotype to phenotype: a diagrammatic representation of human sex determination and differentiation. Intrinsic or extrinsic factors adversely affecting any stage of these processes can lead to anomalies of sex. Source: Grumbach and Conte (1998, Figure 29-28, p. 1329). Reprinted, with permission, from M. M. Grumbach and F. A. Conte. 1998. *In: Williams Textbook of Endocrinology*, 9th ed. J. D. Wilson, D. W. Foster, H. M. Kronenberg, and P. R. Larsen, eds. Philadelphia: W. B. Saunders Co. Copyright by W. B. Saunders Company, Philadelphia.

E. Heard, March 13th 2023

Sex Hormones

Sex hormones (sex steroids, gonadocorticoids, gonadal steroids) are steroid hormones that interact with steroid hormone receptors. They include **androgens**, **estrogens**, and **progestogens**:

Androgens and **estrogens** give rise to **testosterone** and **estradiol**, respectively.

Progestogens are distinct from androgens and estrogens and progesterone is the only naturally occurring human progestogen.

All estrogen is obligatorily synthesized from androgen: starting with either acetate or cholesterol, androstenedione and testosterone are synthesized in both the ovary and testis and then partially converted to the estrogens, estrone and estradiol.

Sex hormones are naturally produced by the gonads (ovaries / testes), by adrenal glands, or by conversion from other sex steroids in other tissues eg liver or fat.

Sex hormones are involved in the regulation of sexual functions, such as the regulation of reproductive cycle, development of accessory reproductive organs.

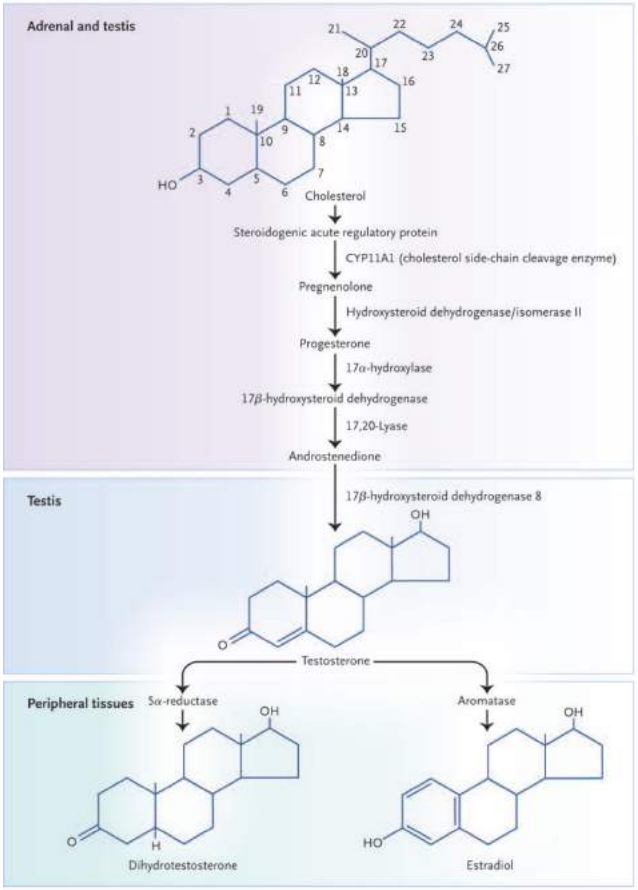
Sex hormones influence the secondary sex characteristics, e.g. body shape and contour, mammary development, and pitch of voice.

All types of hormones are present in both sexes - at different levels: androgens are considered male sex hormones, since they have masculinizing effects, while estrogens and progestogens are considered female sex hormones.

Testosterone is present at higher levels in men, but also present in women. Variable testosterone levels explain some differences in human traits and disease prevalence.

Effects of hormones are mediated by slow genomic mechanisms through nuclear receptors, as well as by faster mechanisms through membrane-associated receptors and signalling cascades.

E. Heard, March 13th 2023

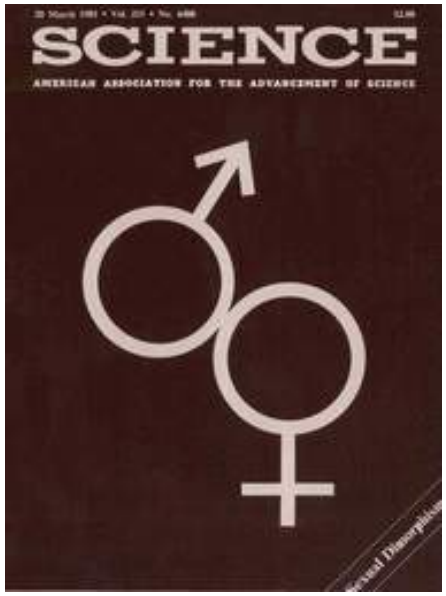


The Biosynthesis of Gonadal Steroids

The total amount of testosterone synthesized differs between the sexes, as do the relative amounts of testosterone and dihydrotestosterone produced and the relative amounts of estradiol and testosterone produced by metabolism.

CYP11A1 denotes cytochrome P-450 11A1. Adapted from Griffin and Wilson.

Sex Hormones control Sexual Development



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63. Supported in part by NIH grant 5R01 HD 12289-02, American Cancer Society grant [W]1T-9 (to F.P.H.), and NIH grants ROI AD 00042 and ROI ATT 5620 (to S.O.). F.P.H. thanks F. Naftolin and L. A. Steiner for critical discussion.

The Hormonal Control of Sexual Development

Jean D. Wilson, Fredrick W. George, James E. Griffin

Human embryos of both sexes develop in an identical fashion for the first 2 months of gestation, and only thereafter do anatomical and physiological development diverge to result in the formation of the male and female phenotypes. The fundamental mechanism of sexual differentiation was elucidated between 1947 and 1952 by Alfred Jost (*1*). He established that the castrated mammalian embryo develops as a female. Male development is induced in the embryo only in the presence of specific hormonal signals arising from the fetal testis. According to the Jost formulation—now the central dogma of sexual development—sexual differentiation is a sequential, ordered, and relatively simple process. Chromosomal sex, established at the time of conception, directs the development of either ovaries or testes. If testes develop, their hormonal secretions elicit the development of the male secondary sex characteristics, collectively known as the male phenotype. If an ovary develops or if no gonad is present, anatomical development is female in character.

Summary. Male and female human embryos develop identically during the first phase of gestation. The indifferent gonads then differentiate into ovaries or testes and soon begin to secrete their characteristic hormones. If ovaries (or no gonads) are present the final phenotype is female; thus no gonadal hormones are required for female development during embryogenesis. Two hormones of the fetal testis—Müllerian regression hormone and testosterone—are responsible for the formation of the male phenotype. Analysis of fibroblasts from the skin of patients with abnormalities of sexual development due to single gene defects shows that testosterone is responsible for virilization of the male internal genital tract, that its derivative dihydrotestosterone causes development of the male external genitalia, and that both hormones act in the embryo by the same receptor mechanisms operative in postnatal life.

Stimulated by this paradigm, subsequent investigators have sought to identify the specific hormones that are secreted by the fetal testis and to elucidate the control mechanisms that regulate the rates of secretion of these hormones at the crucial moment in embryonic development. They have also attempted to characterize, at the molecular and genetic level, the mechanisms by which the testicular hormones act to induce the conversion of the sexually indifferent embryo into the male phenotype. As a consequence, the original formulation of Jost has been refined and expanded, and insight has been obtained into the pathogenesis of many derangements of sexual development in humans which result from single gene defects that impede either the formation or the cellular actions of the hormones of the fetal testis.

Other authors have described the chromosomal basis for sex determination (*2*) and the mechanism by which the X and Y chromosomes cause the differentiation of the gonad into a testis or ovary (*3*). In this article we describe current concepts of the processes by which the fetal gonads acquire the capacity to function as endocrine organs and of the mechanisms by which the endocrine secretions of the fetal testis modulate male development. We focus first on the anatomical events involved in the formation of the sexual phenotypes and then on the factors that mediate this development.

Formation of the Sexual Phenotypes

The temporal relation between the differentiation of the ovary and testis and the development of the sexual phenotypes in the human embryo is shown schematically in Fig. 1. The germ cells do not originate in the embryo itself but rather in the yolk sac (*4*). By about the stage at which the embryo reaches 10 to 20 millimeters in crown-to-rump length, the germ cells migrate to their ultimate destination in the genital ridges of the embryo. After this migration, the primitive gonads in male and female embryos appear identical, and each such gonad has three components: (i) the primordial germ cells, (ii) the mesenchyme of the genital ridge, and (iii) a covering layer of epithelium. Histological differentiation begins when the germ cells in the testis

The authors are in the Department of Internal Medicine at the University of Texas Southwestern Medical School, Dallas 75235.

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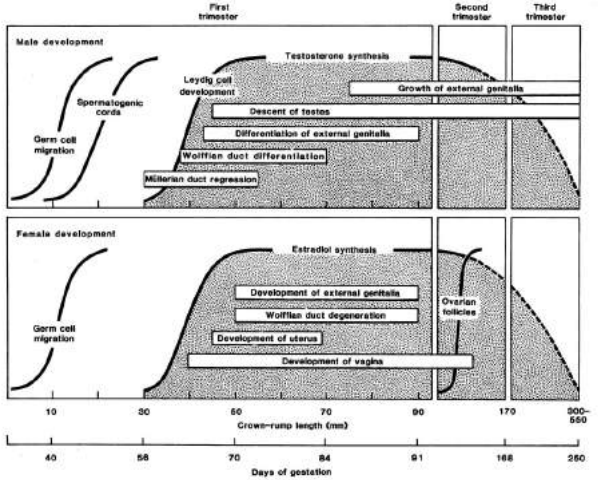
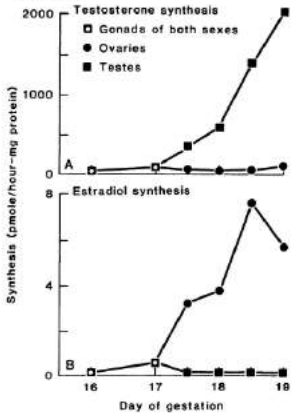


Fig. 1. Relation between differentiation of the gonads and the anatomical differentiation of the human male and female embryos. [Drawn from data in (*3*, *14*, *19*).]



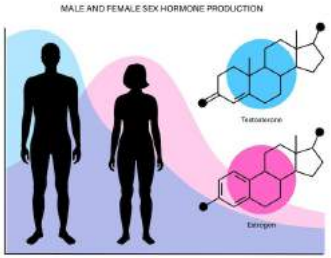


Fig. 4. Onset of endocrine function in the fetal testis and ovary of the rabbit embryo. Each gonad begins to synthesize its characteristic hormone at approximately the same time, beginning on day 17.5 (*17*).

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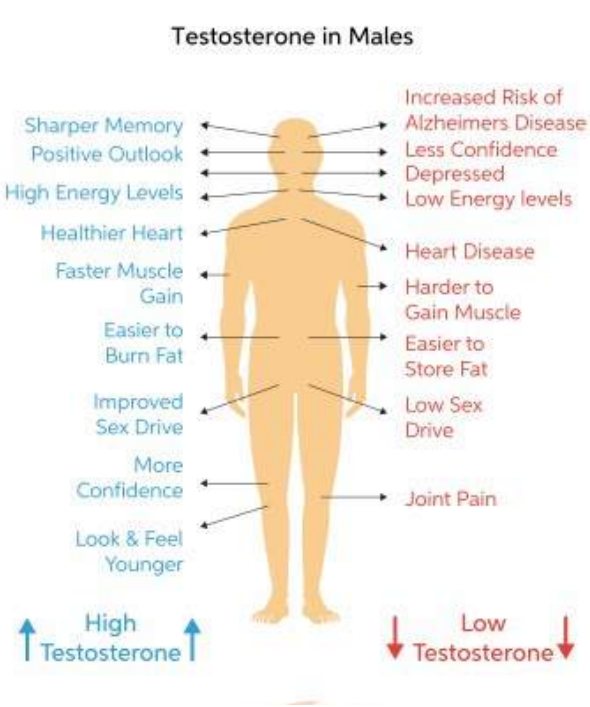
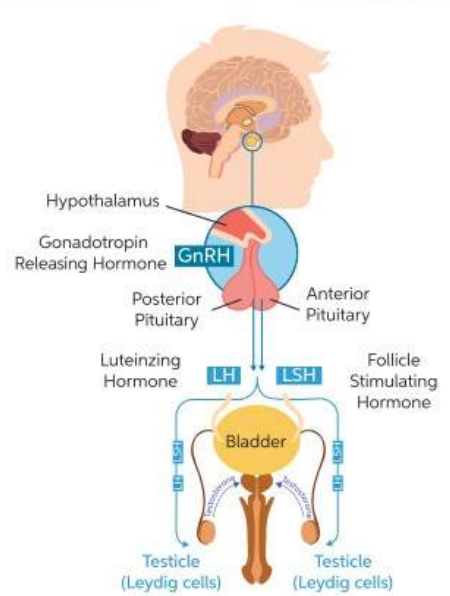
E. Heard, March 13th 2023

Sex Hormones: Testosterone in Males

Testosterone governs male sexual development and physique. It is responsible for the growth of male sexual organs. It is responsible for the development of secondary sex characteristics like body hair and the deepening of the male voice. The above image shows the impact of low testosterone and high testosterone levels on the male body. Testosterone is responsible for boosting muscle mass and sex drive in males. Testosterone leads to an increase in lean body mass in males and increase in muscle strength. Testosterone is also known to decrease the risk of osteoporosis. Hence, synthetic development of testosterone is known as anabolic steroids, and these are very often used and abused by athletes to increase their stamina and performance when playing competitive sports. Anabolic steroids are known to increase muscle development and reduce body fat.





https://www.chegg.com/learn/topic/gonadal-hormones

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Sex Hormones: Estrogen and Progesterone in females

Estrogen is the primary female sex hormone. It is responsible for the estrous or reproductive cycle. The main type of estrogen produced is known as estradiol. Estradiol is not just important in reproductive and sexual functioning; it has an important role in maintaining other organs like our bones. Estradiol is responsible for ovulation. Estradiol is important for increased uterine motility. Estradiol is responsible for neuronal growth. Estradiol is responsible for regulating the cardiovascular physiology of the body.

Progesterone is mainly important in maintaining pregnancy. It is produced in the ovaries, adrenal glands, and placenta. It is also important in maintaining the estrous and menstrual cycles. Progesterone is responsible for follicular growth and ovulation. Progesterone is responsible for endometrial secretion. It maintains pregnancy by inhibiting uterine contractions and the aiding in the glandular development of the endometrium. It also helps in the development of the mammary gland.

