CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2017-2018 :

"Le chromosome X -
paradigme de la génétique et l'épigénetique"

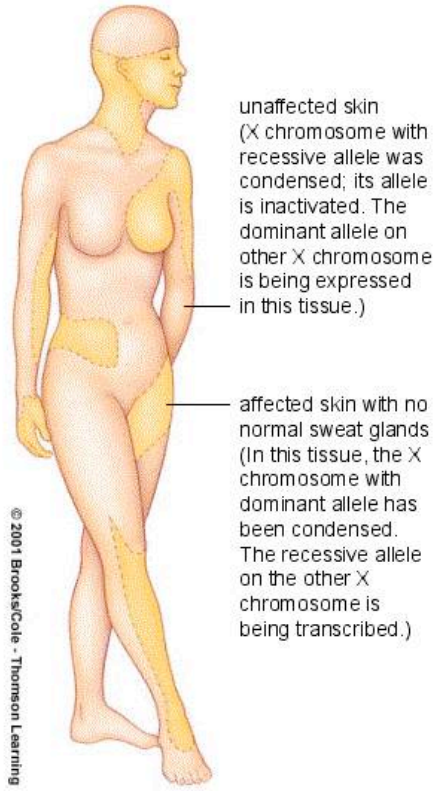
26 février, 2018

Cours V

Le chromosome X et les maladies autoimmunes

SUMMARY

- Random XCI leads to cellular mosaicism within and between individuals
- Every female is a unique mosaic for X-linked gene expression: outcome will depend on interplay of all alleles between the two (active) Xs, as well as the inherent variation in primary "random" XCI, also in cell mixing and migration
- Different organs and lineages show different distributions of clonal populations



R. Magritte

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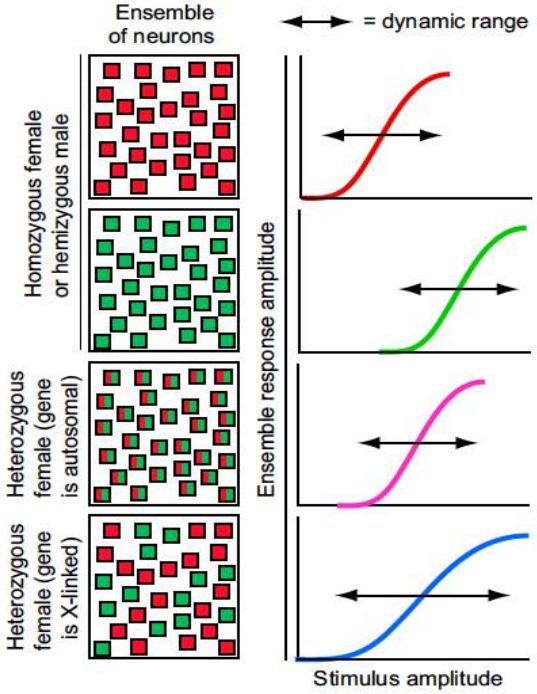
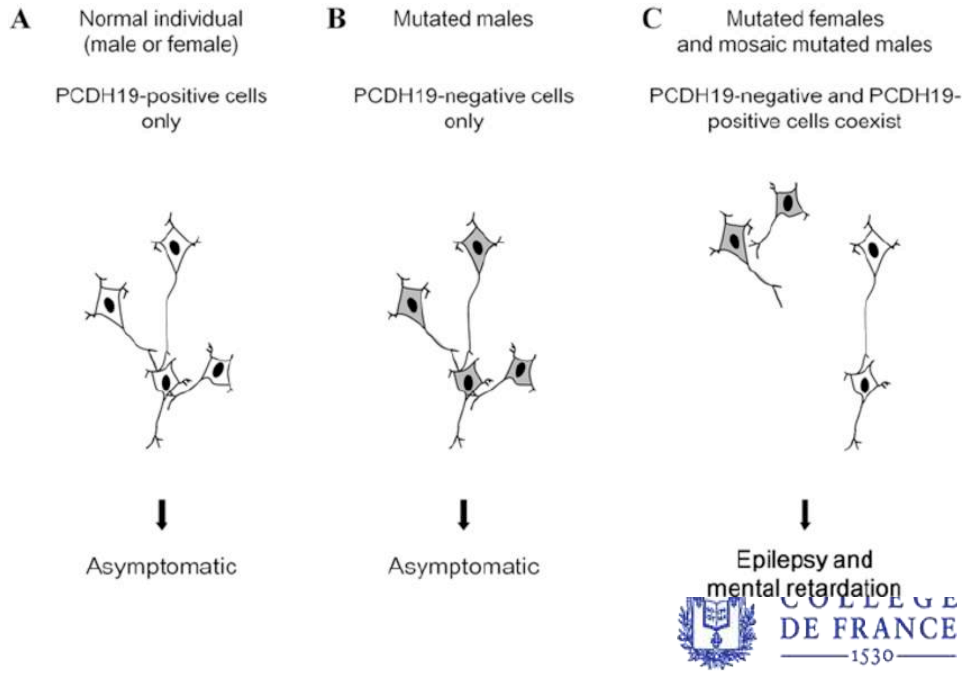
- *Advantages* of random XCI compared to simple heteorozygosity: increased functional possibilities by combining cellular phenotypes (eg in brain)
- Also some *disadvantages* – incompatibility between two cell types (expressing either allele of an X-linked gene) (craniofacial disorder; female-specific epilepsy disorder).



Two biochemically distinct types of neurons => the ensemble's dynamic range is likely to be expanded along the stimulus axis. Wu et al 2015



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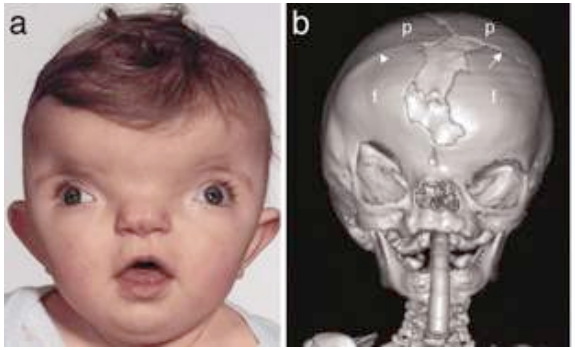

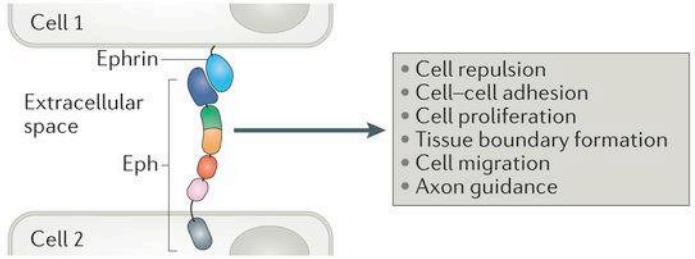

E. Hea

Craniofrontonasal syndrome CFNS: only females +/- affected ; not male -/Y or females -/-
The ephrin receptor and its EFNB1 ligand are both bound to the (trans)membrane of the cell and cascade is activated through cell-cell interactions. Cell-cell interactions are disturbed due to presence of *some* cells with *no* EFNB1 (Xa-) and others with EFNB1 (Xa+), => causing incomplete tissue-border formation.

In heterozygous females (*Ephrin-B1* +/-), patchwork loss of ephrin-B1 disturbs tissue boundary formation at the developing coronal suture, whereas in males deficient in ephrin-B1, an alternative mechanism maintains the normal boundary. This is the only known mutation in the ephrin/Eph receptor signaling system in humans and provides clues to the biogenesis of craniosynostosis.

Twigg et al (2004) Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. PNAS 101 (23) 8652-8657

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- X-linked escapee genes – some ALWAYS escape, others VARY between and within individuals
- Constitutive escapees are conserved with ubiquitous escape (eg *Jarid1c*, *Utx*)
- Variable escape may not be so conserved?

Genes That Escape X-Inactivation in Humans Have High Intraspecific Variability in Expression, Are Associated with Mental Impairment but Are Not Slow Evolving

Yuchao Zhang,[1,2] Atahualpa Castillo-Morales,[3] Min Jiang,[1] Yufei Zhu,[1] Landian Hu,[1] Araxi O. Urrutia,[3] Xiangyin Kong,[*,1] and Laurence D. Hurst[*,3]

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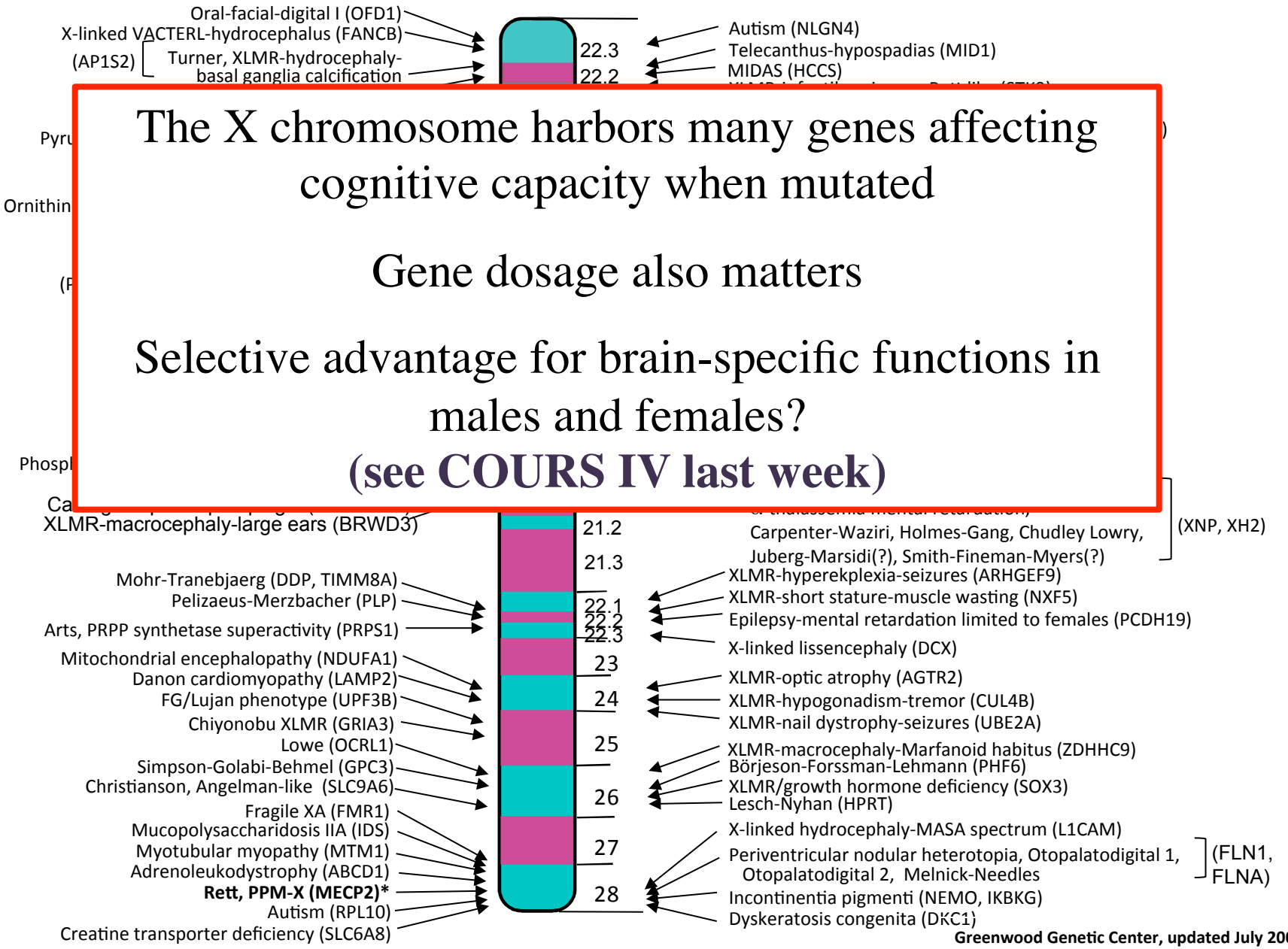
- X-linked escapee genes – some ALWAYS escape, others VARY between and within individuals
- Constitutive escapees are conserved with ubiquitous escape (eg *Jarid1c*, *Utx*)
- Variable escape may not be so conserved?
- But the time and location of escape needs to be examined carefully: dosage might vary during development and in adults
- Escapee genes are implicated in mental impairment (eg *Mecp2*) and in other diseases (autoimmune…)
- *Many* X-linked genes are involved in intellectual deficiencies (XLIDs)

XLID genes



The X chromosome harbors many genes affecting cognitive capacity when mutated

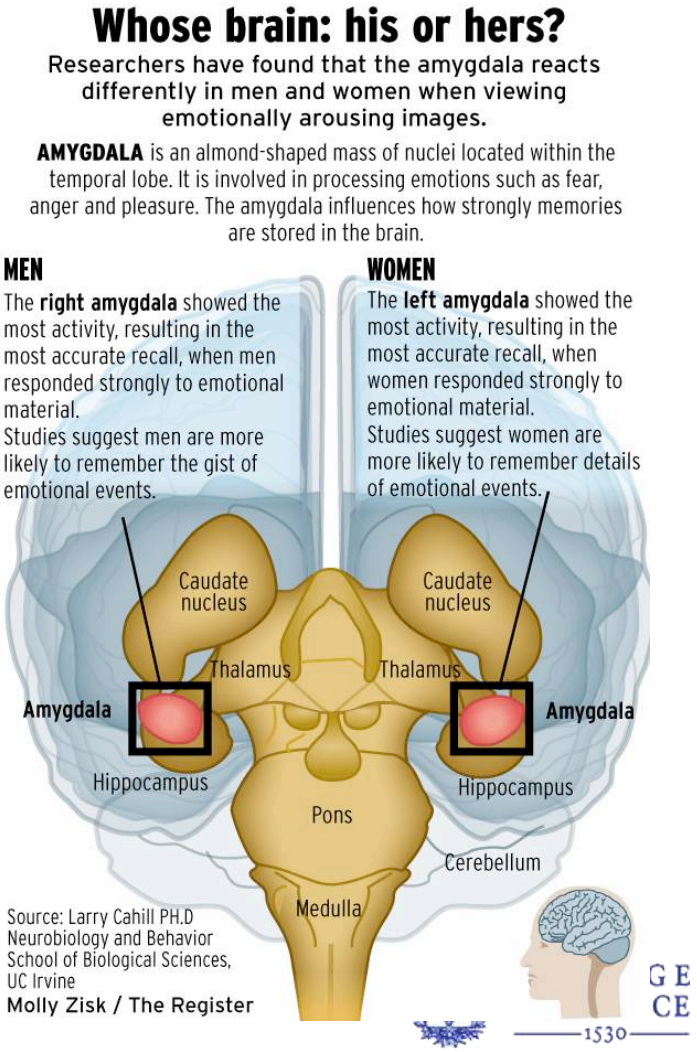
Gene dosage also matters

Selective advantage for brain-specific functions in males and females?

(see COURS IV last week)

Sex Differences in Neurological functions?

• Some scientists argue that sex differences in the brain are robust and widespread (Cahill), whereas others argue that much of the science is flawed and that inherent long-standing bias has stepped in to replace objectivity (Rippon et al, 2014; Eliot 2011)



McCarthy MM. 2016. Multifaceted origins of sex differences in the brain. *Phil. Trans. R. Soc. B 371: 20150106.* http://dx.doi.org/10.1098/rstb.2015.0106

- Cahill L *2006 Why sex matters for neuroscience. Nat. Rev. Neurosci. 7, 477–484*
- Rippon G et al . *2014 Recommendations for sex/gender neuroimaging research: key principles and implications for research design, analysis, and interpretation. Front. Hum. Neurosci. 8, 650*
- Eliot L *2011 The trouble with sex differences. Neuron 72, 895–898.*
- Maney DL *2015 Just like a circus: the public consumption of sex differences. Curr. Top. Behav. Neurosci. 19, 279–296.*

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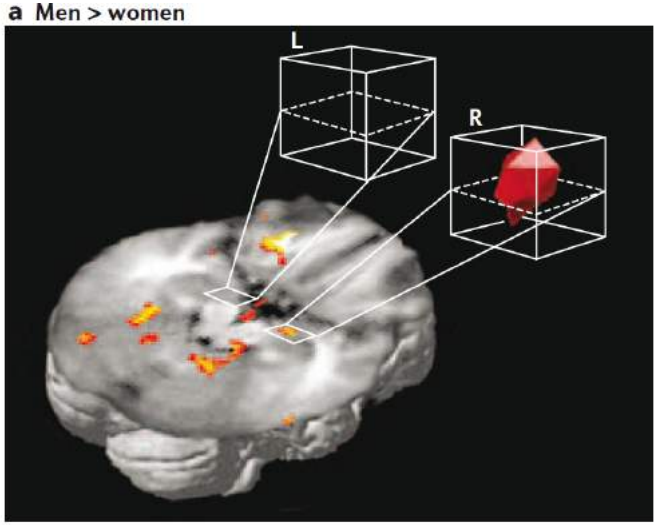
• Sex differences in brain size, structure, activity and function appear to be numerous Advanced imaging techniques have defined highly sex-specific features

• How (and whether) neuro-anatomical differences are connected to behaviour and disease remain open and active areas of research by neurobiologists, psychologists and psychiatrists



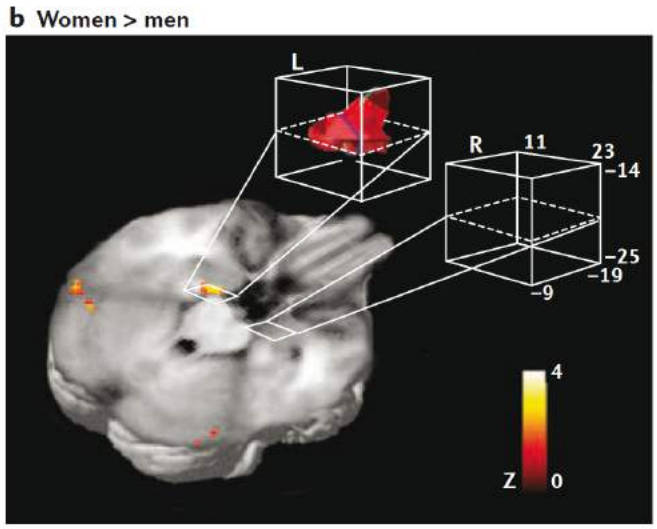
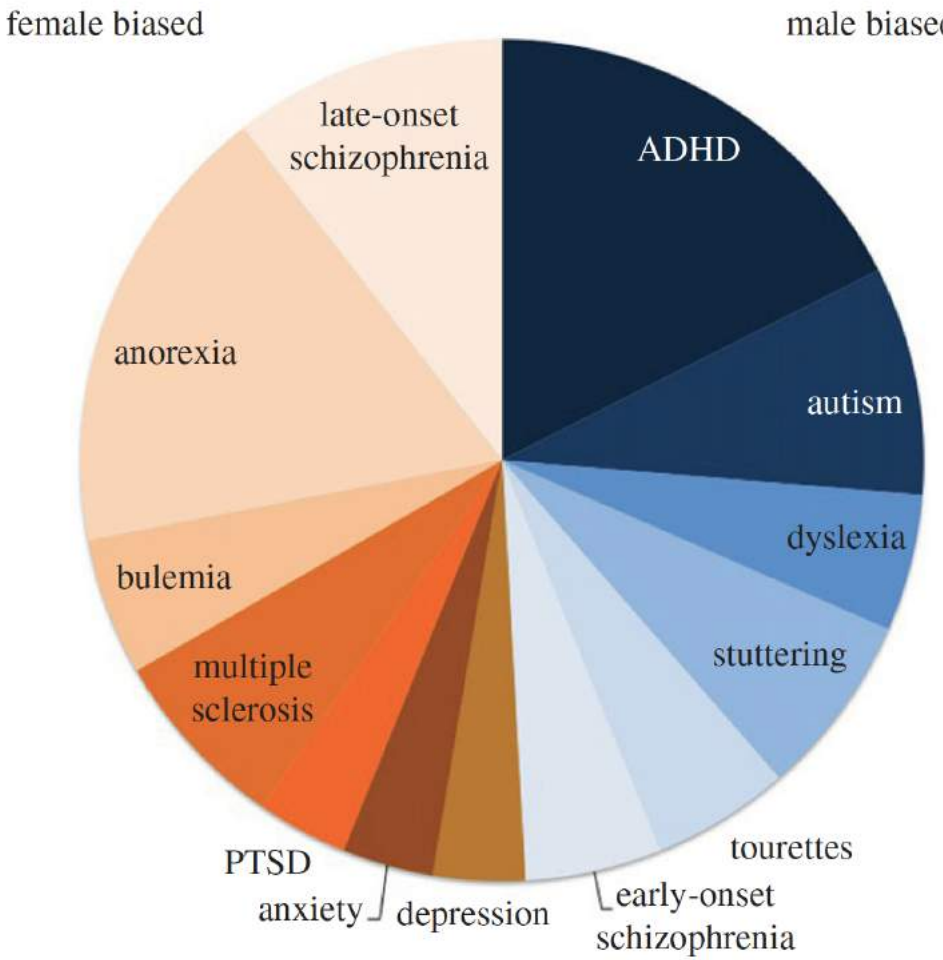


Figure 3 | **Sex differences in the relationship between amygdala activity during emotional experiences and memory for those experiences.** Extensive evidence indicates that the amygdala modulates memory storage for emotionally arousing events. However, findings from several studies now demonstrate sex differences in the relationship between amygdala activity during emotional experiences and memory for those experiences. As illustrated here, activity in the right hemisphere amygdala while viewing emotionally arousing images is more significantly related to subsequent memory for the images in men than it is in women (**a**), whereas the converse is true for the left hemisphere amygdala (**b**). The reasons for this hemispheric laterality, and what it means for the qualities of memories for emotional events in men and women, are now important areas of study. Reproduced, with permission, from REF. 41 © (2004) Cold Spring Harbour Laboratory Press.