Both estrogen and progesterone collectively work in preparing the uterus for pregnancy and the mammary gland for the process of lactation.

https://www.chegg.com/learn/topic/gonadal-hormones

E. Heard, March 13th 2023

The Role of Estrogen and Progesterone

ESTROGEN EFFECTS
Builds up uterine lining
Increases body fat
Depression, headache/migraine
Interferes with thyroid hormone
Increases blood clotting
Decreases libido
Impairs blood sugar control
Increases risk of endometrial cancer
Increases risk of breast cancer

PROGESTERONE EFFECTS
Maintains uterine lining (secretory)
Helps use fat for energy
Anti-depressant
Facilitates thyroid hormone action
Normalizes blood clotting
Restores libido
Regulates blood sugar control
Protects risk of endometrial cancer
Probable risk of breast cancer

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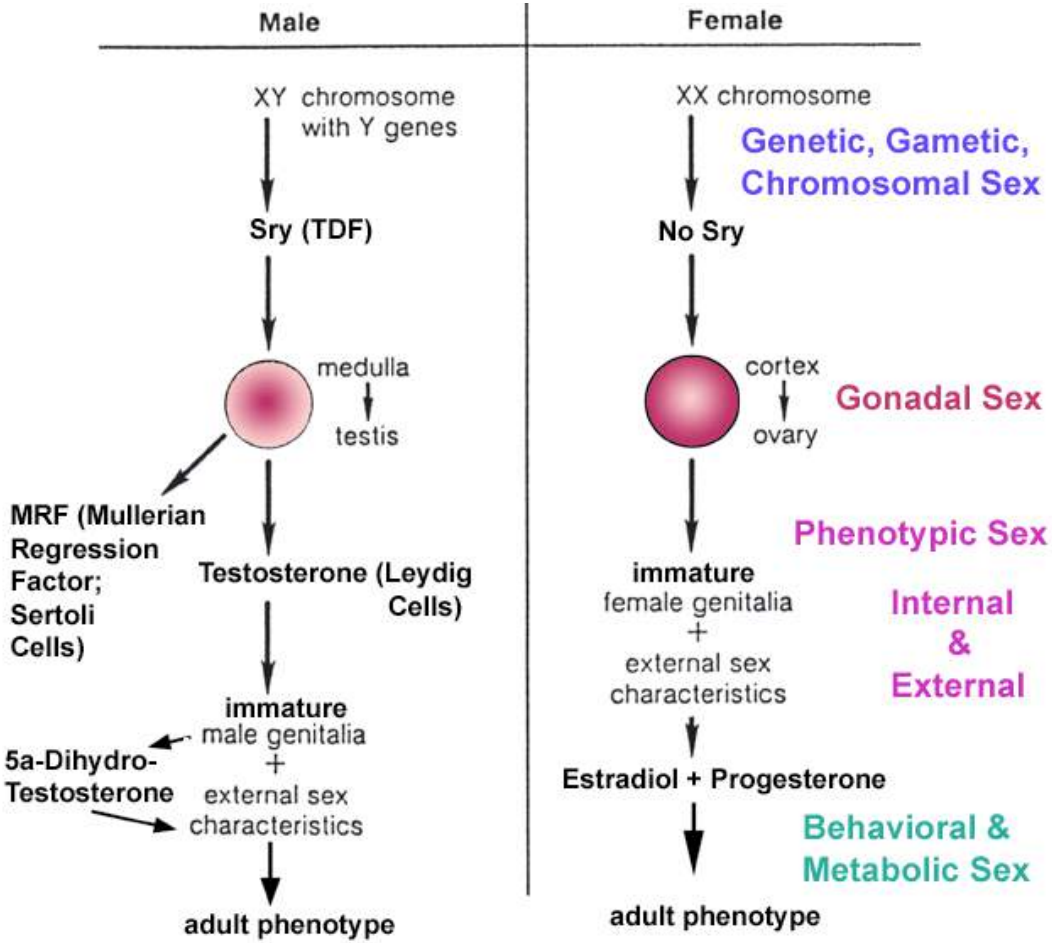
How much hormonal control is there of the human body in non-gonadal tissues?



For example: Sex Hormones and the Human Brain?

McCarthy MM. 2016. Mul
Phil. Trans. R. Soc. B 371:

Male	Female
In utero	***In utero***
• Testosterone and its aromatization to estrogen cause masculinization of the fetal brain	• Absence of androgen production and estrogen-binding activity of alpha-fetoprotein cause feminization of the fetal brain
Adolescence	**Adolescence**
• More between-network connectivity • Larger grey matter volume • Lower grey matter density	• More within-network connectivity • Less grey matter volume • Higher grey matter density
Adulthood	**Adulthood**
• More total brain volume • More grey matter volume • More white matter volume • More cerebrospinal fluid volume • Higher proportion of white matter • Larger volume of the central subdivision of the bed nucleus stria terminalis • Better visuospatial and mathematical ability • Weaker right-hand preference	• Less total brain volume • Less grey matter volume • Less white matter volume • Less cerebrospinal fluid volume • Higher proportion of grey matter • Thicker cortex • Higher global cerebral blood flow • Better perceptual speed and fine manual dexterity • Stronger right-hand preference

In mammals, many sex differences are downstream of the unequal effects of XX vs. XY sex chromosomes and are influenced by hormones and environmental factors

Need to distinguish:

- Effects of gonadal hormones on development and in adulthood eg Xu et al
- Effects of sex chromosomes operating outside of the gonads.
- Effect of maternal factors and early life environment

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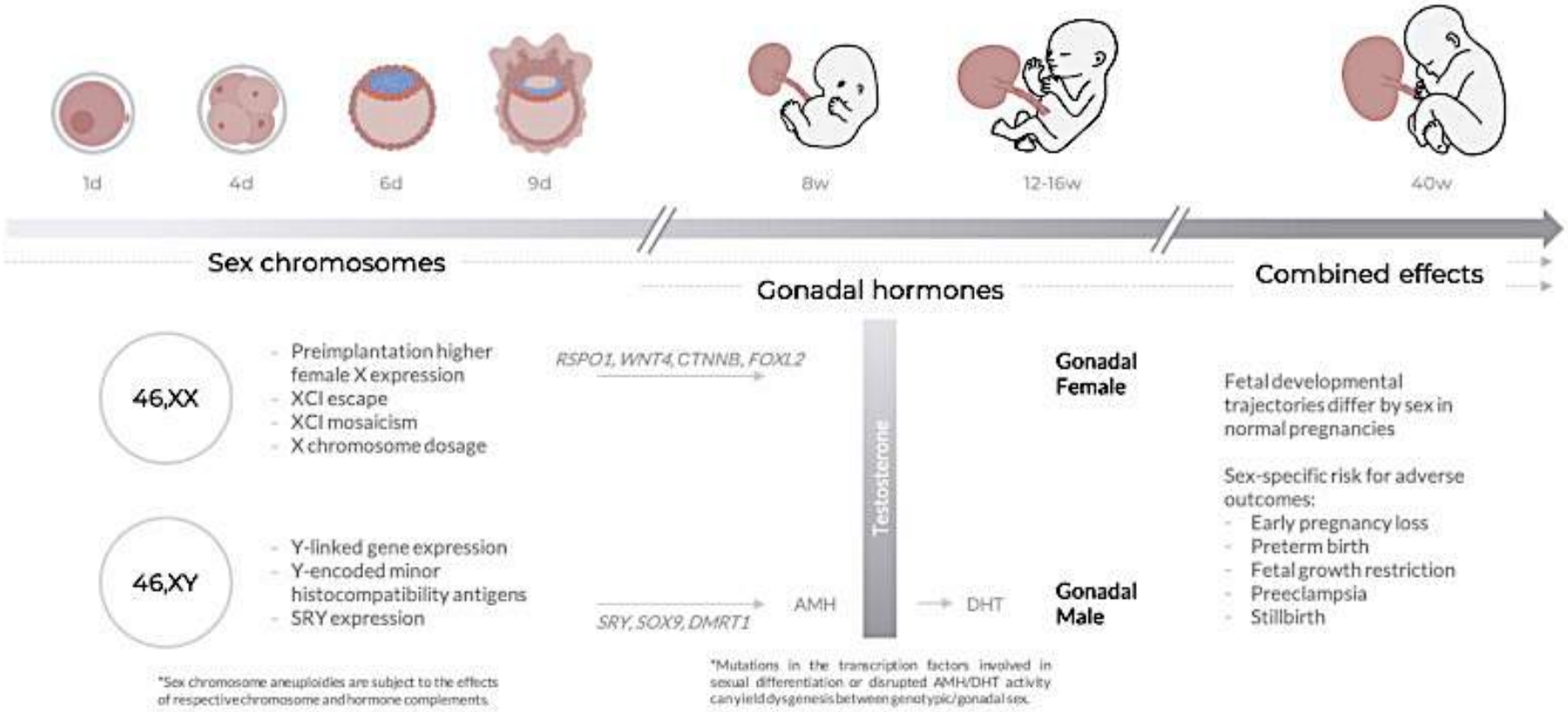
Sex differences exist from the beginning of life



Typical sex differences across gestational age

Throughout pregnancy, sex differences may arise as a consequence of both sex chromosome and sex hormone (testosterone) biology.
The combined effects of sex chromosomes and hormones on placental function may contribute to sex differences in healthy development and risk for adverse pregnancy outcomes.

E. Heard, March 13th 2023

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Maternal diet and susceptibility to sex-specific neuropsychiatric disorders

nature metabolism

Article https://doi.org/10.1038/s42255-022-00693-8

Maternal diet disrupts the placenta–brain axis in a sex-specific manner

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Check for updates

Alexis M. Ceasrine[1], Benjamin A. Devlin[1], Jessica L. Bolton[2], Lauren A. Green[1], Young Chan Jo[1], Carolyn Huynh[1], Bailey Patrick[1], Kamryn Washington[1], Cristina L. Sanchez[1], Faith Joo[1], A. Brayan Campos-Salazar[4], Elana R. Lockshin[4], Cynthia Kuhn[2], Susan K. Murphy[3], Leigh Ann Simmons[4] & Staci D. Bilbo[1,4,7]

Here, we demonstrate a fundamental, sex-biased mechanism through which mHFD increases offspring susceptibility to neuropsychiatric disorder development. Our results in mice demonstrate that, in the context of mHFD, endotoxin accumulation mediates in utero inflammation through the pattern recognition receptor TLR4 in males and females, leading to increased macrophage reactivity in both the placenta and the fetal brain. In female mice, TLR4-dependent inflammation causes diminished social preference through a 5-HT-independent mechanism. In human females, maternal decidual triglyceride accumulation is negatively associated with nervous system development, suggesting that inflammation impacts neuronal development in females, although the target neuronal population is not yet known. In male mHFD offspring, embryonic microglia aberrantly phagocytose 5-HT neurons in the DRN, leading to diminished brain 5-HT from embryonic stages through adulthood, and offspring anhedonia. In human tissues, maternal decidual triglyceride accumulation is associated with pro-inflammatory signalling pathways in both sexes, and negatively correlated with brain 5-HT levels in males only, reinforcing our findings in mice, even though the human tissue analyses were limited by small sample size and relied on correlational analyses. Correlational analyses were chosen because no clinical data were available regarding maternal diet for the human tissue analysed here, and thus we could not group the tissues into LFD and HFD groups as we could with the mouse tissue. Future studies expanding on these findings by including the collection of maternal data will be important.

E. Heard, March 13th 2023

High maternal weight is associated with detrimental outcomes in offspring, including increased susceptibility to neurological disorders such as anxiety, depression and communicative disorders. Despite widespread acknowledgement of sex biases in the development of these disorders, few studies have investigated potential sex-biased mechanisms underlying disorder susceptibility.

A maternal high-fat diet causes endotoxin accumulation in fetal tissue, and subsequent perinatal inflammation contributes to sex-specific behavioural outcomes in offspring.

In male offspring exposed to a maternal high-fat diet, increased macrophage Toll-like receptor 4 signalling results in excess microglial phagocytosis of serotonin (5-HT) neurons in the developing dorsal raphe nucleus, decreasing 5-HT bioavailability in the fetal and adult brains. Bulk sequencing from a large cohort of matched first-trimester human samples reveals sex-specific transcriptome-wide changes in placental and brain tissue in response to maternal triglyceride accumulation (a proxy for dietary fat content). Further, fetal brain 5-HT levels decrease as placental triglycerides increase in male mice and male human samples. **These findings uncover a microglia-dependent mechanism through which maternal diet can impact offspring susceptibility for neuropsychiatric disorder development in a sex-specific manner.**

Also see studies of Sonia Garel

(More next WEEK COURS III and IV)

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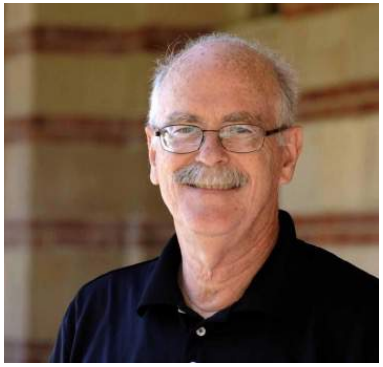
Non-gonadal tissue sex phenotypes



Arthur P. Arnold (UCLA)

Rethinking sex determination of non-gonadal tissues

Arthur P. Arnold*
Department of Integrative Biology & Physiology, Laboratory of Neuroendocrinology of the Brain Research Institute, University of California, Los Angeles, CA, United States
*Corresponding author: e-mail address: arnold@ucla.edu

"From the moment of our conception, each of us has a sex. Sex has a major role in determining the physical attributes of our bodies, the structure of our brains, our behavioral tendencies, our susceptibility and reaction to diseases, the environment in which we grow up, our place in society, the attitudes of others towards us, and our conception of self. Although sex may be considered to be determined primarily biologically, our gender (i.e. the social perception and implications of our sex) is arguably equally or more important for our lives. Sex and gender differences are created by an intricate reciprocal interaction of numerous biological and environmental forces." *AP Arnold 2010*

For the last 50 years, students have been taught that outside the gonads — where sperm and eggs are produced — cells with XX and XY pairs are functionally equivalent because there is nothing on the X or Y chromosome that acts outside the testes. They've been taught that hormones secreted by the testes and the ovaries, where eggs are produced, are entirely responsible for making the body more masculine or feminine.
However we now know that there are intrinsic biochemical differences between XX and XY cells that affect tissues and organs across the entire body and have a significant impact independent of sex hormones. And medical practitioners must understand these differences to properly treat their patients. *D Page 2016*

Sex differences exist from the beginning of life

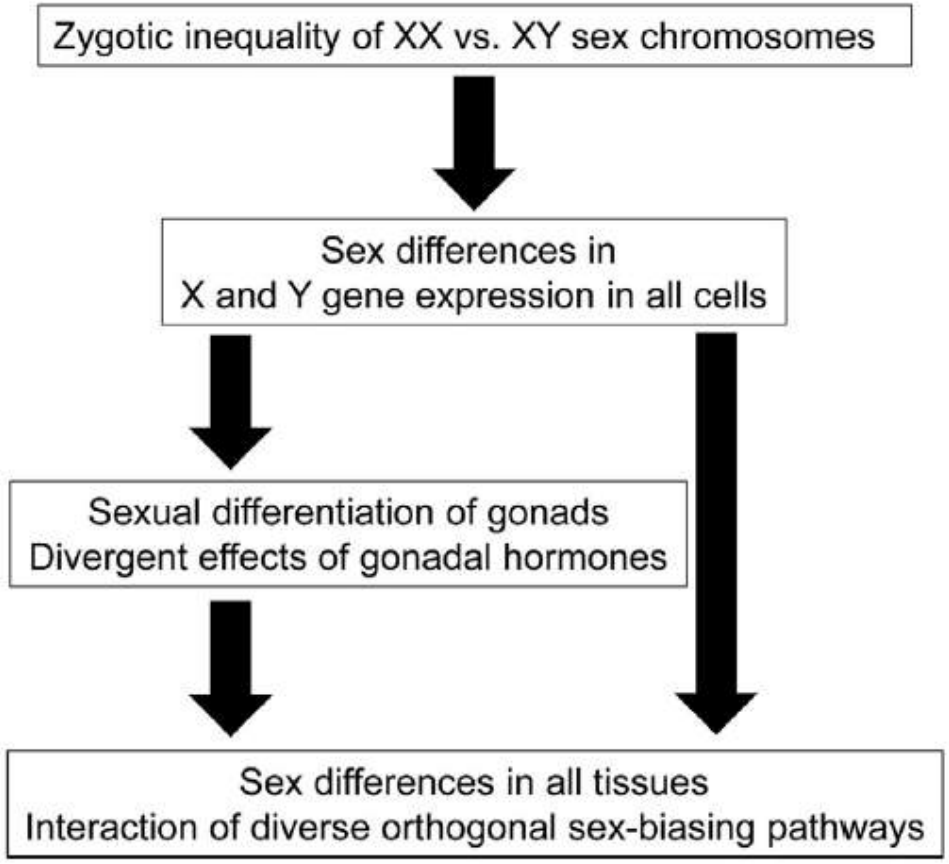
A widely-held tenet of sex determination is that "in most mammals, sex determination is initiated by transient expression of Sry …," the Y gene initiating testis differentiation.

Arnold challenges the thinking behind this narrative, because
(1) ubiquitous sex differences in cell phenotypes emerge well before the expression of Sry and
(2) after differentiation of gonads, significant sex differences in cells are not the result of the this "sex determination" process but this excludes some components of sexual differentiation.

Arnold considers that all biological sex differences stem originally from the inherent imbalance of sex chromosome factors, present in the zygote, acting in the embryo and throughout life.

Arnold defines "sex determination" to include **any factors that cause sex differences in cells, tissues, and individuals**.

Factors that determine the sex of cells and individuals can be primary (encoded by the sex chromosomes of the zygote) or secondary factors downstream of the primary X and Y factors (Arnold, 2011). In this view, Sry is a primary sex-determining factor, and testicular hormones secreted because of the differentiation of testes are secondary sex determinants.