Sex Differences in Neurological functions?

• Striking sex (or gender) bias in the frequency of diagnosis of numerous disorders.
• Identification of biological variables between the sexes provides diagnostic value and insight into disease aetiology.

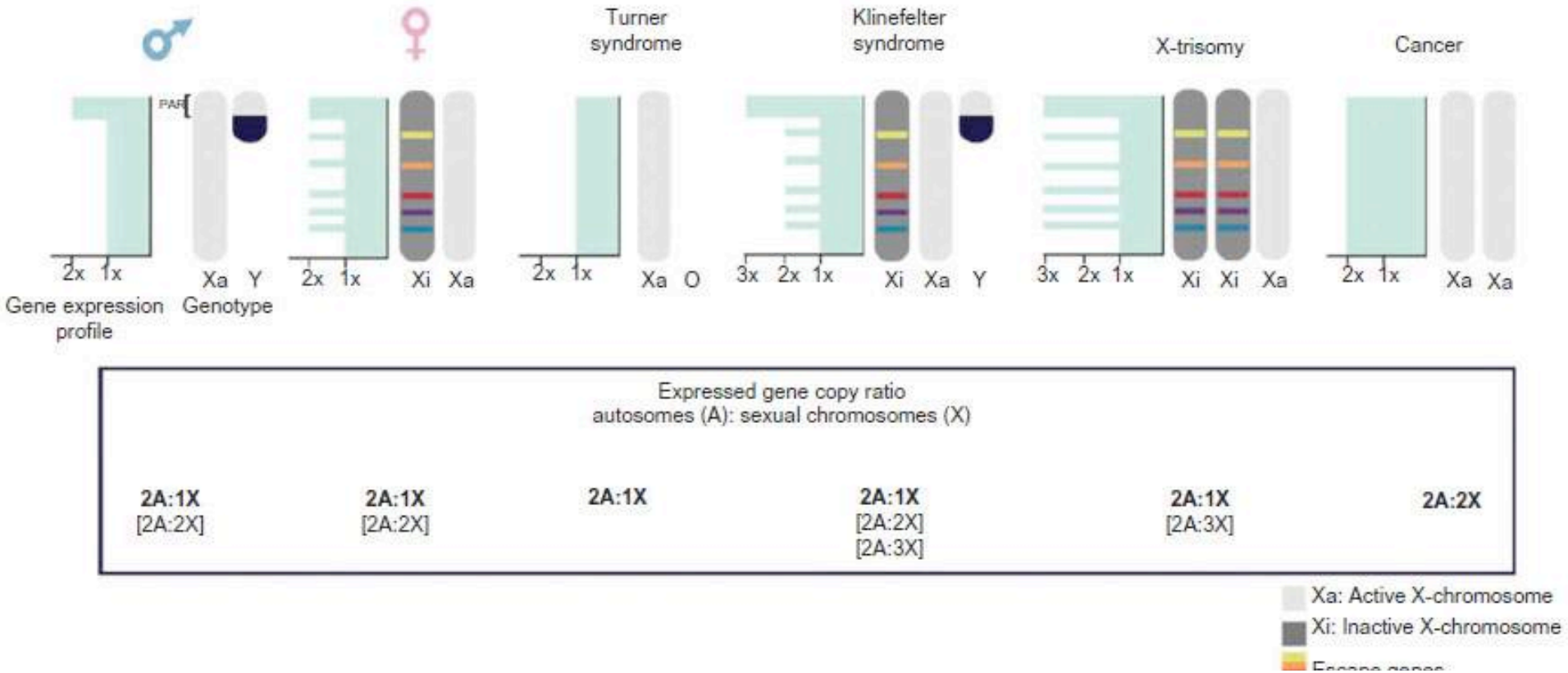


- Autism spectrum disorder is diagnosed in boys 4-5 times more often than girls
- Schizophrenia manifests differently in men and women across lifespan
- Unipolar depression and PTSD are up to twice as frequent in women and girls. *But, may be skewed by social factors such as willingness to seek treatment.*
- Differences in drug and alcohol abuse in men and women are speculated to be based in sex differences in risk-seeking and reward systems.

Sex, Gender, Culture

In humans these can be inextricably linked…

Sex Chromosome Aneuploidies: Impact of aberrant X-linked gene dosage?



- Many X chromosome ploidy alterations (including XXY Kleinfelter Syndrome and XXX, XXXX, XXXXX) are associated with learning impairments (Rooman et al. 2002).
- The phenotype of X polysomies is thought to reflect the action of genes that escape X-inactivation.
- Genes Escaping X-inactivation are often related to Mental Impairment

Cognitive, Behavioral, and Neural Consequences of Sex Chromosome Aneuploidy

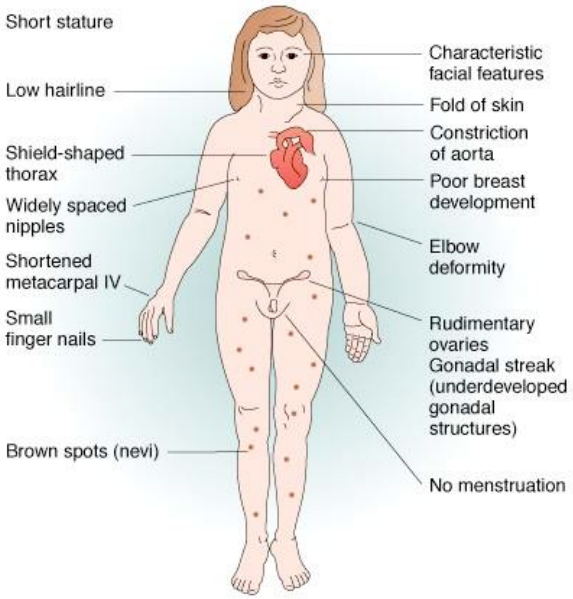
IQ SCORE MEAN VALUES, ACROSS STUDIES, BY SEX CHROMOSOME ANEUPLOIDY

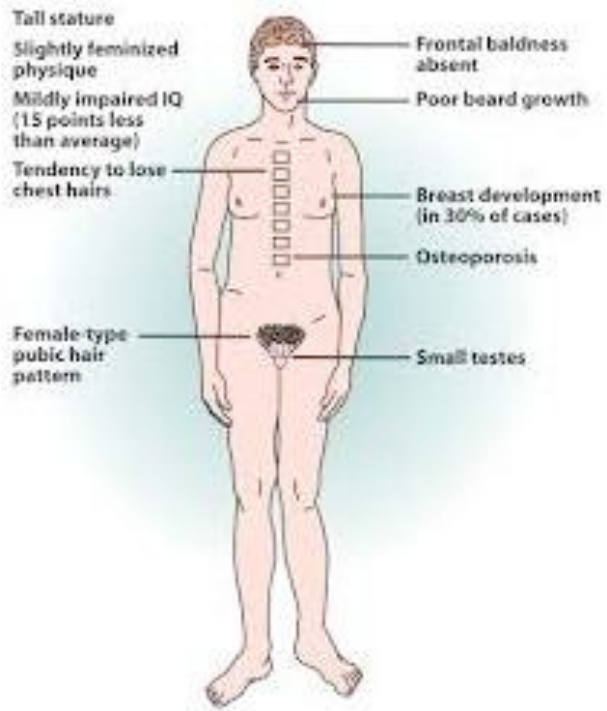
	Full-scale IQ: general intelligence	Verbal IQ: verbal skills	Performance IQ: nonverbal skills
XO	90–94 (Hong et al., 2011; Rovet, 1990, 1993)	93–99 (Hong et al., 2011; Rovet, 1993)	88–91 (Rovet, 1993; Hong et al., 2011)
XYY	91–97 (Bardsley et al., 2013; Tartaglia et al., 2012)	88–92 (Bardsley et al., 2013; Tartaglia et al., 2012)	95–102 (Bardsley et al., 2013; Tartaglia et al., 2012)
XXY	92–98 (Rovet et al., 1995; Tartaglia et al., 2012)	84–93 (Rovet et al., 1995; Tartaglia et al., 2012)	98–99 (Rovet et al., 1995; Tartaglia et al., 2012)
XXX	83–93 (Tartaglia et al., 2012; Tartaglia et al., 2010b)	82–87 (Tartaglia et al., 2012; Rovet et al., 1995)	87–100 (Tartaglia et al., 2012; Rovet et al., 1995)
XXYY	78–79 (Tartaglia et al., 2008b; Tartaglia et al., 2012)	74–77 (Tartaglia et al., 2008b; Tartaglia et al., 2012)	84–87 (Tartaglia et al., 2008b; Tartaglia et al., 2012)

% CASES OF AUTISM SPECTRUM DISORDER AND ATTENTION-DEFICIT/ HYPERACTIVITY DISORDER

	XO	XXY	XYY	XXX	XXYY
ASD	3%–4% (Creswell and Skuse, 1999; Saad et al., 2013)	11%-27% (Bruining et al., 2009; Bishop et al., 2011)	19%–36% (Bishop et al., 2011; Tartaglia et al., 2012)	No increased risk (Bishop et al., 2011)	28%–34% (Tartaglia et al., 2008a; Tartaglia et al., 2012)
ADHD	20%–50% (Green et al., 2015; Saad et al., 2013)	36%–63% (Bruining et al., 2009; Tartaglia et al., 2010a)	46%–76% (Ross et al., 2012; Tartaglia et al., 2012)	25%–52% (Bender et al., 1993; Tartaglia et al., 2012)	72.2% (Tartaglia et al., 2012)

Turner's Sydrome (XO) and Klinefelter's Syndrome (XXY)

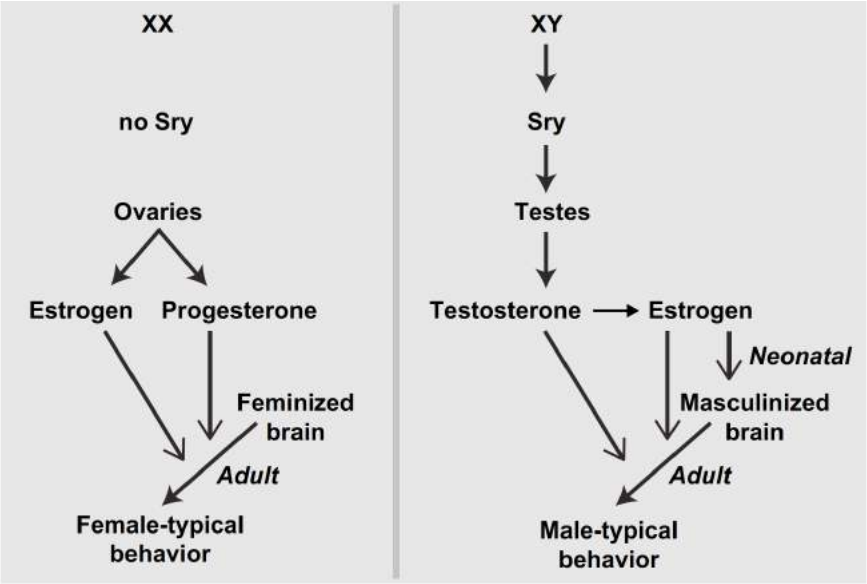


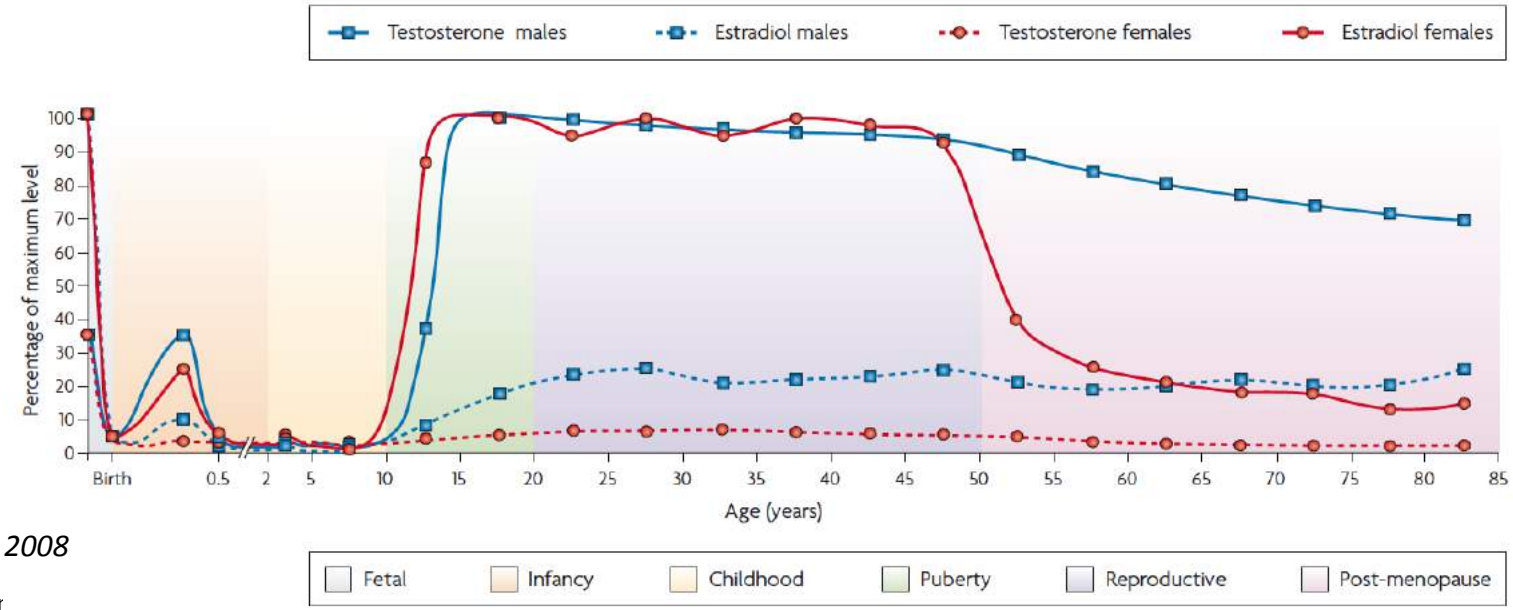


- Turner syndrome appears to confer specific advantages in the development of language
- In childhood, affected girls can perform better than typically developing girls in terms of receptive and expressive language tasks, phonological tasks, and lexico-semantic language tasks, but perform worse on some executive function tasks eg speeded number naming (Temple &Shephard, 2012).
- This suggests that X-linked gene haploinsufficiency in Turner's individuals may confer an early advantage for receptive and expressive language skills, phonological skills, and lexicosemantic processing.
- In the **male** aneuploidies (XXY, XYY, and XXYY), there is a relative deficit in verbal compared with visuospatial ability, in contrast to X monosomy which is associated with better verbal than visuospatial skills.
- However, there are also hormonal consequences: for example, X monosomy leads to atretic ovaries, so sex hormone abnormalities could be a contributory factor to the unusual pattern of cognitive skills and weaknesses.

Sex Differences in Neurological functions?

- In the twentieth century, gonadal hormones emerged as the primary proximate factors that act on tissues to cause sex differences in phenotypes.
- Only hormones were incorporated into theories of the origins of sex differences in phenotype.





Ober et al, NRG 2008

Sex Differences in Neurological functions?

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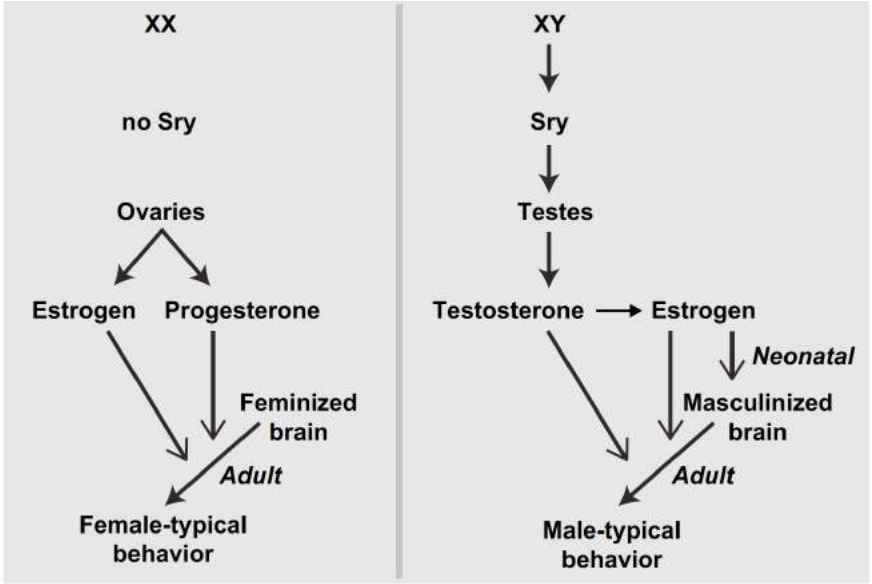
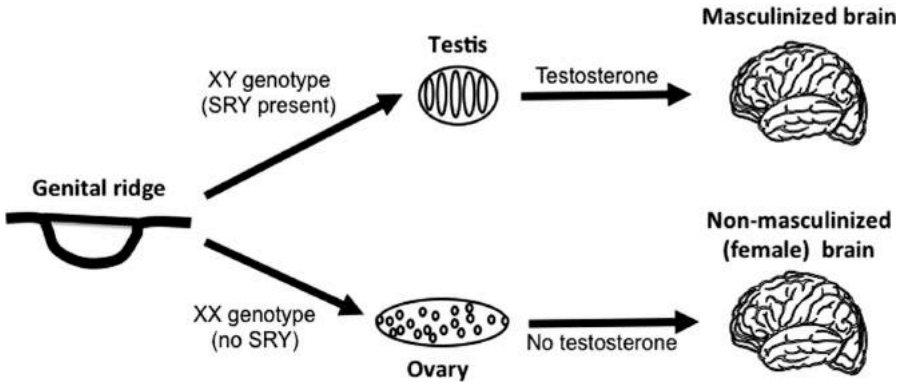
- Only hormones were incorporated into theories of the origins of sex differences in phenotype.
- However, circulating sex hormones in adult animals do NOT fully account for all sex differences in the brain
- In the past two decades, the sexual imbalance of effects of the X and Y chromosomes have been clearly shown to cause sex differences in non-gonadal tissues that are not mediated by gonadal hormones – effects mainly localized to the X



Classical theory of brain sexual differentiation



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Sex Differences in Neurological functions?

Sex differences in physiology and disease: need to know which sex-biasing factors cause sex differences in phenotype and how.