COLLÈGE DE FRANCE 1530

Cell-autonomous sex identity in some Birds

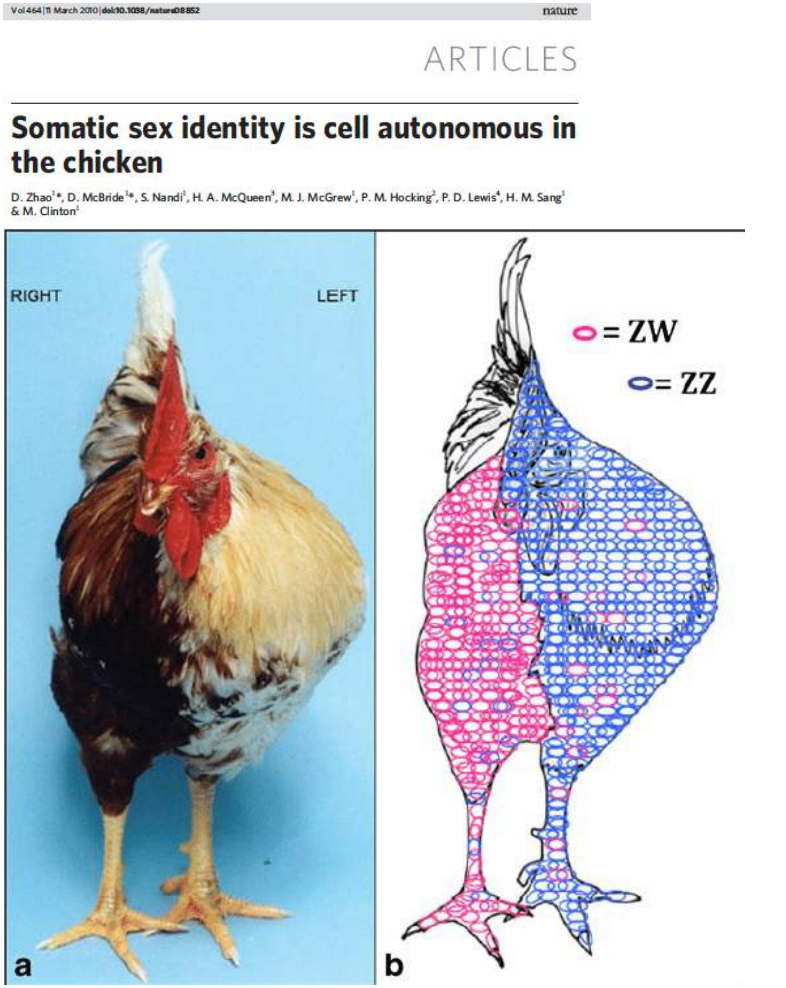
Gynandromorph birds are mixed-sex (ZZ/ZW) chimaeras in which the male side of the chickens had a high proportion of ZZ cells, and the female side had a high proportion of ZW cells.

Based on the Jost paradigm, circulating sex hormones would be expected to pattern differentiation as uniformly male or female, regardless of the genotype of the cells.

However, the gynandromorph results suggest that the chromosomal constitution of cells in birds influences their perception of the hormone environment ie individual cells across the animal "know their sex" by their sex chromosome constitution.

Most of the phenotypes that show a sex chromosome effect in birds (different phenotype in ZZ vs. ZW) are often *also* influenced by gonadal hormone levels.

Eg chicken's comb and wattle, large in males and small in females. These structures have long been known to be sensitive to androgens, so that the sex difference was thought to be downstream of gonadal determination. Yet, the chicken gynandromorph has a larger comb and wattle on the male side, indicating that sex chromosome complement also contributes to the sex difference.



COLLÈGE DE FRANCE 1530

E. Heard, March 13th 2023

Avian somatic cells possess a cell-autonomous sex identity

Birds depend on a ZW system with variations

- Birds also use a stable pair of sex chromosomes for determining sex.
- Unlike mammals, they have a ZZ/ZW chromosomal system, in which the female is the heterogametic sex.
- These sex chromosomes evolved from a completely different set of autosomes than the XY chromosomes in mammals.
- In birds, sex determination is controlled by the dosage of a gene on the Z chromosome known as doublesex and mab-3 related transcription factor 1 (*DMRT1*): males have two copies of *DMRT1*, whereas females have only one.
- Despite the presence of a strong ZW genetic system in chickens, ZZ male eggs can be sex-reversed to female by the application of oestrogen during the critical period of gonad formation and commitment to testis or ovarian fate. Sensitivity to oestrogen is a characteristic of most egg-laying species.
- Birds develop rarely as **gynandromorphs** in which plumage, genitalia and other sexual dimorphisms are divided bilaterally into male characteristics on one side and female characteristics on the other
- Implies that **sex determination is cell autonomous.**
- Birds appear to lack a chromosome-wide mechanism of Z gene dosage compensation comparable to X inactivation (Arnold et al. 2008) although this is still under investigation.

Vol 464 | 11 March 2010 | doi:10.1038/nature08852 nature

ARTICLES

Somatic sex identity is cell autonomous in the chicken

D. Zhao[1]*, D. McBride[1]*, S. Nandi[1], H. A. McQueen[3], M. J. McGrew[1], P. M. Hocking[2], P. D. Lewis[4], H. M. Sang[1] & M. Clinton[1]

Figure 1 | Image of gynandromorph bird (G1). ISA brown bird where the right side has female characteristics and left side has male characteristics (white colour and larger wattle, breast musculature and spur).

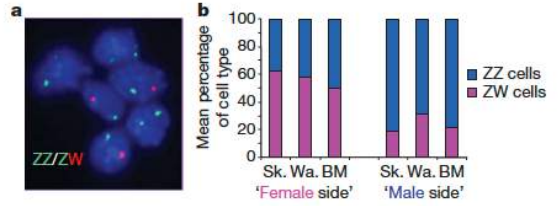


Figure 2 | Male and female cells in gynandromorph birds. a, FISH analysis

- Gynandromorph birds are mixed-sex chimaeras
- Sex differences precede gonadal hormone influences
- Chimaeras confirm cell-autonomous sexual differentiation
- Avian somatic cells possess a cell autonomous sex identity
- NB in mammalian mixed-sex chimaeras, XX cells can become functional Sertoli cells and XY cells can become functional granulosa cells.

COLLÈGE DE FRANCE 1530

E. Heard, March 13th 2023

The impact of sex on gene expression across 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.

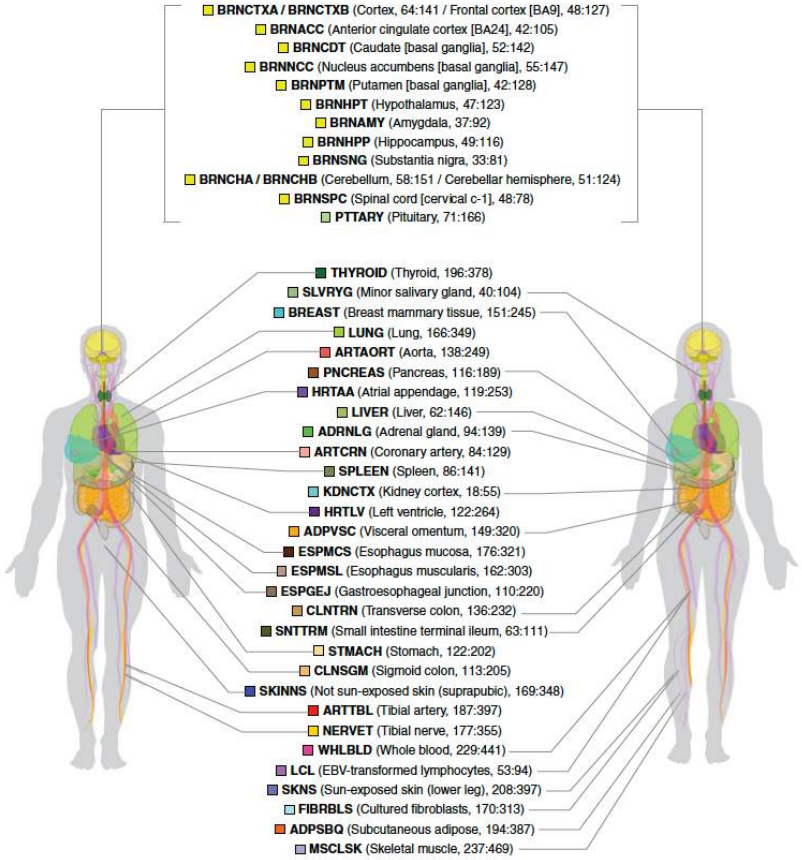


Fig. 1. Sample, data types, and discovery sets in the study of sex differences in GTEx v8. Tissue types (including 11 distinct brain regions and two cell lines) are illustrated, with sample numbers from GTEx v8 genotyped donors (females:males, in parentheses) and color coding indicated for each. This study included N = 44 tissue sources present in both sexes with ≥70 samples. Tissue sources comprised two cell lines, 40 tissues, and two additional replicates for brain cerebellum and cortex tissues. Tissue name abbreviations are shown in bold. See (9) for specific numbers of donors used in each analysis.

E. Heard, March 13th 2023

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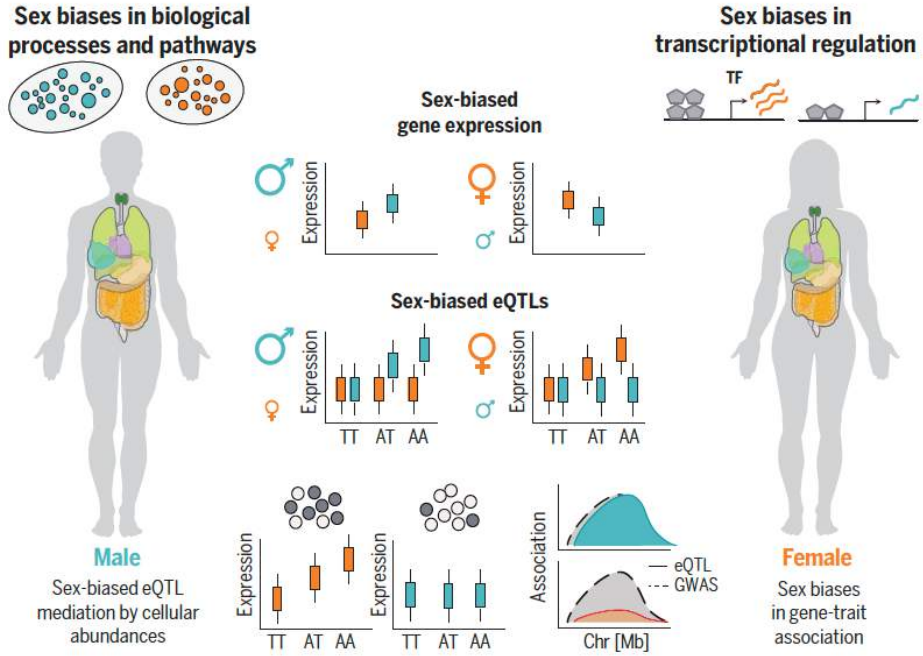
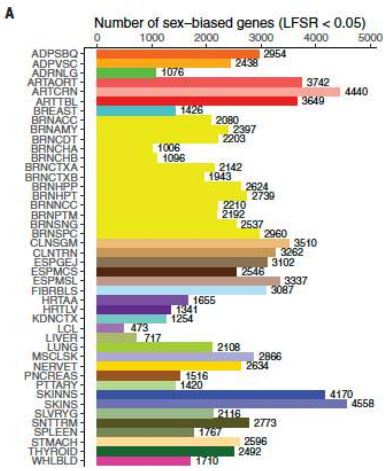




- Sex affects gene expression and its genetic regulation across tissues.
- Sex effects on gene expression were measured in 44 GTEx human tissue sources and integrated with genotypes of 838 subjects.
- A total of 37% genes exhibit sex-biased expression in at least 1 tissue.
- Sex-biased expression is present in numerous biological pathways and is associated to sex-differentiated transcriptional regulation.
- Sex-biased expression quantitative trait loci in cis (sex-biased eQTLs) are partially mediated by cellular abundances and reveal gene trait associations.

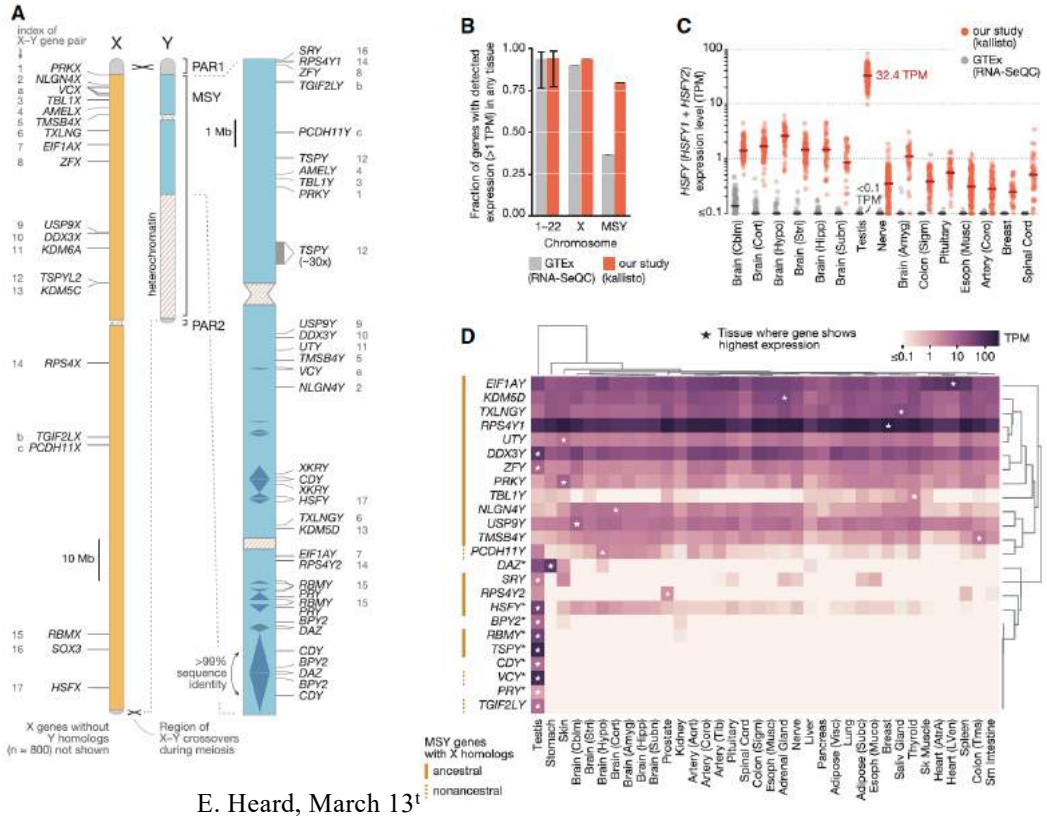
Oliva et al., Science 369, 1331 (2020)

COLLÈGE DE FRANCE 1530

The impact of the Y Chromosome across human tissues

Quantitative analysis of Y-Chromosome gene expression across 36 human tissues

Alexander K. Godfrey,[1,2] Sahin Naqvi,[1,2] Lukáš Chmátal,[1] Joel M. Chick,[3] Richard N. Mitchell,[4] Steven P. Gygi,[3] Helen Skaletsky,[1,5] and David C. Page[1,2,5]



E. Heard, March 13t

How do human Y-Chromosome genes contribute to differences between XX and XY individuals beyond the reproductive system?

The Y Chromosome retains conserved, dosage-sensitive regulatory genes expressed in tissues throughout the body (Bellott et al. 2014), which might underlie associations between the Y Chromosome and disease (Tartaglia et al. 2012; Cannon-Albright et al. 2014; Eales et al. 2019).

The divergence of MSY genes from their X homologs is likely to be in regulatory (i.e., noncoding) sequences .
In this way the Y Chromosome can directly give rise to differences between XX and XY individuals.

Because the X–Y gene pairs encode regulators of transcription, translation, and protein stability that are highly dosage sensitive (Bellott et al. 2014; Naqvi et al. 2018), small differences in their expression levels could contribute significantly to the widespread sex differences in gene expression observed across tissues (Naqvi et al. 2019) and ultimately to phenotypic differences between the sexes.

COLLÈGE DE FRANCE 1530

The landscape of X-chromosome gene activity across human tissues

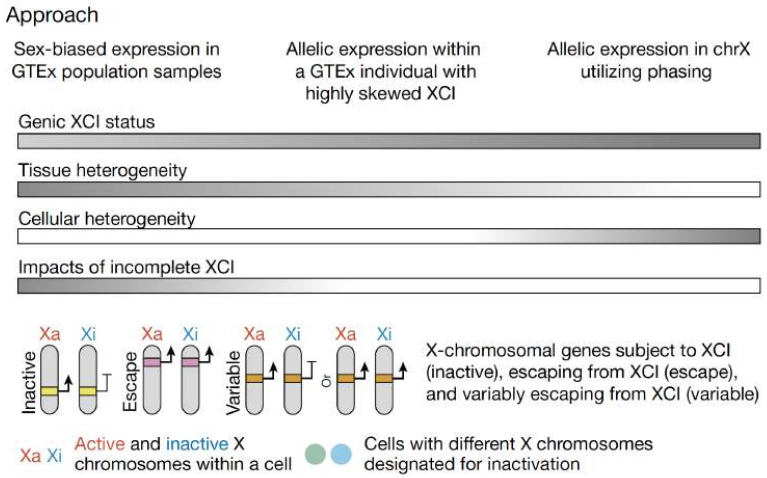
LETTER

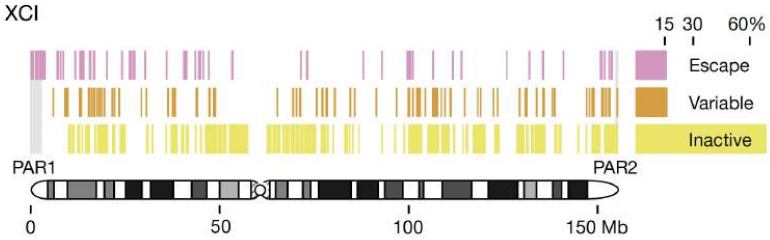
OPEN

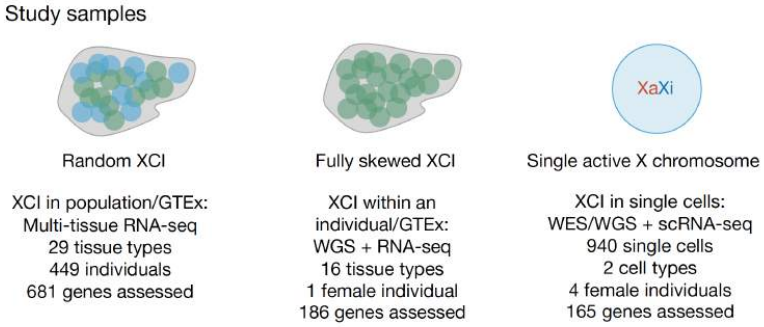
doi:10.1038/nature24265

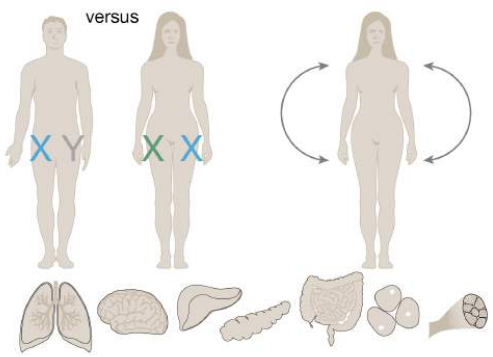
Landscape of X chromosome inactivation across human tissues

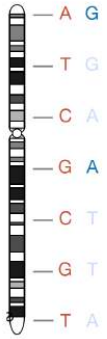
Taru Tukiainen[1,2], Alexandra-Chloé Villani[2,3], Angela Yen[2,4], Manuel A. Rivas[1,2,5], Jamie L. Marshall[1,2], Rahul Satija[3,6,7], Matt Aguirre[1,2], Laura Gauthier[1,2], Mark Fleharty[2], Andrew Kirby[8,2], Beryl B. Cummings[1,2], Stephanie E. Castel[9,8], Konrad J. Karczewski[1,2], François Aguet[2], Andrea Byrnes[1,2], GTEx Consortium†, Tuuli Lappalainen[8,9], Aviv Regev[2,9], Kristin G. Ardlie[2], Nir Hacohen[2,3] & Daniel G. MacArthur[1,2]











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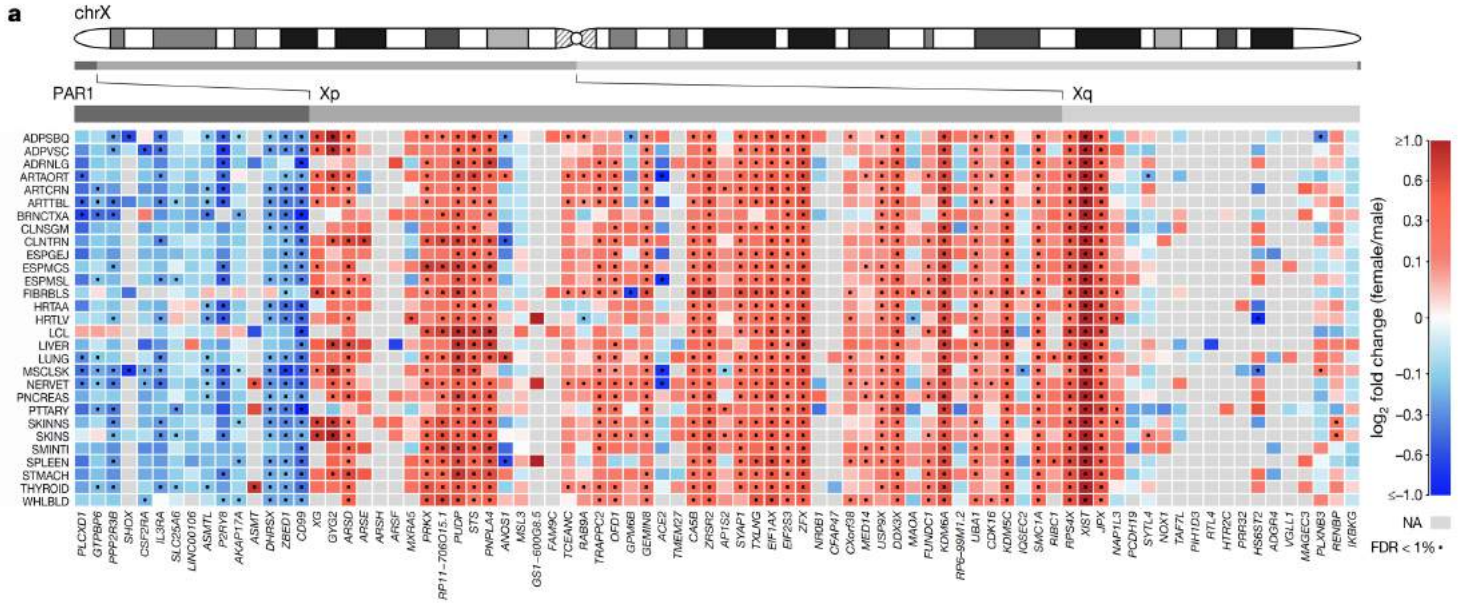
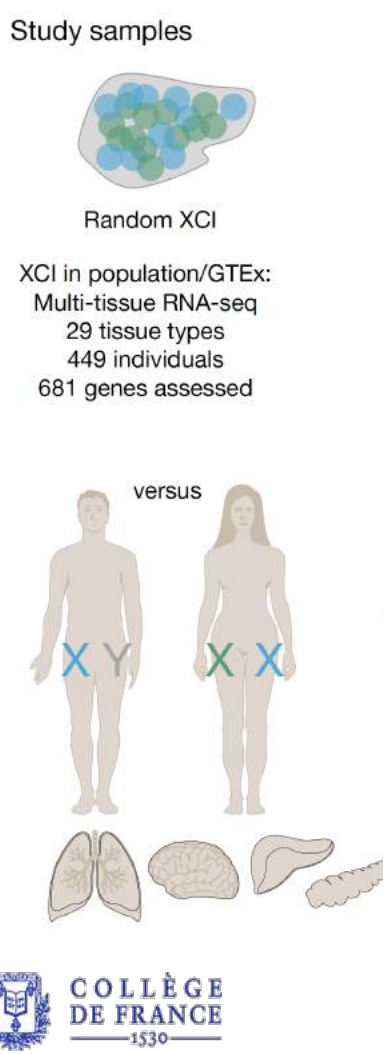
E. Heard, March 13th 2023

The landscape of X-chromosome gene activity across human tissues

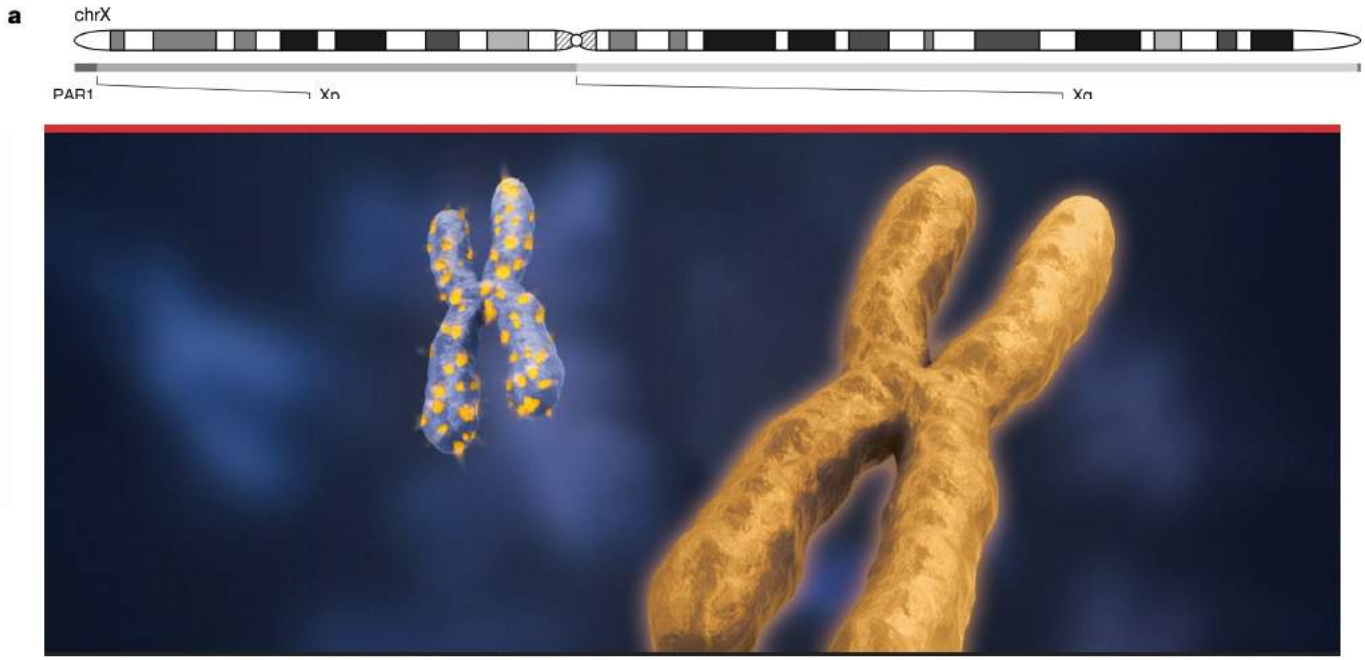




- Multiple regions show female bias: ie **escape from XCI** – this is variable between tissues and individuals
- Sex bias pattern of nine genes not previously classified as full escape genes that follow a similar profile to established escape genes
- Genes in the pseudoautosomal region show higher expression in XY males than in XX females => lower activity of PAR region on Xi?

COLLÈGE DE FRANCE 1530

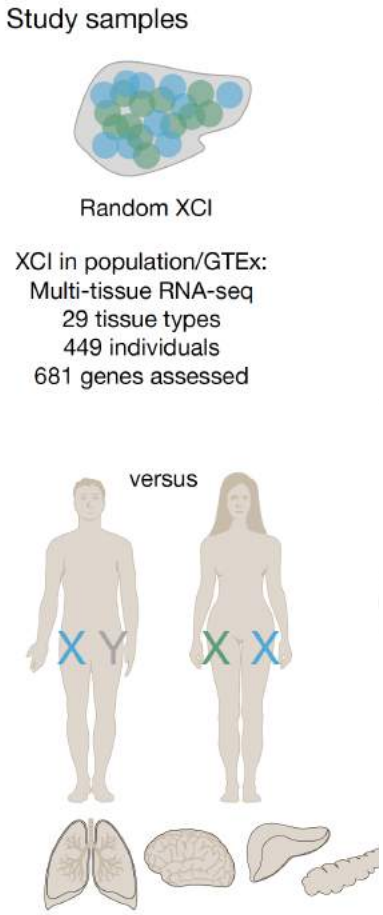
The landscape of X-chromosome gene activity across human tissues



Genes that Escape Silencing on the Second X Chromosome May Drive Disease

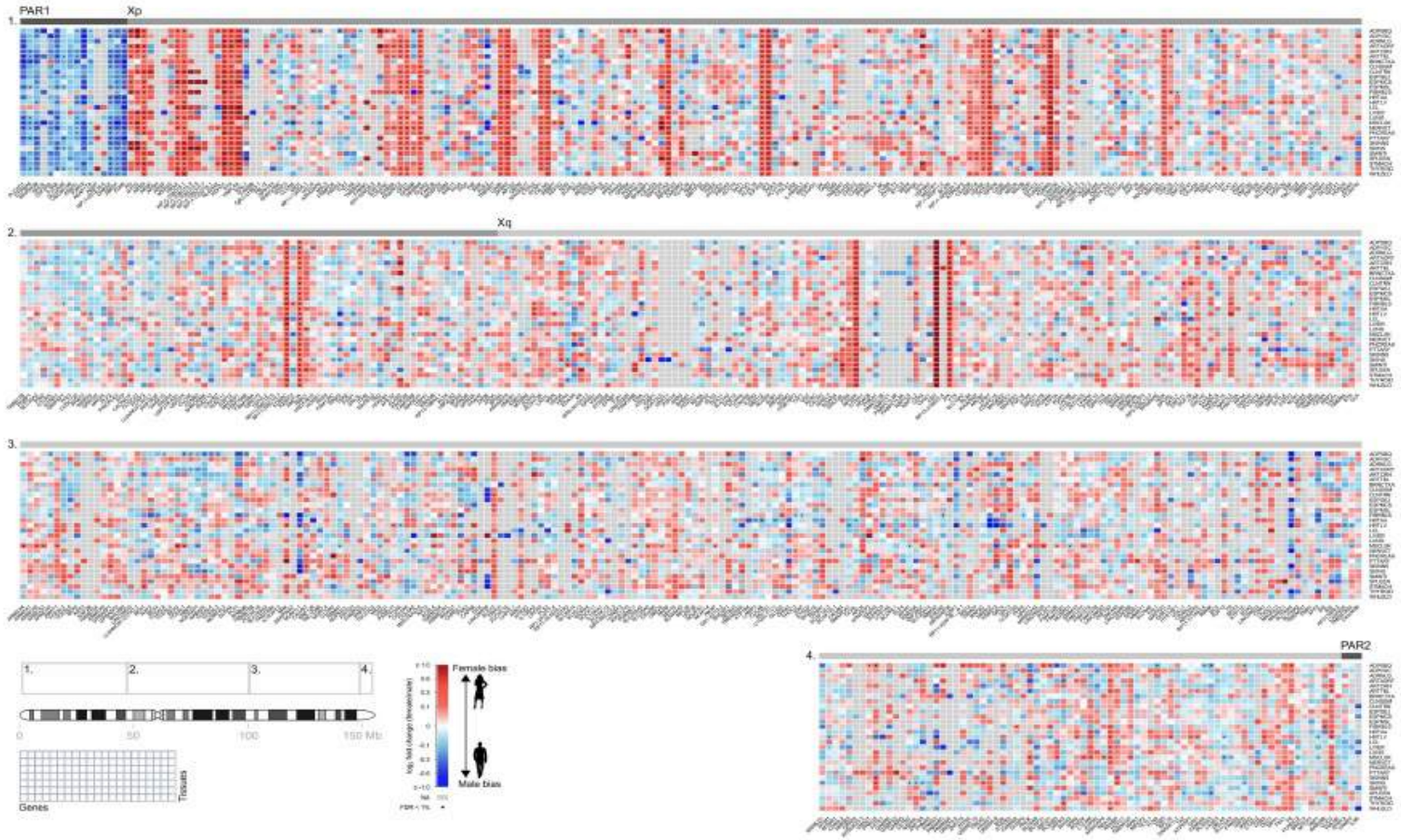
When X-linked genes evade silencing on the "inactive" chromosome in XX cells, some protect women from diseases such as cancer, but others seem to promote conditions such as autoimmunity.