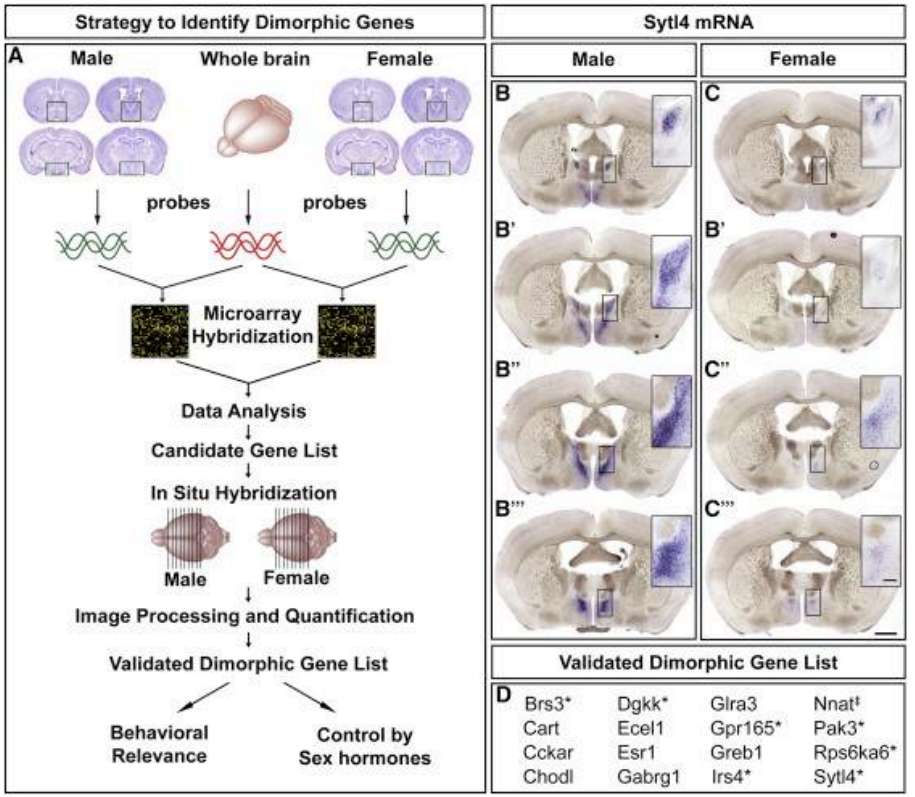
In mammals, sex differences are downstream of the unequal effects of XX vs. XY sex chromosomes. Need to distinguish:

- Effects of gonadal hormones on development and in adulthood eg Xu et al
- Effects of sex chromosomes operating outside of the gonads.

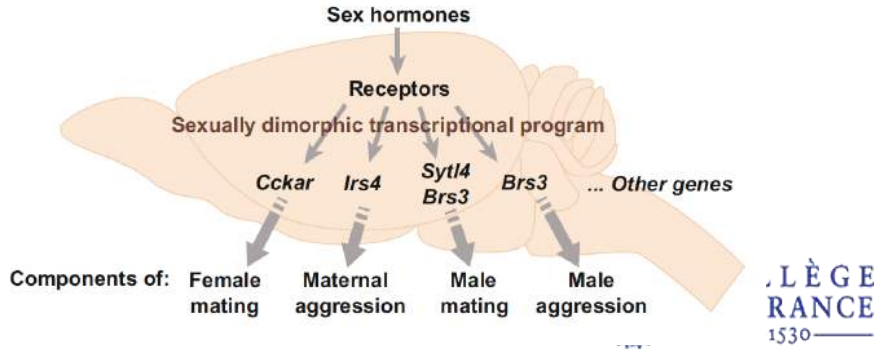
Need to use appropriate experimental design and animal models.

Two important mouse models allow conclusions on the sex-biasing effects of sex chromosome complement, interacting with gonadal hormone effects

1) Four Core Genotypes model
2) XY* model.

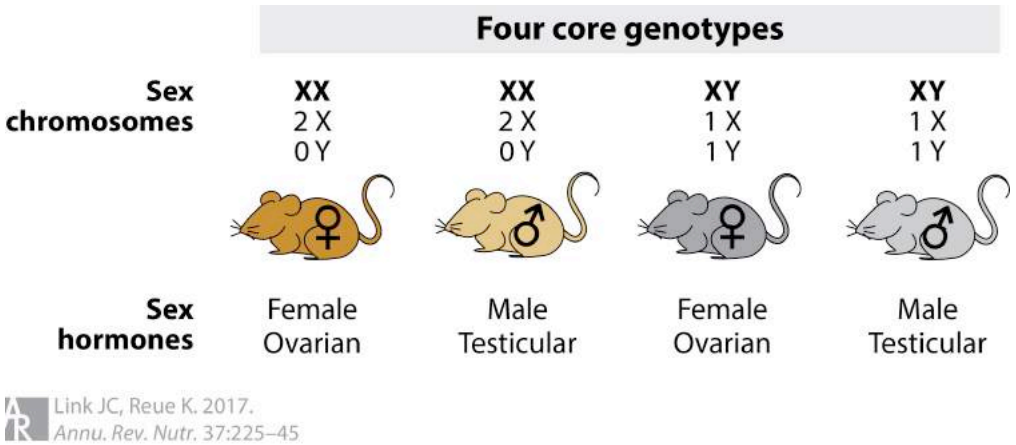


DOI: https://doi.org/10.1016/j.cell.2011.12.018

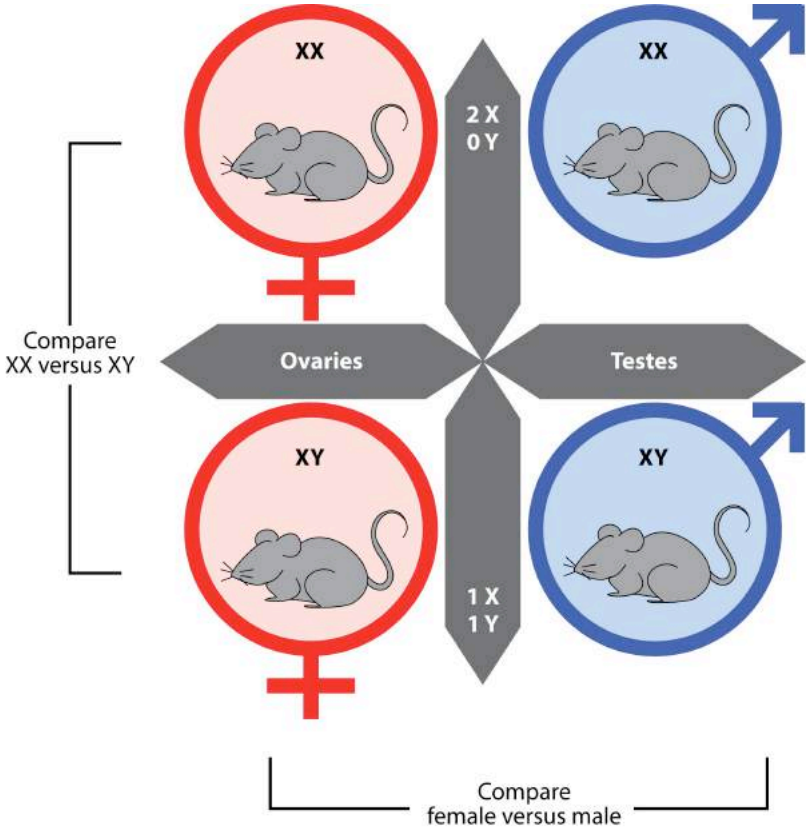


Methods for distinguishing effects of X chromosome number from effects of sex hormones

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



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



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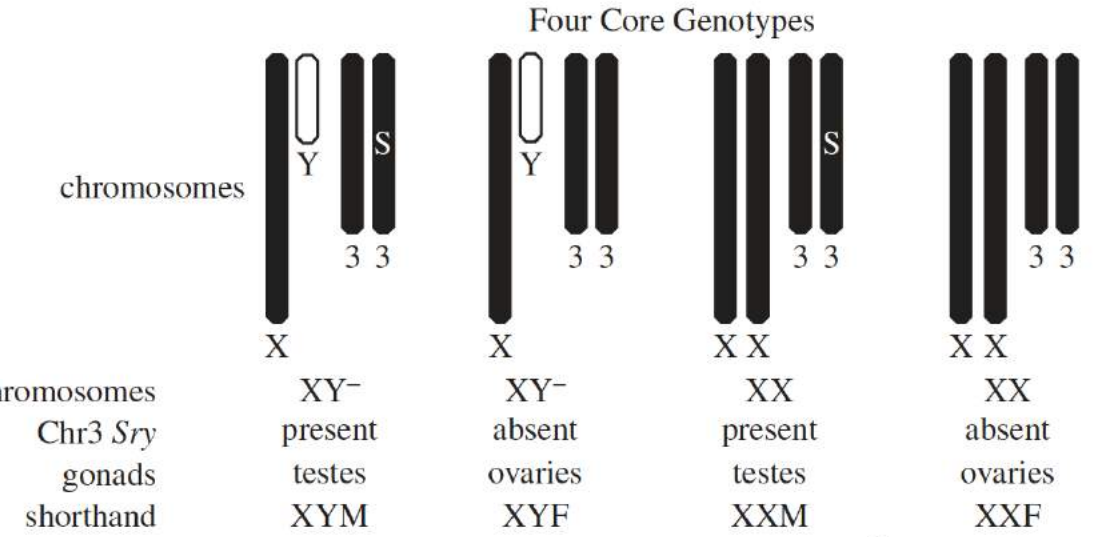
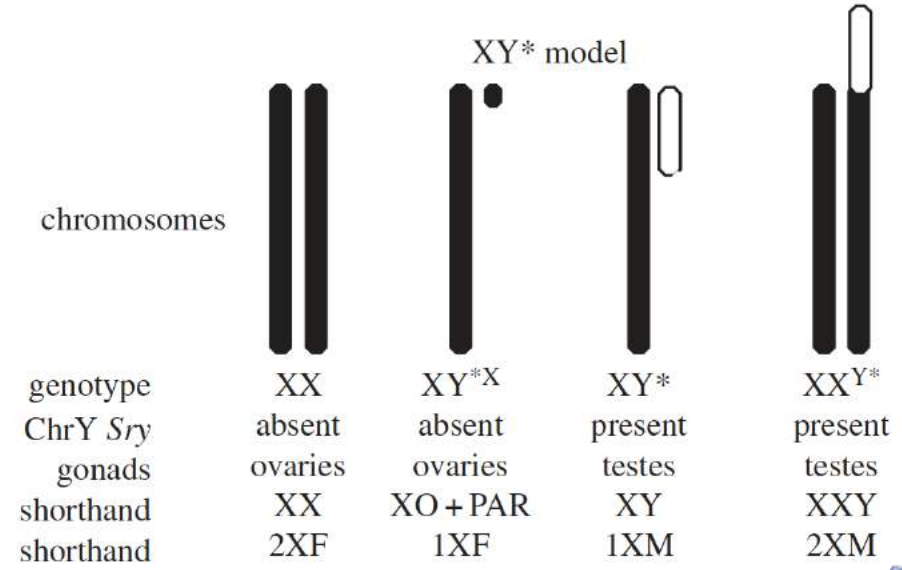
Methods for distinguishing effects of X chromosome number from effects of sex hormones

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).