E. Heard, March 13th 2023



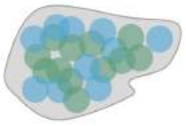
COLLÈGE DE FRANCE 1530

The landscape of X-chromosome gene activity across human tissues



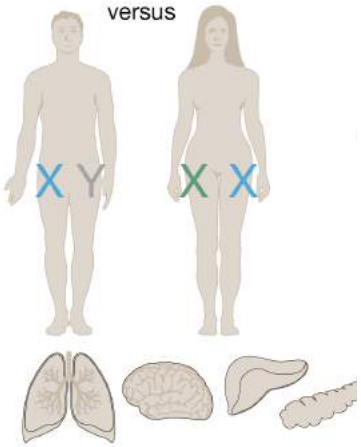
Extended Data Figure 4 | Heat map representation of male–female expression differences in all assessed X-chromosomal genes ($n = 681$) across 29 GTEx tissues. The colour scale displays the direction of sex bias, with red colour indicating higher female expression. Genes that were too weakly expressed to be assessed in a given tissue type in the sex bias analysis are coloured grey. Dots mark the observations where sex bias was significant at FDR < 1%.

Study samples



Random XCI

XCI in population/GTEx:
Multi-tissue RNA-seq
29 tissue types
449 individuals
681 genes assessed



E. Heard, March 13th 2023

COLLÈGE DE FRANCE 1530

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

Different types of sexual inequality that are currently known or suspected in XX vs. XY cells because of the inherent imbalance of representation of sex chromosome genes (Arnold, 2017):

(1) The male-specific expression of Y genes causes XY cells to differ from XX cells (Arnold, 2017b; Case & Teuscher, 2015).

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

- X genes escaping X inactivation are expressed higher in XX than XY cells (Carrel et al., 1999; Tukiainen et al., 2017). For example, sex differences in trophoblast expression of the putative X escapee gene Ogt are reported to cause sex differences in resilience to prenatal insults (Nugent, O'Donnell, Epperson, & Bale, 2018).
- 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 (Tukiainen et al., 2017)
- X genes that experience a parental imprint can be expressed at a higher or lower level in XX vs. XY cells because a paternal imprint affects only XX cells (Arnold, 2017a).
- The difference in epigenetic regulation of the X and Y chromosomes could impact the epigenetic status of the autosomes. The large heterochromatic X chromosome, present only in XX cells, might sequester heterochromatizing factors and reduce their availability for autosomal heterochromatin, affecting autosomal gene expression (Wijchers & Festenstein, 2011).

Other forces cause sex differences in **populations** of humans and other animals, because of natural genetic heterogeneity not modelled in inbred strains (reviewed by Arnold, 2017a).

These factors cause average differences between groups of males and females.
(1) XY males have **hemizygous exposure of X alleles**, so that they express X allelic variations more prominently than XX females (Migeon, 2007).
XY individuals with X-linked lethal alleles are removed from the population, and shift the mean phenotype of males vs. females.

(2) 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. Eg in XX mice with a null mutation in one allele of the X-linked Hccs gene, cells expressing the null allele are selectively removed from the heart during embryonic development (Drenckhahn et al., 2008).

(3) Some autosomal alleles are likely to be better adapted to one sex than the other, and thus will be found in greater frequency in one sex, creating an average genetic difference in autosomes of males and females.

(4) The different modes of inheritance of the mitochondrial and nuclear genomes lead to sex-biasing effects. Because the mitochondrial genome is inherited in the female lineage, mitochondrial alleles might arise that are beneficial to females but harmful to males. Such a mismatch of mitochondrial genomes of the mother and nuclear genomes of her sons can have differential harmful effects in males relative to females (Camus, Clancy, & Dowling, 2012; Frank, 2012; Innocenti, Morrow, & Dowling, 2011).

X-Chromosome Variation in Mosaicism and Escape

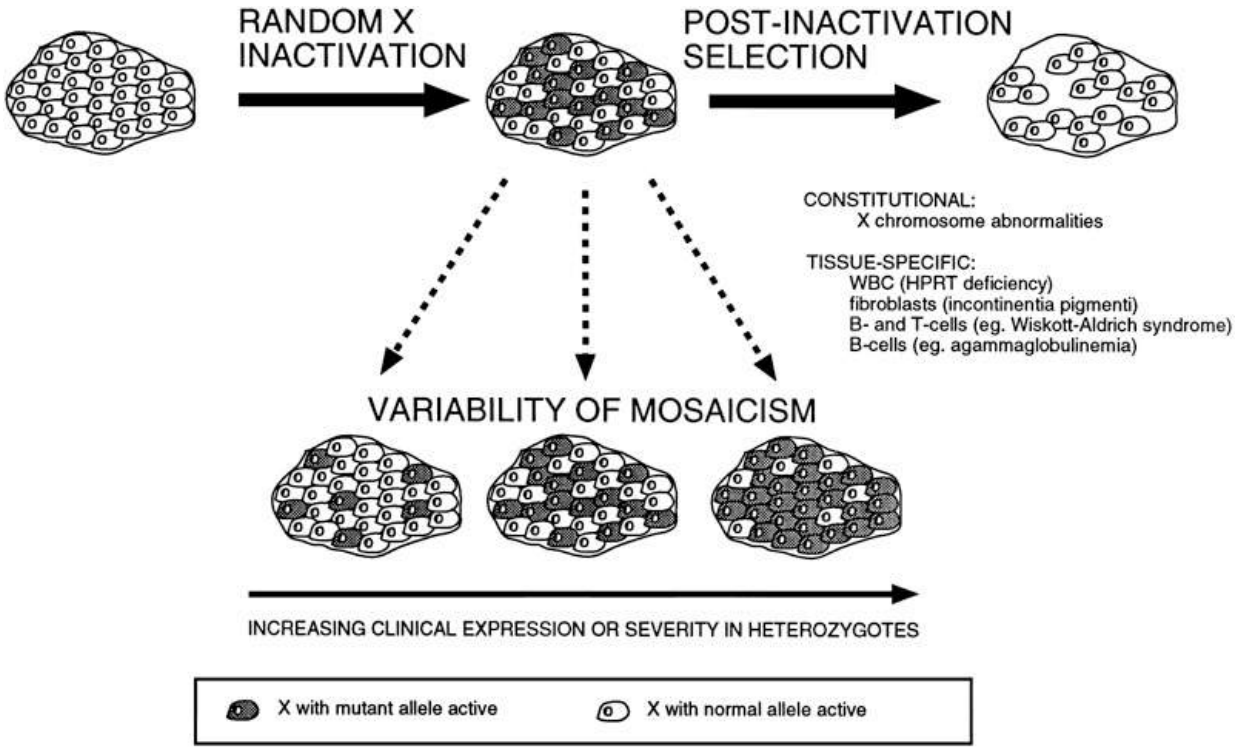
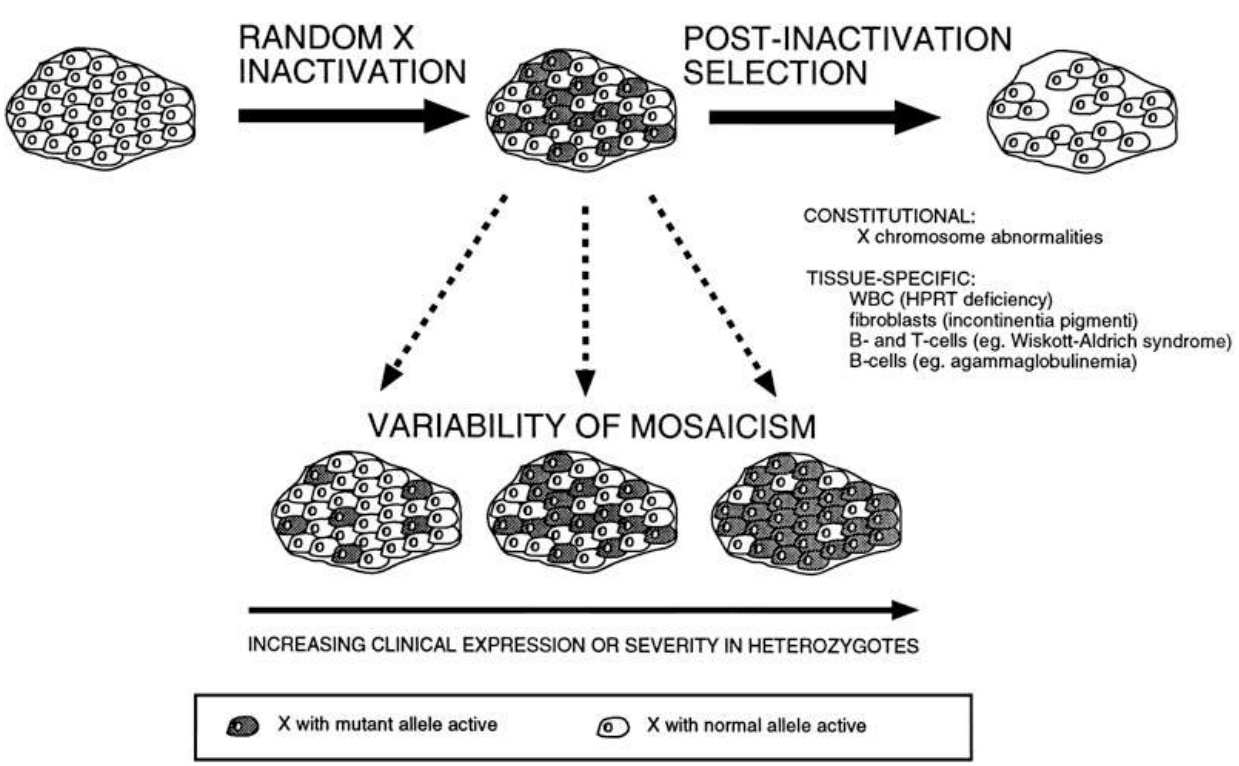
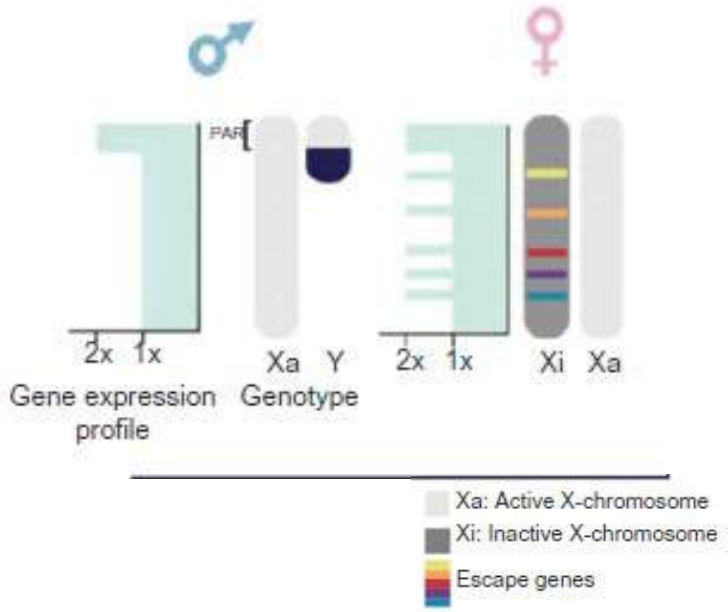


Table 1. Examples of inherited X-linked diseases affected by XCI patterns

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

X-Chromosome Variation in Mosaicism and Escape





COLLÈGE DE FRANCE 1530

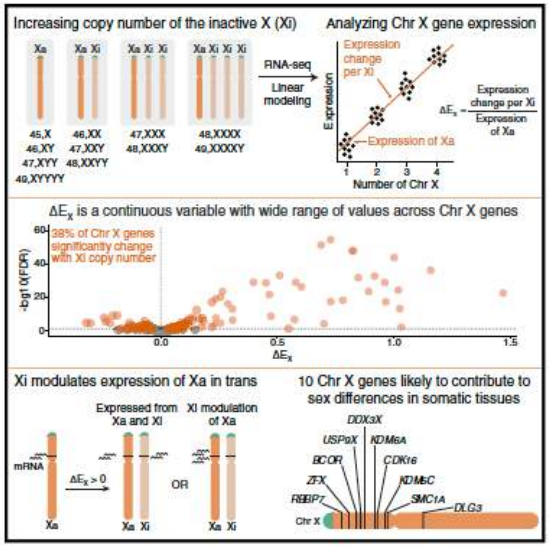
The inactive X chromosome contributes to male-female differences in common disease

Cell Genomics

Article

The human inactive X chromosome modulates expression of the active X chromosome

Graphical abstract



Authors

Adrianna K. San Roman, Alexander K. Godfrey, Helen Skaletsky,..., Carole Samango-Sprouse, Maximilian Muenke, David C. Page

Correspondence

dcpage@wi.mit.edu

In brief

Through RNA sequencing of individuals with sex chromosome aneuploidy, San Roman et al. identify modular "active" (Xa) and "inactive" (Xi) X chromosome transcriptomes. Looking beyond classical X inactivation, which acts in *cis*, they find that Xi modulates Xa transcript levels in *trans*. They identify 10 X chromosome genes most likely to contribute to male-female differences in common disease.

- Analyzed gene expression in sex chromosome aneuploidy
- samples using linear models
- Xi and Xa transcriptomes are modular
- 38% of X chromosome genes are affected by Xi copy number—in cis and in trans
- Ten X-chromosome genes likely contribute to male-female differences in somatic tissues

E. Heard, March 13th 2023

The ''inactive''X chromosome (Xi) has been assumed to have little impact, in trans, on the ''active''X (Xa).
To test this, we quantified Xi and Xa gene expression in individuals with one Xa and zero to three Xis. Our linear modeling revealed modular Xi and Xa transcriptomes and significant Xi-driven expression changes for 38% (162/423) of expressedXchromosomegenes.By integrating allele-specific analyses,we found thatmodulation of Xa transcript levels by Xi contributes to many of these Xi-driven changes (R121 genes). By incorporating metrics of evolutionary constraint, we identified 10 X chromosome genes most likely to drive sex differences in common disease and sex chromosome aneuploidy syndromes. We conclude that human X chromosomes are regulated both in cis, through Xi-wide transcriptional attenuation, and in trans, through positive or negative modulation of individual Xa genes by Xi. The sum of these cis and trans effects differs widely among genes.

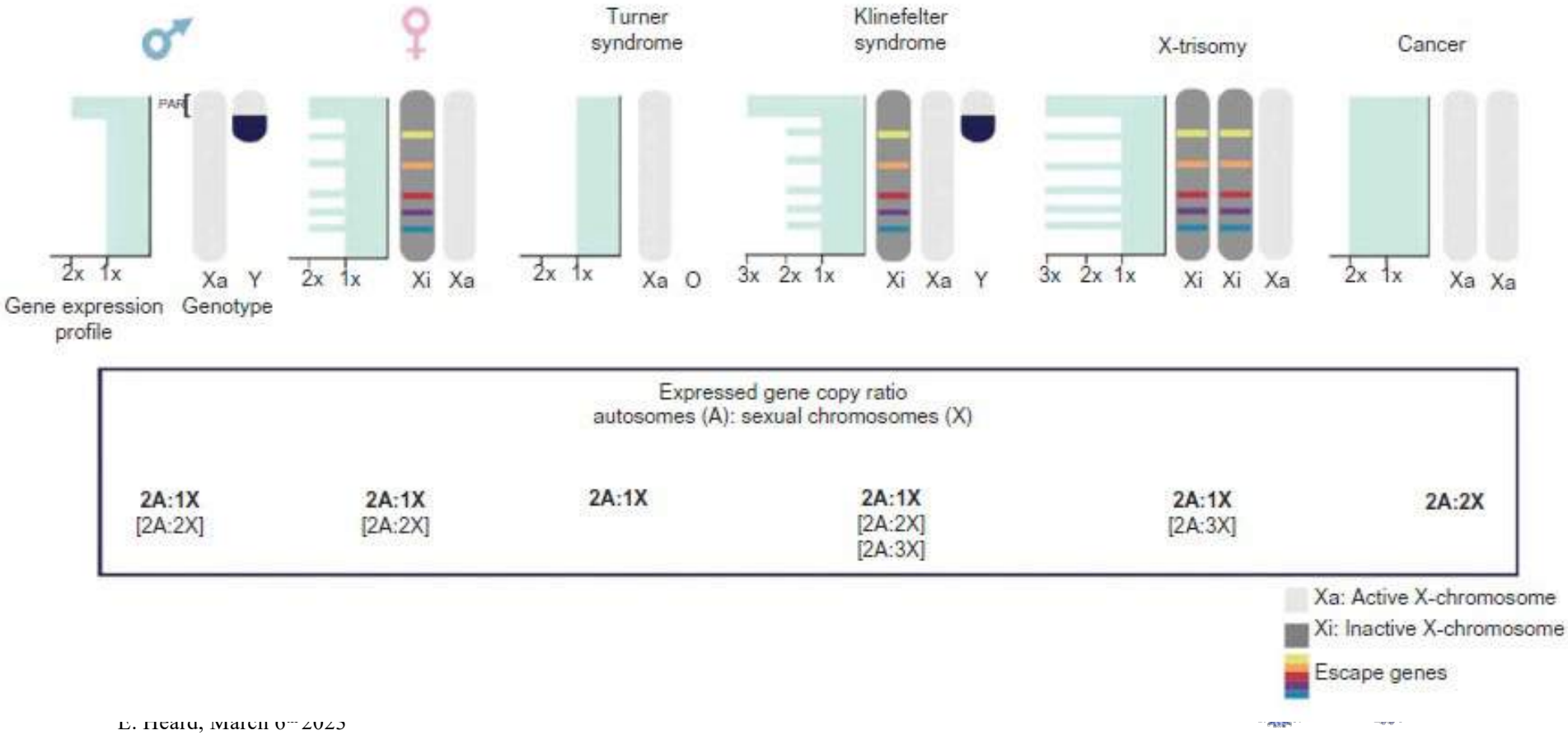
More in COURS III and IV

COLLÈGE DE FRANCE
1530

The inactive X chromosome contributes to male-female differences in disease

Lessons from X-chromosome anueploidies?