XY* model (effects due to Y chromosome):
breed an XX mother with XY* father produces four genotypes, based on the abnormal recombination of the Y* chromosome with the X chromosome



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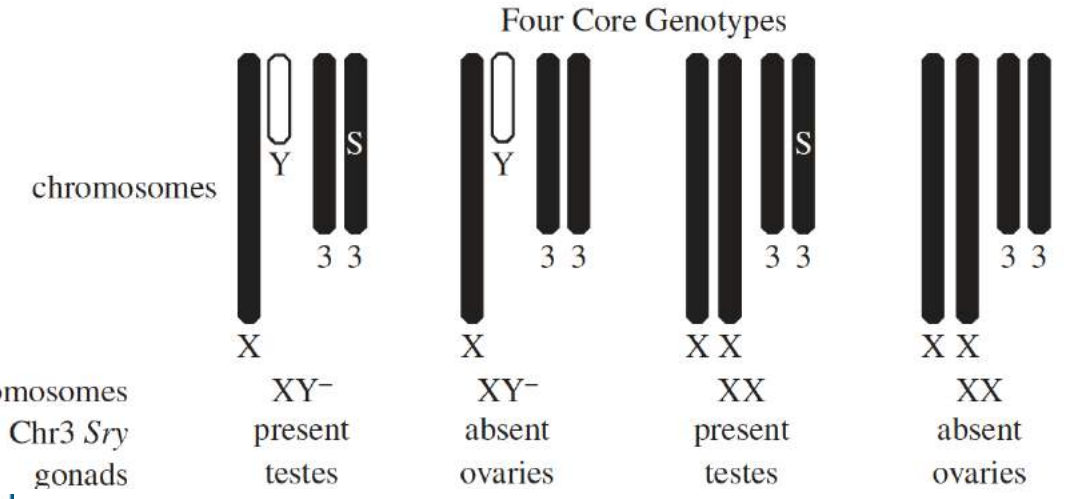
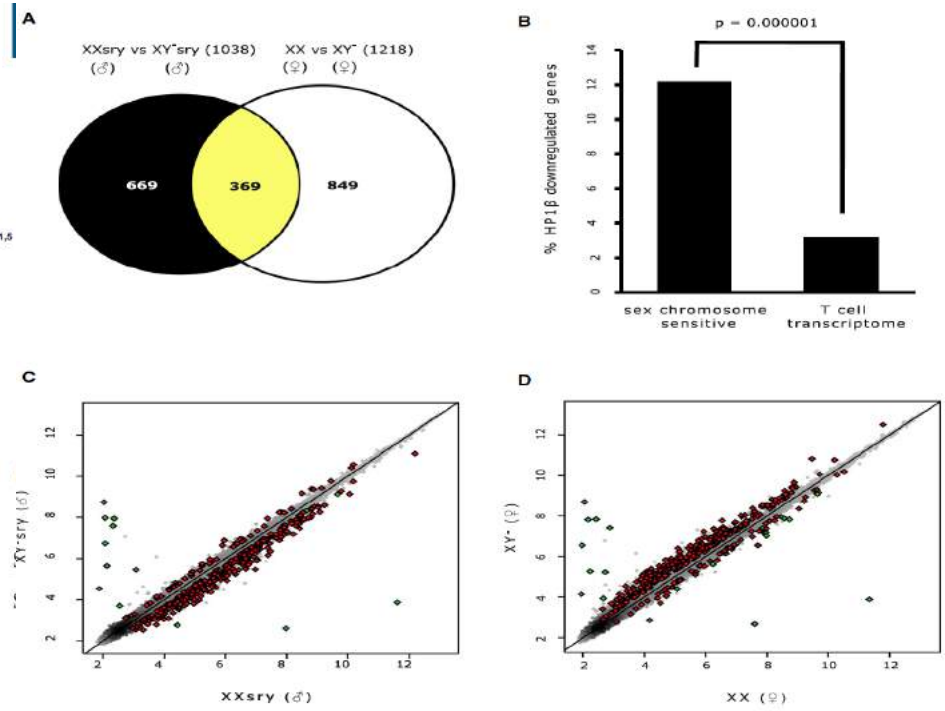


Developmental Cell
Short Article

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,*]



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


The importance of having two X chromosomes

Arthur P. Arnold[1,8], Karen Reue[3,4], Mansoureh Eghbali[5], Eric Vilain[4,6,7], Xuqi Chen[1,8], Negar Ghahramani[4,8], Yuichiro Itoh[1,8], Jingyuan Li[5], Jenny C. Link[3,4], Tuck Ngun[4,8] and Shayna M. Williams-Burris[1,2,8]

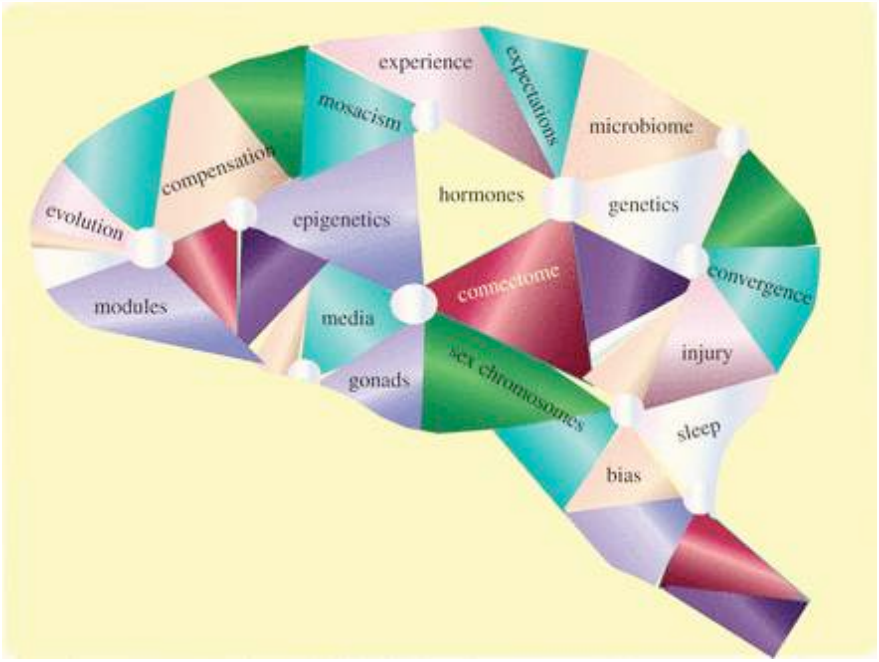
Use of these models to identify mechanisms (and genes) that contribute to sex differences in traits such as obesity, cardiovascular disease and **behaviour**.

Studies of mouse behaviour provide evidence that the number of X chromosomes contributes to sex differences:

- 1) Fear reactivity in gonad-intact adult mice is greater in XO than XX mice (reluctance of the mouse to venture into an open area of an elevated plus maze).
- 2) Sexual behaviour: XY* mice are GDX as adults and treated equally with testosterone, then tested with a receptive female, they show male sex behaviour differently depending on the number of X chromosomes
- 3) Social behaviour: mice with one X chromosome show less social behaviour when paired with another mouse, compared with mice with two X chromosomes. Mice with one X investigate their cage partners more frequently than mice with two X chromosomes, but spend less total time in proximity to or interacting with the partner.
- 4) Anxiety - greater anxiety-like behaviour, found in adult mice with one X chromosome also found in juvenile mice and may explain the tendency of mice with one X chromosome to avoid novel mice or social partners, more than mice with two X chromosomes.

Hormones versus Sex Chromosomes on Brain Function

- Although Hormones dominated theories of the origins of sex differences, over the past two decades, the sexual imbalance of effects of the X and Y chromosomes have been clearly shown to cause sex differences in non-gonadal tissues that are not mediated by gonadal hormones
- Specific X-linked genes are prime candidates for these effects
- Do not yet know yet <u>which X genes</u> are responsible, or <u>how they act</u>…
- Escapee genes – often involved in chromatin and/or transcription – control of regulatory networks?
- NB escapees with a Y copy –appear to have diverged in function or pattern of expression, compensation by the Y paralogue is not complete.



Sex-biasing factors can **counteract** the effects of each other, **reducing** rather than producing sex differences in **phenotype** (de Vries and others). This change in viewpoint requires a change in experimental strategies for dissecting sex chromosome effects.
Ultimately need to define the "sexome": the sum of effects of sex-biasing factors on gene systems and networks

Many XLID genes encode proteins with Epigenetic Functions

And many of these epigenetic X-linked factors are constitutive or

- Specific requirements in female brains /CNS or cognitive functions?
- Chromatin complexes involved in handling the inactive X in the brain?
- Accidental re-expression – which may even be deleterious?