Difficult to disentangle hormonal and genetic/epigenetic differences

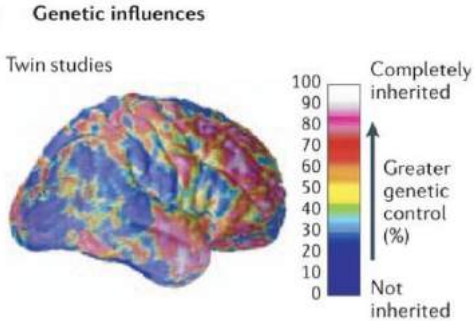


Twin studies: X-linked gene expression variation between genetically identical individuals

X Inactivation as a Source of Behavioural Differences in Monozygotic Female Twins

Caroline S. Loat, Kathryn Asbury, Michael J. Galsworthy, Robert Plomin, and Ian W. Craig
Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, Kings College, London, UK

Although members of monozygotic twin pairs are identical in genome sequence, they may differ in patterns of gene expression. One early and irreversible process affecting gene expression, which can create differences within pairs of female monozygotic twins, is X inactivation — one twin can express mainly paternally-received genes on the X chromosome while the other twin expresses mainly maternally-received genes. It follows that non-identical X chromosome expression may cause female monozygotic twins to correlate less strongly than male monozygotic twins on complex behavioural traits affected by X-linked loci. We tested this hypothesis using data from around 4000 same-sex twin pairs on 9 social, behavioural and cognitive measures at ages 2, 3 and 4. Consistent with our hypothesis, monozygotic males were generally more similar than monozygotic females. Three of four significant differences were in traits showing higher correlations in males than females, and these traits — prosocial behaviour, peer problems, and verbal ability — have all been proposed previously in the literature as being influenced by genes on the X chromosome. Interestingly, dizygotic twins showed the reverse pattern of correlations for similar variables, which is also consistent with the X inactivation hypothesis; taken together, then, our monozygotic and dizygotic results suggest the presence of quantitative trait loci on the X chromosome.



Human twin studies are powerful means to distinguish genetic from hormonal and environmental variations.
Yet they are also limited by confounding effects including upbringing, life style choices as well as genetic and epigenetic changes during life....
(COURS 2017)

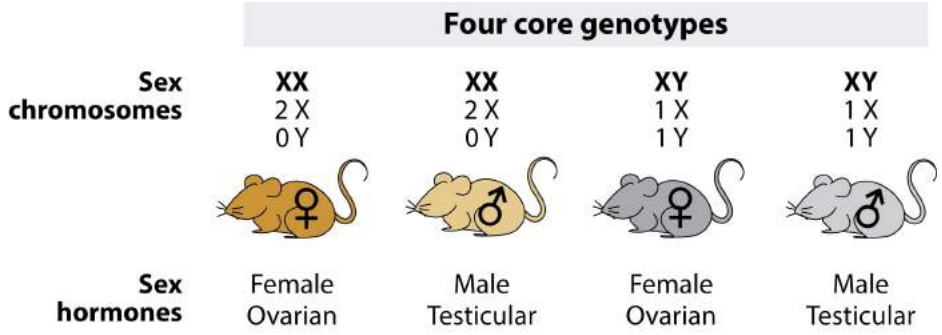
"Identical twins have identical genes but they don't have identical brains and that is because learning leads to anatomical changes in the brain and even identical twins will have different social experiences, different learning experiences, and therefore will end up having different brains." E Kandel

Methods for distinguishing effects of X chromosome number from effects of sex hormones

XX and XY mice with testes (XXM and XYM) or with ovaries (XXF and XYF)

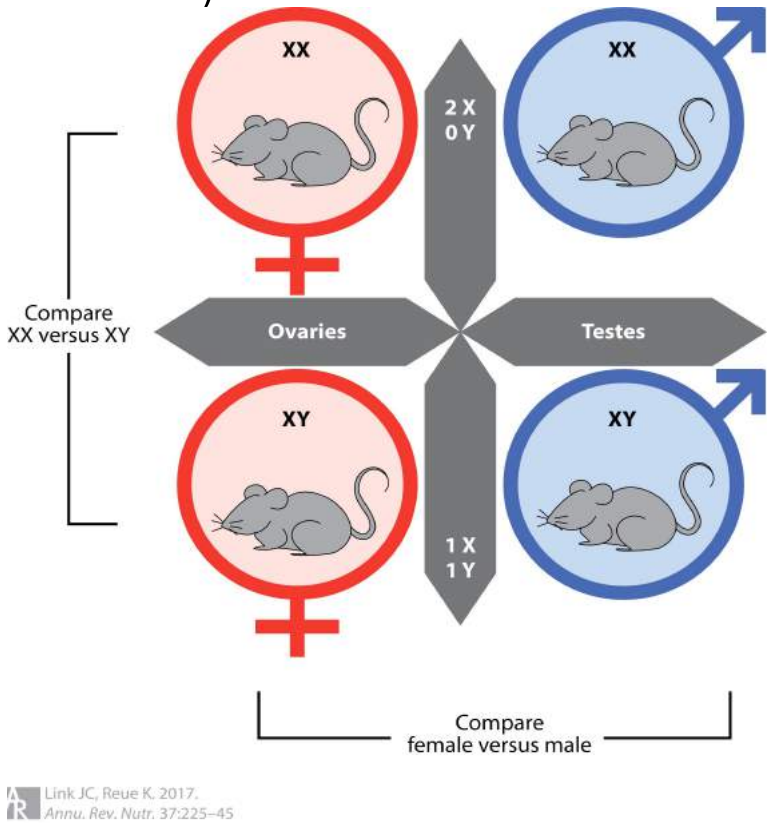
A major goal has been to distinguish sex differences caused by gonadal hormones versus sex chromosome complement (XX versus XY).

Hormonal effects are most often detected by manipulating hormone levels, synthesis, or action to find which hormones account for sex differences.



Link JC, Reue K. 2017. *Annu. Rev. Nutr.* 37:225–45

Test for phenotypic effects of the number of X chromosomes, mirroring the natural difference between females and males, in a manner that will reveal X gene effects and distinguish from hormonal effects.



Link JC, Reue K. 2017. *Annu. Rev. Nutr.* 37:225–45

E. Heard, March 13th 2023

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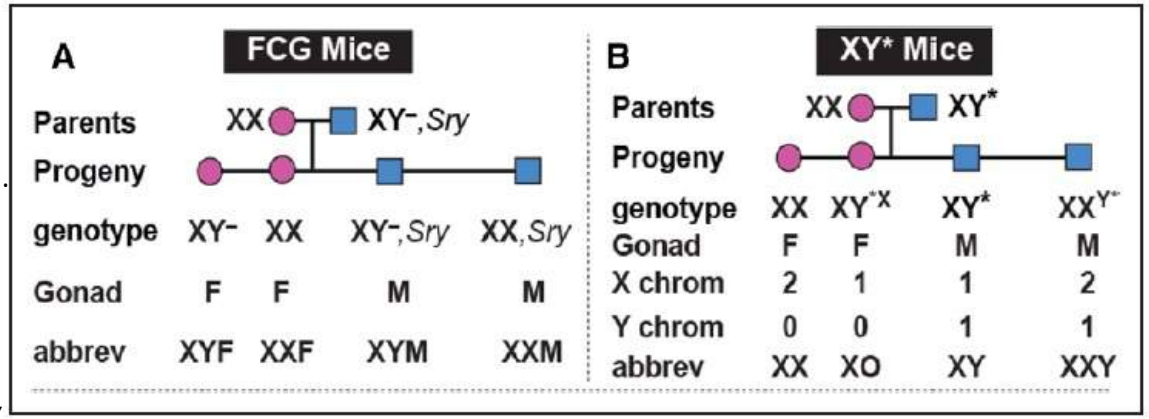
Mouse Models to Discriminate Hormonal and Sex Chromosome Effects That Cause Sex Differences

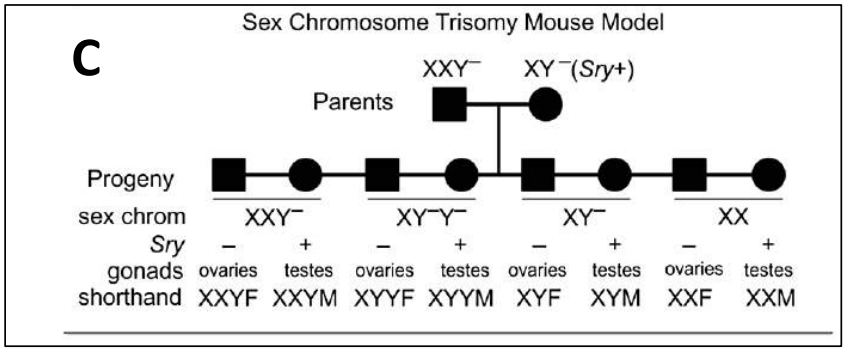
Mouse models for measuring sex chromosome effects:

A, In the four core genotypes (FCG) model, the testis-determining gene (Sry) is removed from Y chromosome to make the "Y–" chromosome, and an Sry transgene is inserted into chromosome 3. XY– mice have ovaries and are called XY females (XYF) here. Mice with an Sry transgene have testes and are called males, XXM or XYM.

B, The XY* model is useful for figuring out whether a difference between XX and XY is because of effects of the X chromosome or Y chromosome. It compares groups that differ in the number of X chromosomes (female mice with XX or XO and male mice with XY or XXY) or compares mice that differ in the presence/absence of Y chromosome (XO vs XY and XX vs XXY), to determine which causes XX vs XY differences.

C, Sex Chromosome Trisomy (SCT) mouse model produces XXY, XYY, XY, and XX mice in the same litters, each genotype with either testes or ovaries. Model for Klinefelter syndrome (KS), caused by XXY karyotype.





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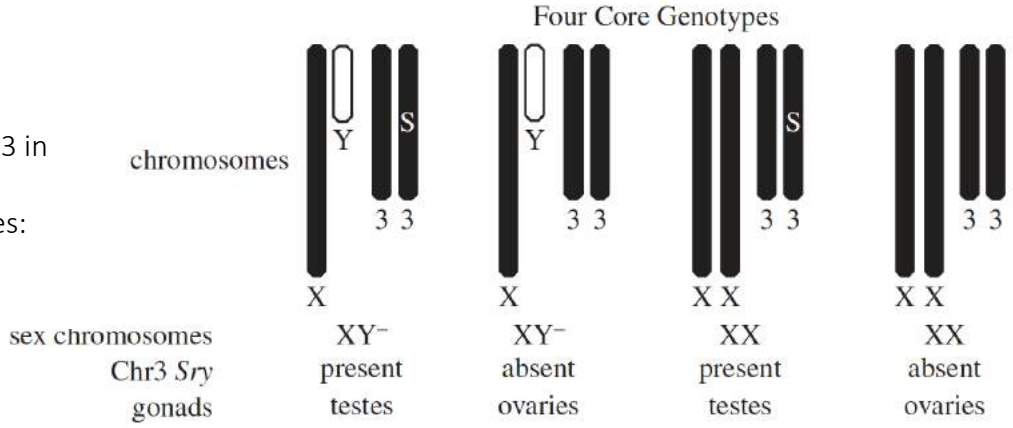
Methods for distinguishing effects of X chromosome number from effects of sex hormones

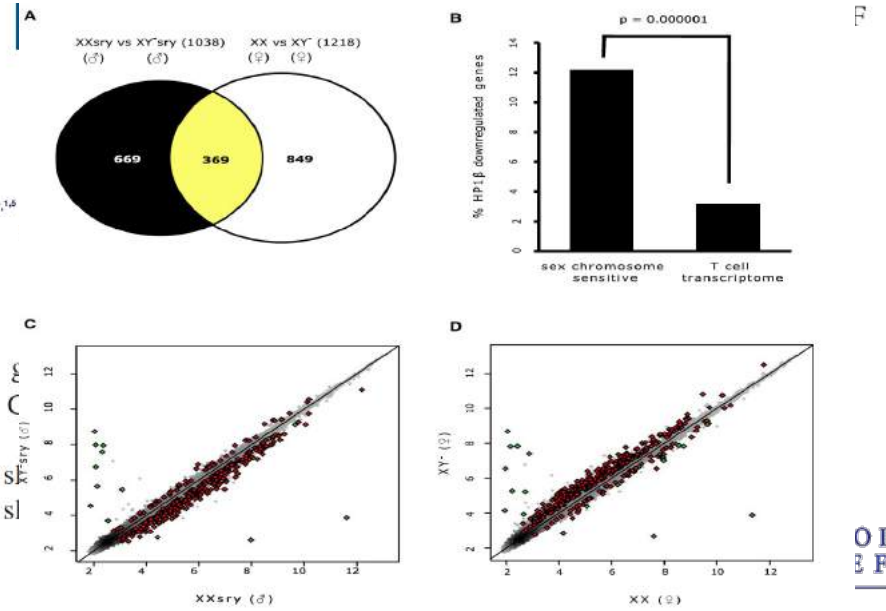
Four Core Genotype Model:
(effects due to XX vs X number)
Y chromosome is deleted for Sry
An Sry transgene (S) is present on chrom 3 in some groups.
Breeding XYM x XXF produces 4 genotypes:
XX and XY mice with testes (XXM, XYM)
XX and XY mice with ovaries (XXF, XYF).



Developmental Cell
Short Article

Sexual Dimorphism in Mammalian Autosomal Gene Regulation Is Determined Not Only by *Sry* but by Sex Chromosome Complement As Well

Patrick J. Wijchers,[1,4] Cihangir Yandim,[1] Eleni Panousopoulou,[1] Mushfika Ahmad,[1] Nicky Harker,[2] Alexander Saveliev,[1,5] Paul S. Burgoyne,[3] and Richard Festenstein[1,*]



1218 autosomal genes that were different between XX and XY females (Figure 2A, white), 369 of which were affected by sex chromosome complement in both males and females (yellow).

''sex chromosome complement-sensitive set'' of 369 autosomal genes (SCS) from here on.