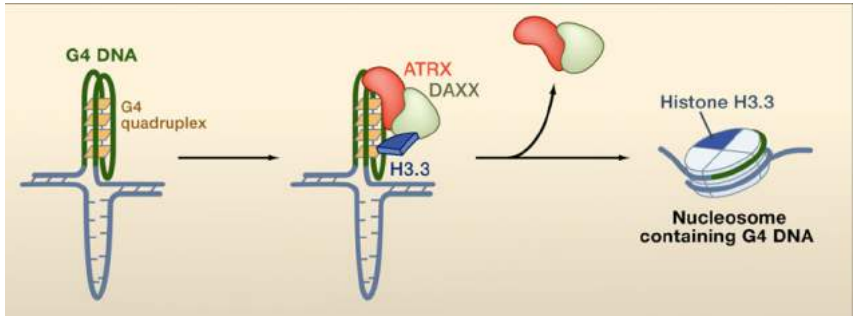
Loss of Kdm5c causes spurious transcription and prevents the fine-tuning of activity-regulated enhancers in neurons

Marilyn Scandaglia[1], Jose P. Lopez-Atalaya[1], Alejandro Medrano-Fernandez[1], Maria T. Lopez-Cascales[1], Beatriz del Blanco[1], Michal Lipinski[1], Eva Benito[1,#], Roman Olivares[1], Shigeki Iwase[2], Yang Shi[3], and Angel Barco[1,4]

A mouse model of X-linked intellectual disability associated with impaired removal of histone methylation

Shigeki Iwase[1,2,*,#], Emily Brookes[1,§,#], Saurabh Agarwal[2,#], Aimee I Badeaux[1], Hikaru Ito[3], Christina N Vallianatos[2], Giulio Srubek Tomassy[4], Tomas Kasza[2], Grace Lin[5], Andrew Thompson[6], Lei Gu[1], Kenneth Y. Kwan[5], Chinfei Chen[6], Maureen A. Sartor[7], Brian Egan[8], Jun Xu[3,*], and Yang Shi[1,*]

ATRX cause alpha thalassaemia, microcephaly and mental retardation



European Journal of Human Genetics (2018) 26:64–74
https://doi.org/10.1038/s41431-017-0038-6

ESHG

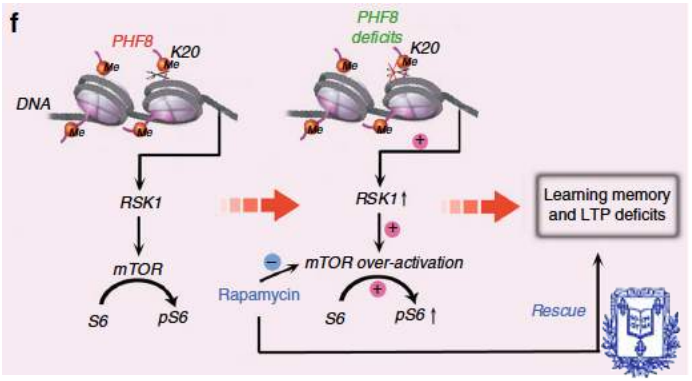
ARTICLE

***HUWE1* variants cause dominant X-linked intellectual disability: a clinical study of 21 patients**

ARTICLE
DOI: 10.1038/s41467-017-02531-y OPEN

***Phf8* histone demethylase deficiency causes cognitive impairments through the mTOR pathway**

Xuemei Chen[1,2,3], Shuai Wang[1], Ying Zhou[1], Yanfei Han[1,4], Shengtian Li[1], Qing Xu[2], Longyong Xu[2], Ziqi Zhu[2], Youming Deng[2], Lu Yu[2], Lulu Song[1], Adele Pin Chen[2], Juan Song[5], Eiki Takahashi[6], Guang He[1], Lin He[1], Weidong Li[1] & Charlie Degui Chen[2]



E. Heard, February 26h, 2018

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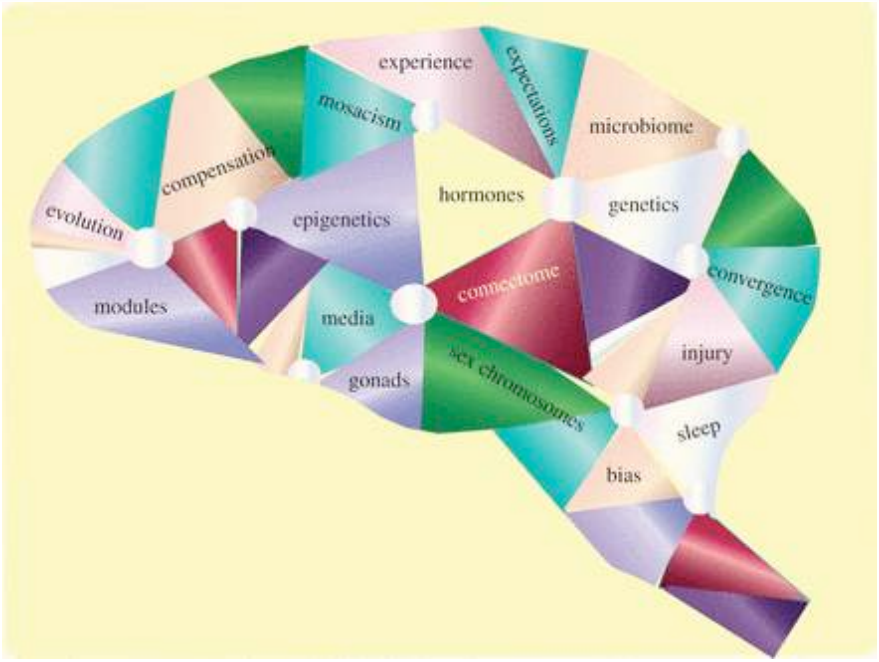
Hormones versus Sex Chromosomes on Brain Function

- Multifaceted origins of sex differences in the brain. Many variables impacting sex differences in the brain: biological (hormones, sex chromosomes), experience, environment.

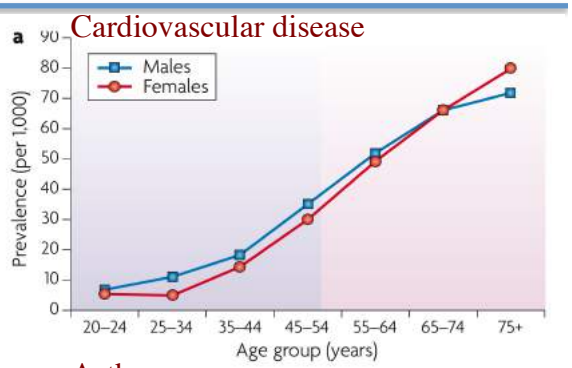
- Cultural and societal expectations may also exert biological influences on the brain but determining these is a challenge

- Media reports exaggerating the significance of sex differences confound efforts to have reasoned data-based discussions by the diverse community of scientists addressing this topic.

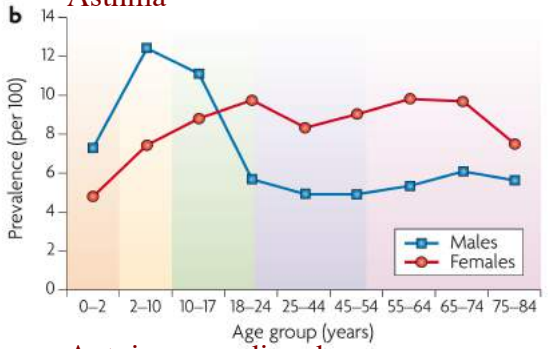


Sex bias in Human Disease

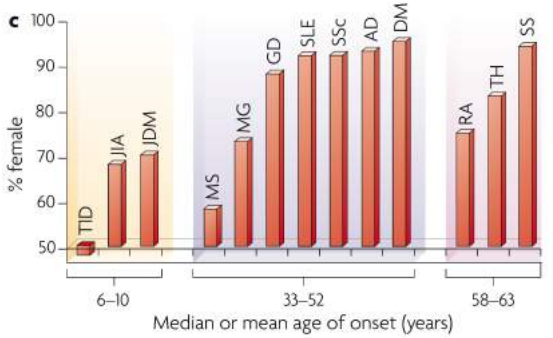
Cardiovascular disease



Asthma



Autoimmune disorders



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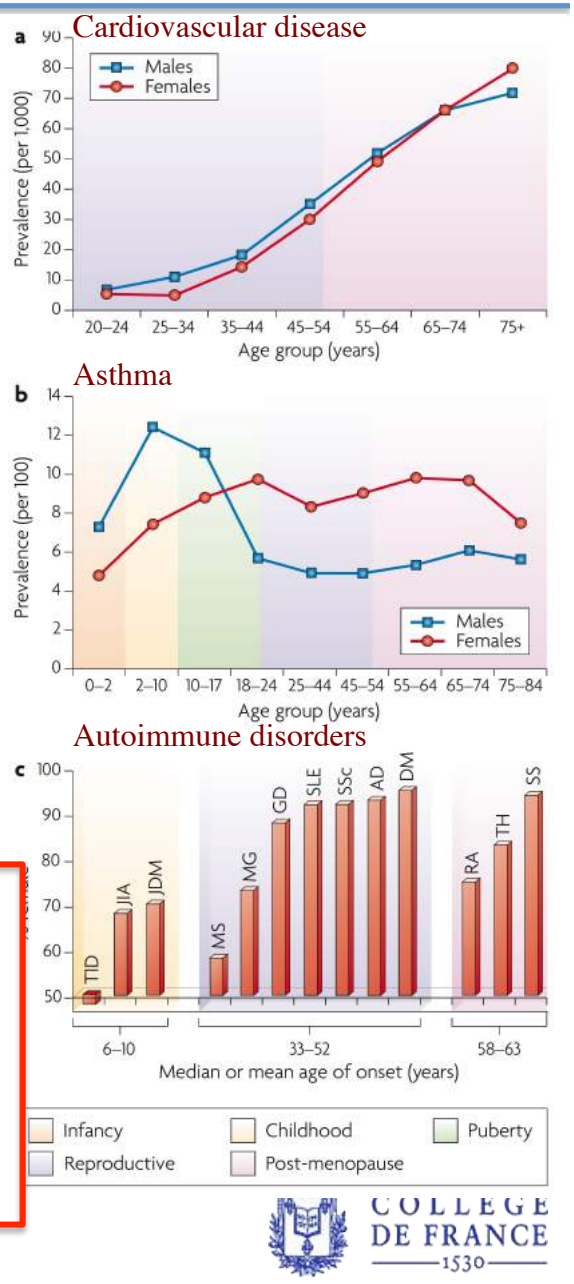
Sex bias in Human Disease

Accumulating evidence suggests that nearly all human diseases have sex-specific differences in prevalence, age of onset and/or severity:

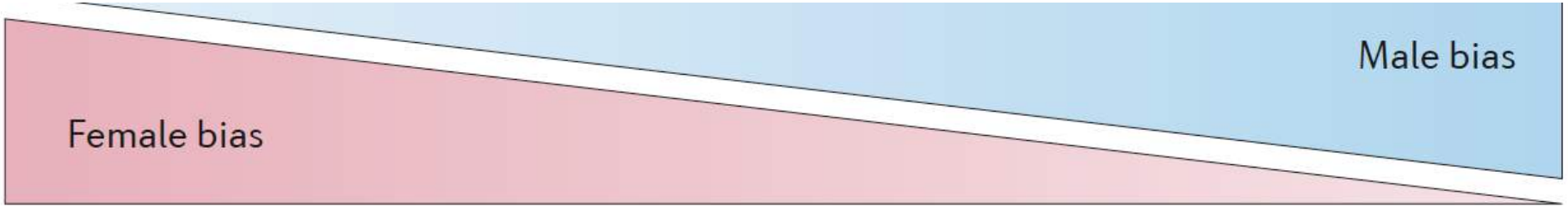
- Neurological and psychiatric disorders
- Cardiovascular disease: predominant in men throughout adulthood but has a higher rate of occurrence in post-menopausal women compared with men
- Asthma, which is more prevalent among boys in childhood but shows a higher occurrence of new cases among girls around and following puberty
- Several common cancers
- Autoimmune diseases are more prevalent in women throughout life, but particularly for diseases that begin during or immediately following the reproductive years

Up to 6,000 genes (of 23,000 total in humans), are differentially expressed in men and women, due to hormones and genes on the X and Y.