E. Heard, March 13th 2023

Identification of Sex differences caused by sex chromosome effects

Metabolism and body weight

- The greater body weight of male mice, relative to females, is reduced by gonadectomy of adult mice, indicating that gonadal hormones largely are responsible for this difference.
- After gonadectomy of FCG mice, the body weight of XX mice increases slowly over weeks until it is much greater than that of XY mice, irrespective of their type of gonad (Chen et al., 2012).
- The sex chromosome effect is eventually as large as the effect of gonadal hormones in young mice at the outset of the experiment.
- Use of the XY* model allows mice with different numbers of X or Y chromosomes to be tested.
- These studies show that the XX-XY difference is caused by X genes, not Y genes. The greater body weight is mostly the result of an X chromosome effect on the amount of body fat.
- In mice eating a high fat diet, sex chromosomes cause sex differences in the level of plasma cholesterol, and accumulation of liver triglycerides (Chen et al., 2012; Link et al., 2015).
- In the case of body weight, the gonadal hormonal effect counteracts the sex chromosome effect: male hormones are associated with greater body weight, but male sex chromosomes (XY) with lower body weight. To date, little is known about the molecular site of interaction of the two effects, and whether they influence the same molecular pathways or distinct pathways that both impinge on the same emergent phenotype.

E. Heard, March 13th 2023

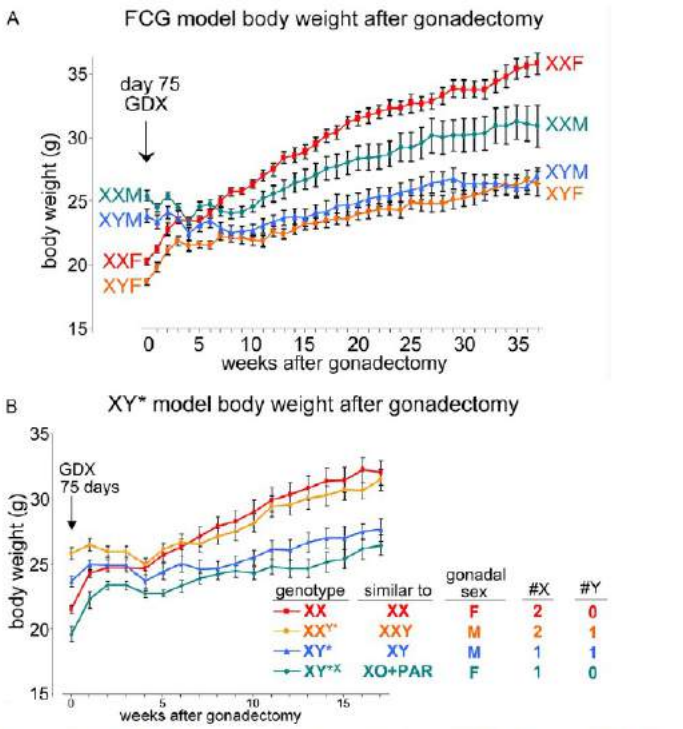


Fig. 3 The number of X chromosomes influences body weight in FCG and XY* mice. (A) In FCG mice, gonadal males at postnatal day 75 (week 0) weighed about 25% more than gonadal females. When gonads were removed on that day, the sex differences disappeared by 4 weeks after gonadectomy (GDX). After that, XX mice slowly gained much more body weight than XY mice, irrespective of previous gonadal type. (B) In the XY* model, the same experiment replicates the larger body size of two groups with testes, relative to two groups with ovaries. After gonadectomy, the groups with two X chromosomes gained more weight than those with one X chromosome, illustrating that the body weight is affected by the number of X chromosomes. M = gonadal male, F = gonadal female, PAR = pseudoautosomal region. *Reprinted from Chen, X., McClusky, R., Chen, J., Beaven, S.W., Tontonoz, P., Arnold, A.P., et al. (2012) The number of X chromosomes causes sex differences in adiposity in mice.* PLoS Genetics, 8, *e1002709 under terms of the Creative Commons Attribution License.*

Identification of Sex differences caused by sex chromosome effects

Sex chromosome regulation of brain sensitivity to gonadal hormones

Recent discovery in FCG mice that sex chromosomes influence the metabolism o
gonadal hormones in the brain, and regulate the
sensitivity of specific brain regions to sex steroid hormones.

The **amygdala** has long been recognized as a site of action of gonadal steroids, tc
cause sex differences in function. In the anterior amygdala of E16 mouse embryc
XY mice, relative to XX, have greater expression of aromatase, the enzyme
converting androgens to estrogens, irrespective of gonadal type (Cisternas
et al., 2015).

The same sex chromosome effect on aromatase is found in cultures of embryoni
amygdala neurons (Cisternas, Cabrera Zapata, Arevalo, Garcia-Segura, &
Cambiasso, 2017; Cisternas et al., 2015). In vitro, estrogen receptor beta (Esr2) is
expressed higher in XY than XX cells (Cisternas et al., 2017). Treatment of neuron
with estradiol or dihydrotestosterone increases expression of aromatase and Esr
in XX cells only, and abolishes the difference in Esr2 expression caused by sex
chromosomes. Esr1 expression in vitro is also regulated by both sex chromosome
complement and by gonadal hormones. This example is particularly interesting
because it demonstrates a regulation by sex chromosomes both of local synthesi
of estradiol, and of the sensitivity to estradiol, to regulate amygdalar phenotypes

The regulation of aromatase by sex chromosomes could shift the balance of actic
of estrogens and their androgenic precursors, contributing to hormonally driven
sex differences in amygdala development. It will be exciting to discover the
molecular pathways by which X or Y genes regulate steroid sensitivity of this
and other brain regions (Cambiasso et al., 2017; Cisternas, Garcia-Segura, &
Cambiasso, 2018).

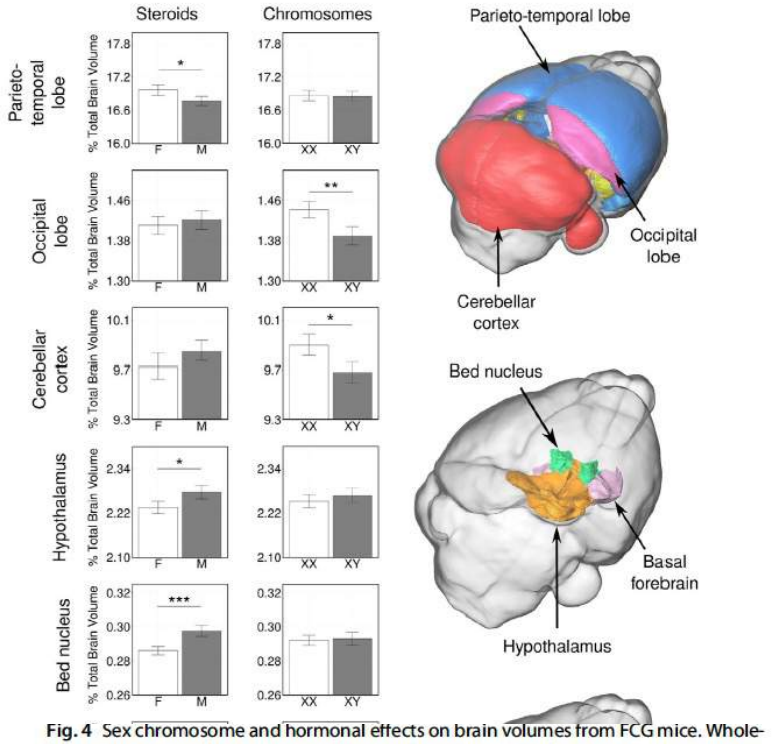


Fig. 4 Sex chromosome and hormonal effects on brain volumes from FCG mice. Whole-brain MRI was used to measure volumetric variation in gonad-intact FCG mice. Among seven brain regions illustrated, some showed significant effects of gonadal hormones (parieto-temporal lobe of the cerebral cortex, the hypothalamus, and the bed nucleus of the stria terminalis). Others showed effects of sex chromosomes (basal forebrain, occipital lobe of the cerebral cortex, cerebellar cortex, corpus callosum). Asterisks indicate changes were significant at false discovery rate of 5% or less. Error bars represent 95% confidence intervals. *Reprinted by permission of Springer Nature from Corre, C., Friedel, M., Vousden, D.A., Metcalf, A., Spring, S., Qiu, L.R., et al. (2016) Separate effects of sex hormones and sex chromosomes on brain structure and function revealed by high-resolution magnetic resonance imaging and spatial navigation assessment of the Four Core Genotype mouse model.* Brain Structure & Function, 221, *997–1016.*

Methods for distinguishing effects of X chromosome number from effects of sex hormones

Cardiovascular and pulmonary disease

Women and men differ in the susceptibility and progression of numerous cardiovascular diseases, including atherosclerosis, aneurysms, ischemia/ reperfusion injury, and systemic and pulmonary hypertension (Arnold et al., 2017).

In animal models, the sex differences appear to be caused by a combination of sex chromosome and hormonal effects.

These diseases have quite diverse molecular mechanisms, indicating that a wide range of cellular processes are influenced by sex chromosome complement.

In models of abdominal aortic aneurysms, which affect men much more than women, testosterone exacerbates disease, and XY sex chromosome complement is worse than XX (Alsiraj et al., 2016). Thus, both male hormones and male sex chromosomes appear to promote disease.

In a model of ischemia/reperfusion injury, estrogens are generally protective, but XX sex chromosome complement is worse that XY, indicating compensatory or counteracting effects of estrogens and sex chromosomes (Li et al., 2014).

Hypertension affects men more than women. Ovarian hormones appear to be protective, but XX mice are more affected in mouse models than XY mice, again suggesting counteracting hormonal/sex chromosomal effects (Ji et al. 2010).

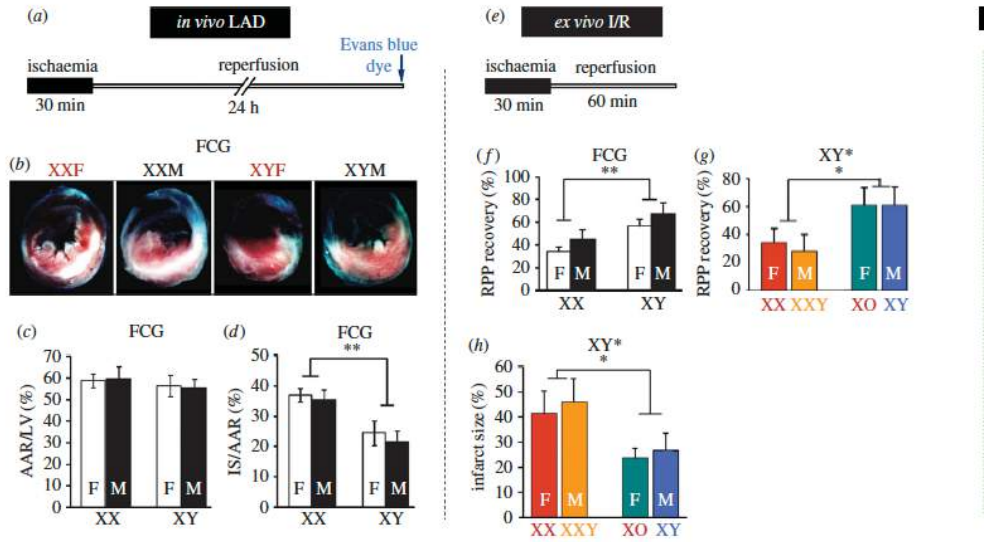


Figure 3. Use of the FCG model shows that after ischaemia/reperfusion injury, GDX XX mice have worse recovery and larger myocardial infarct area compared with GDX XY mice, irrespective of gonadal type. (*a*) Experimental protocol *in vivo*: the left anterior descending artery was occluded in GDX FCG mice for 30 min followed by 24 h of reperfusion. (*b*) Representative cross sections of heart muscle stained with triphenyl tetrazolium chloride. The white area represents the infarcted area, blue shows the non-infarcted area, red plus white areas show risk area. (*c*) Percentage of area at risk (AAR) divided by left ventricle area. (*d*) Infarct size (IS) divided by AAR. $^{**}p < 0.01$, $n = 6–7$. (*e*) Experimental protocol *ex vivo*: perfusion of the heart is shut off for 30 min, then reperfused for 60 min before measuring heart function. (*f*) The rate pressure product (RPP), a measure of recovery after injury, was worse in XX than XY mice. $^{**}p < 0.01$. (*g*) Use of the XY* model shows that in the *ex vivo* system, mice with two X chromosomes (XX, XXY) have worse recovery (lower RPP) than mice with one X chromosome (XO, XY). (*h*) The infarct size as the percentage of total ventricular area in hearts *ex vivo*. $^{*}p < 0.05$. Adapted from [61] with permission from Oxford University Press.

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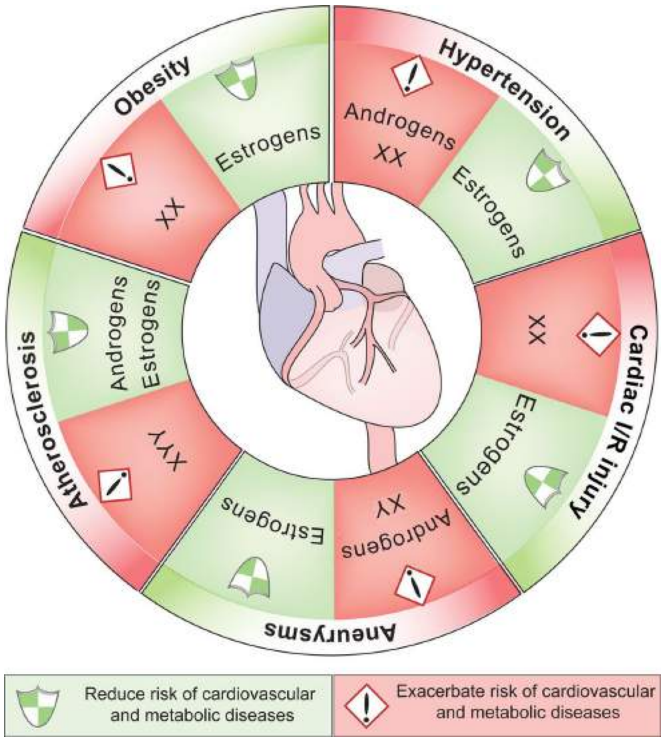
Sex Differences in Cardiovascular Diseases

Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases

Arthur P. Arnold, Lisa A. Cassis, Mansoureh Eghbali, Karen Reue, Kathryn Sandberg

Hormonal and sex chromosome effects in obesity, atherosclerosis, aneurysms, ischemia/reperfusion injury, and hypertension.

Cardiovascular diseases occur and progress differently in the 2 sexes, due to biological factors differing between the sexes having sex-specific protective and harmful effects.



Arthur P. Arnold. Arteriosclerosis, Thrombosis, and Vascular Biology. Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases, Volume: 37, Issue: 5, Pages: 746-756, DOI: (10.1161/ATVBAHA.116.307301)

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E. Heard, March 13th 2023

Sex Differences in Cardiovascular Diseases

Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases

Arthur P. Arnold, Lisa A. Cassis, Mansoureh Eghbali, Karen Reue, Kathryn Sandberg

Hormonal and sex chromosome effects in obesity, atherosclerosis, aneurysms, ischemia/reperfusion injury, and hypertension.

Cardiovascular diseases occur and progress differently in the 2 sexes, due to biological factors differing between the sexes having sex-specific protective and harmful effects.

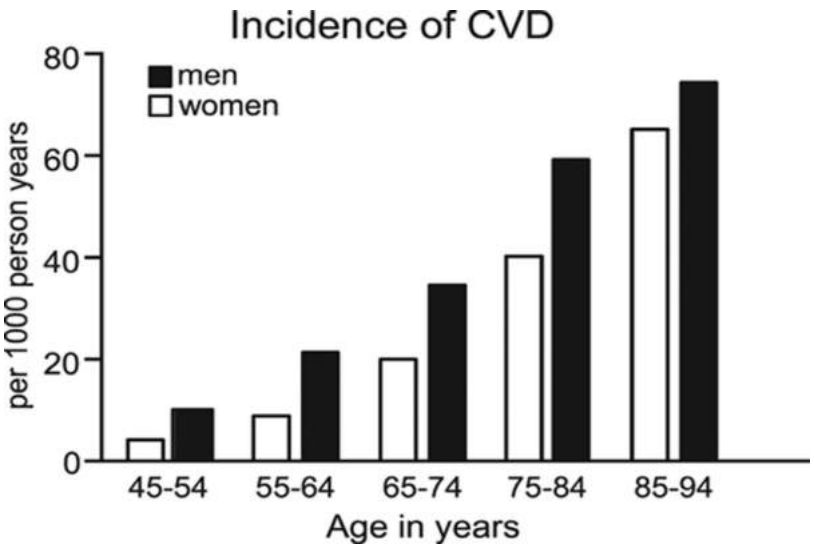
The overall lifetime risk of CVD is similar in the 2 sexes, but men develop CVD earlier than women. At 55 years of age, the lifetime risk of a first incident coronary heart disease is higher in men than in women, but the risk of first incident cerebrovascular disease or heart failure is higher in women than in men.
These sex differences suggest that biomedical principles, learned from the study of males, may not apply equally to females.

By comparing the 2 sexes directly, and breaking down sex into its component parts, one can discover sex-biasing protective mechanisms that might be targeted in the clinic.

Gonadal hormones, especially estrogens and androgens, have long been found to account for some sex differences in cardiovascular diseases.

More recently, the inherent sexual inequalities in effects of sex chromosome genes have also been implicated as contributors in animal models of cardiovascular diseases, especially a deleterious effect of the second X chromosome found in females but not in males. Hormonal and sex chromosome mechanisms interact in the sex-specific control of certain diseases, sometimes by opposing the action of the other.

E. Heard, March 13th 2023



Data on the incidence of cardiovascular disease defined as coronary heart disease, heart failure, stroke, and intermittent claudication in women (white bars) and men (black bars) from 45 to 94 years of age. Data are derived from the Framingham Heart Study as reported by the National Heart, Lung and Blood Institute[138] and adapted from Mozaffarian et al[4] with permission of the publisher. Copyright © 2015, American Heart Association, Inc.

Arthur P. Arnold. Arteriosclerosis, Thrombosis, and Vascular Biology. Sex Hormones and Sex Chromosomes Cause Sex Differences in the Development of Cardiovascular Diseases, Volume: 37, Issue: 5, Pages: 746-756, DOI: (10.1161/ATVBAHA.116.307301)

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Methods for distinguishing effects of X chromosome number from effects of sex hormones

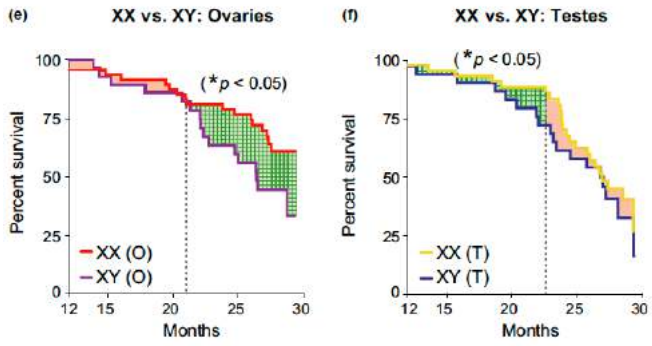
Interaction of sex chromosome effects and age

Female longevity is observed in humans and much of the animal kingdom, but its causes remain elusive.

Using FCG model to produce XX and XY mice, each with either ovaries or testes, the authors show that the **female XX sex chromosome complement increases survival during aging in male and female mice.**

In combination with ovaries, XX statud also extends lifespan.

Understanding causes of sex-based differences in aging could lead to new pathways to counter age-induced decline in both sexes.



In mice with ovaries, XX increased survival after 21 months (Figure 2e). In mice with testes, XX also increased survival, but the benefit was earlier, prior to 23 months, and did not alter maximal lifespan (Figure 2f). Thus, independent of maximal lifespan, the XX genotype increased survival during aging in both male and female mice, albeit at different times.

XX sex chromosomes extended lifespan in combination with ovaries and independently increased survival during aging.

Female XX sex chromosomes increase survival and extend lifespan in aging mice

Emily J. Davis[1] | Iryna Lobach[2] | Dena B. Dubal[1]

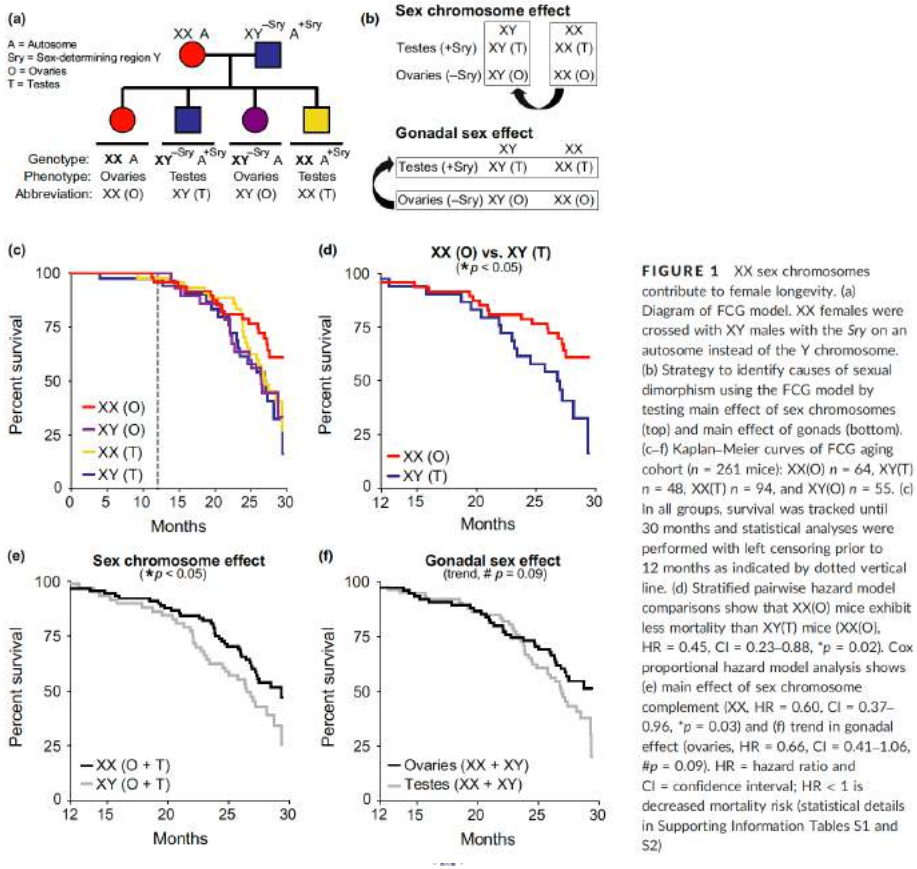
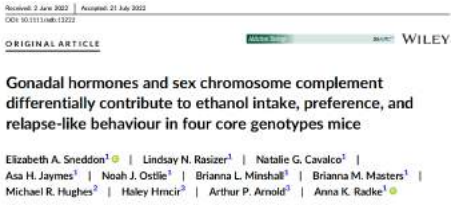


FIGURE 1 XX sex chromosomes contribute to female longevity. (a) Diagram of FCG model. XX females were crossed with XY males with the *Sry* on an autosome instead of the Y chromosome. (b) Strategy to identify causes of sexual dimorphism using the FCG model by testing main effect of sex chromosomes (top) and main effect of gonads (bottom). (c–f) Kaplan–Meier curves of FCG aging cohort (n = 261 mice): XX(O) n = 64, XY(T) n = 48, XX(T) n = 94, and XY(O) n = 55. (c) In all groups, survival was tracked until 30 months and statistical analyses were performed with left censoring prior to 12 months as indicated by dotted vertical line. (d) Stratified pairwise hazard model comparisons show that XX(O) mice exhibit less mortality than XY(T) mice (XX(O), HR = 0.45, CI = 0.23–0.88, $^*p$ = 0.02). Cox proportional hazard model analysis shows (e) main effect of sex chromosome complement (XX, HR = 0.60, CI = 0.37–0.96, $^*p$ = 0.03) and (f) trend in gonadal effect (ovaries, HR = 0.66, CI = 0.41–1.06, $\#p$ = 0.09). HR = hazard ratio and CI = confidence interval; HR < 1 is decreased mortality risk (statistical details in Supporting Information Tables S1 and S2)

Hormones and sex chromosomes differentially contribute to ethanol intake, preference, and relapse-like behaviour

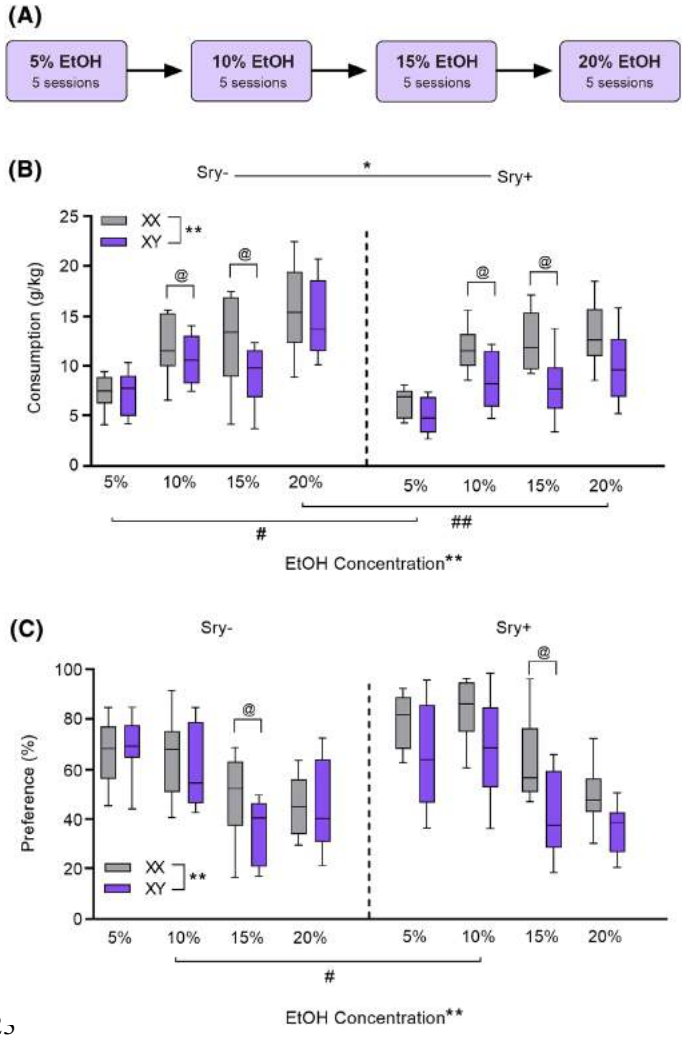


ORIGINAL ARTICLE

WILEY

Gonadal hormones and sex chromosome complement differentially contribute to ethanol intake, preference, and relapse-like behaviour in four core genotypes mice

Elizabeth A. Sneddon | Lindsay N. Rasizer | Natalie G. Cavalco | Asa H. Jaymes | Noah J. Ostlie | Brianna L. Minshall | Brianna M. Masters | Michael R. Hughes | Haley Hrncir | Arthur P. Arnold | Anna K. Radke

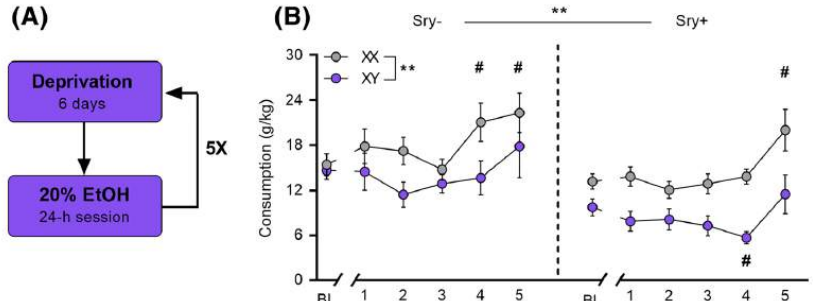


Differential effects of gonadal hormones and sex chromosomes on EtOH intake and preference.

(A) Four core genotypes (FCG) mice drank EtOH, 5%, 10%, 15%, 20% concentrations, for 24 h across five drinking sessions per concn.

(B) Sry- (vs. Sry+) and XX (vs. XY) mice consumed greater amounts of EtOH.

(C) XX chromosomes were associated with heightened preference for EtOH versus water.



XX chromosomes promote the alcohol deprivation effect.

(A) Following the last 20% EtOH session, four core genotypes (FCG) mice underwent six sessions of deprivation and were then re-exposed to 20% EtOH for one 24-h session. This cycle of deprivation and reexposure was repeated five times.

(B) XX/Sry and XX/Sry+ mice escalated intake compared with drinking at baseline (= 5 sessions preceding deprivation; BL). # $p < 0.05$ vs. BL (Dunnett's), ** $p < 0.01$ (main effects three-way ANOVA).

Maternal diet and susceptibility to sex-specific neuropsychiatric disorders

nature metabolism

Article https://doi.org/10.1038/s42255-022-00693-8

Maternal diet disrupts the placenta–brain axis in a sex-specific manner

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Check for updates

Alexis M. Ceasrine[1], Benjamin A. Devlin[1], Jessica L. Bolton[2], Lauren A. Green[1], Young Chan Jo[1], Carolyn Huynh[1], Bailey Patrick[1], Kamryn Washington[1], Cristina L. Sanchez[1], Faith Joo[1], A. Brayan Campos-Salazar[4], Elana R. Lockshin[4], Cynthia Kuhn[3], Susan K. Murphy[3], Leigh Ann Simmons[6] & Staci D. Bilbo[1,4,7]

Here, we demonstrate a fundamental, sex-biased mechanism through which mHFD increases offspring susceptibility to neuropsychiatric disorder development. Our results in mice demonstrate that, in the context of mHFD, endotoxin accumulation mediates in utero inflammation through the pattern recognition receptor TLR4 in males and females, leading to increased macrophage reactivity in both the placenta and the fetal brain. In female mice, TLR4-dependent inflammation causes diminished social preference through a 5-HT-independent mechanism. In human females, maternal decidual triglyceride accumulation is negatively associated with nervous system development, suggesting that inflammation impacts neuronal development in females, although the target neuronal population is not yet known. In male mHFD offspring, embryonic microglia aberrantly phagocytose 5-HT neurons in the DRN, leading to diminished brain 5-HT from embryonic stages through adulthood, and offspring anhedonia. In human tissues, maternal decidual triglyceride accumulation is associated with pro-inflammatory signalling pathways in both sexes, and negatively correlated with brain 5-HT levels in males only, reinforcing our findings in mice, even though the human tissue analyses were limited by small sample size and relied on correlational analyses. Correlational analyses were chosen because no clinical data were available regarding maternal diet for the human tissue analysed here, and thus we could not group the tissues into LFD and HFD groups as we could with the mouse tissue. Future studies expanding on these findings by including the collection of maternal data will be important.

High maternal weight is associated with detrimental outcomes in offspring, including increased susceptibility to neurological disorders such as anxiety, depression and communicative disorders. Despite widespread acknowledgement of sex biases in the development of these disorders, few studies have investigated potential sex-biased mechanisms underlying disorder susceptibility.

A maternal high-fat diet causes endotoxin accumulation in fetal tissue, and subsequent perinatal inflammation contributes to sex-specific behavioural outcomes in offspring.

In male offspring exposed to a maternal high-fat diet, increased macrophage Toll-like receptor 4 signalling results in excess microglial phagocytosis of serotonin (5-HT) neurons in the developing dorsal raphe nucleus, decreasing 5-HT bioavailability in the fetal and adult brains. Bulk sequencing from a large cohort of matched first-trimester human samples reveals sex-specific transcriptome-wide changes in placental and brain tissue in response to maternal triglyceride accumulation (a proxy for dietary fat content). Further, fetal brain 5-HT levels decrease as placental triglycerides increase in male mice and male human samples. These findings uncover a microglia-dependent mechanism through which maternal diet can impact offspring susceptibility for neuropsychiatric disorder development in a sex-specific manner.

E. Heard, March 13th 2023

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Using animal models to gain a better understanding of Sex Bias in Disease

Arnold *Biology of Sex Differences* 2010, **1**:1
http://www.bsd-journal.com/content/1/1/1

BIOLOGY OF SEX DIFFERENCES

EDITORIAL Open Access

Promoting the understanding of sex differences to enhance equity and excellence in biomedical science

Arthur P Arnold

From the moment of our conception, each of us has a sex. Sex has a major role in determining the physical attributes of our bodies, the structure of our brains, our behavioral tendencies, our susceptibility and reaction to diseases, the environment in which we grow up, our place in society, the attitudes of others towards us, and our conception of self. Although sex may be considered to be determined primarily biologically, our gender (i.e., the social perception and implications of our sex) is arguably equally or more important for our lives. Sex and gender differences are created by an intricate reciprocal interaction of numerous biological and environmental forces.

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

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

Investigating sexual dimorphism in:
- Cancers
- Autoimmune diseases
- Neurological and neurodegenerative disorders
- Cardiovascular diseases
- Metabolic disorders
- Behviour

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E. Heard, March 13th 2023

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**