Most clinical trials in the past have been carried out only on men



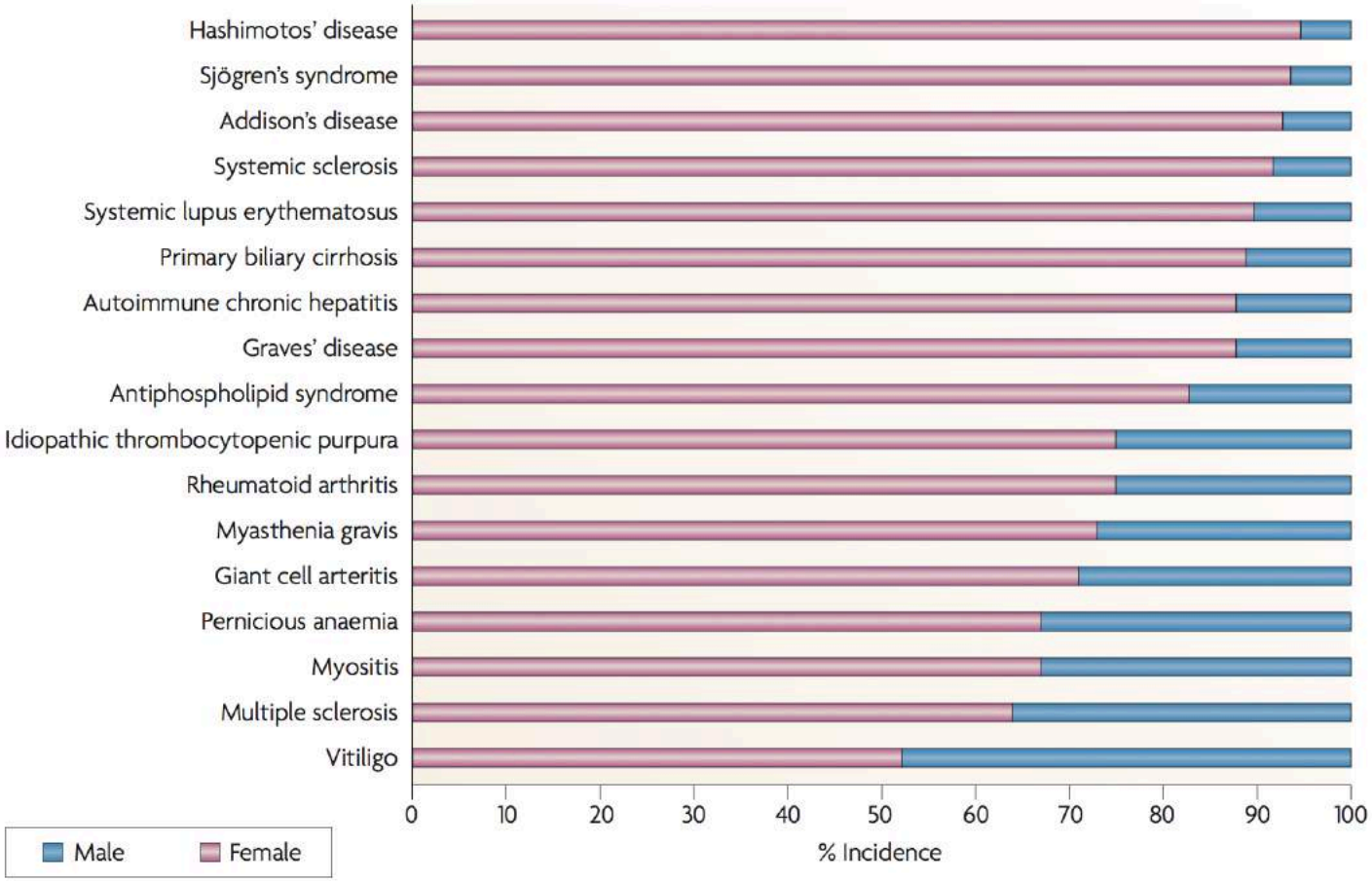
Sex bias in Infectious, Inflammatory Diseases and Cancer



Autoimmune diseases	Infectious diseases		Non-reproductive cancers
• Graves disease	• HIV	• Ebola	• Bladder
• Hashimoto thyroiditis	• Influenza	• MERS	• Bowel
• Multiple sclerosis	• Toxoplasmosis	• Hepatitis B	• Kidney
• Rheumatoid arthritis	• Legionella	• Tuberculosis	• Leukaemia
• Systemic lupus erythematosis	• Malaria	• Leptospirosis	• Liver
• Type 1 diabetes	• Zika	• Campylobacter	• Lung
		• Schistosomiasis	• Malignant melanoma
		• Amebiasis	• Oesophagus
		• Aspergillosis	• Stomach

Figure 2 | **Sex bias in infectious diseases, inflammatory diseases and cancers.**

Sex distribution of autoimmune diseases



- Differences in immune response may underlie sex bias in autoimmune disease.
- Factors linked with reproduction may underlie sex bias in autoimmune disease.
- External factors might influence susceptibility to autoimmune disease.
- Studies in autoimmunity should be stratified according to gender.

E. Hea

The X Chromosome and Autoimmune Disease

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).

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%

Autoimmunity is the result of both environmental and genetic components and, to date, only a few gene defects have been assigned to a particular autoimmune disease.
Breakdown of self tolerance can be caused by hormones, immunological challenge during pregnancy, fetal microchimerism, skewing of X chromosome inactivation or X chromosome-associated abnormalities, such as gene duplication or microdeletions.

X-chromosome number affects lupus & Sjogren's Syndrome susceptibility

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.

Estimated prevalence of SLE and SS in women with 47,XXX is~2.5 and ~2.9 times higher, respectively, than that in women with 46,XX. Liu et al, 2016

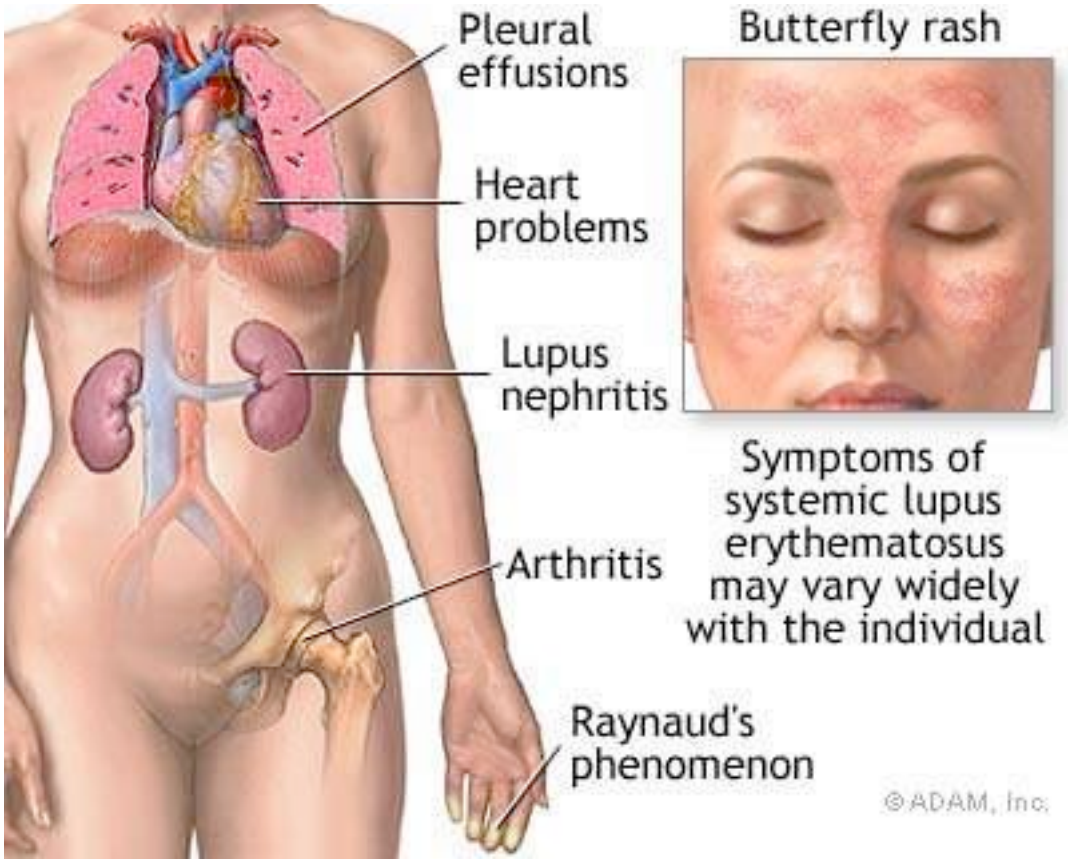
Increased prevalence of 47,XXX among women with either SLE or SS data suggests that the **number of X chromosomes is the key factor** imparting the 10-fold difference in risk between men and women, **because sexual development and sex hormones are normal in women with 47,XXX,**

Systemic Lupus Erythematosus (Lupus)

Table 1. American College of Rheumatology Criteria for the Diagnosis of Systemic Lupus Erythematosus (SLE).*

Criterion	Definition
Malar rash	A rash on the cheeks and nose, often in the shape of a butterfly
Discoid rash	A rash that appears as red, raised, disk-shaped patches
Photosensitivity	A reaction to sunlight that causes a rash to appear or get worse
Oral ulcers	Sores in the mouth
Arthritis	Joint pain and swelling of two or more joints
Serositis	Inflammation of the lining around the lungs (pleuritis) or inflammation of the lining around the heart that causes chest pain, which is worse with deep breathing (pericarditis)
Kidney disorder	Persistent protein or cellular casts in the urine
Neurologic disorder	Seizures or psychosis
Blood disorder	Anemia (low red-cell count), leukopenia (low white-cell count), lymphopenia (low level of specific white cells), or thrombocytopenia (low platelet count)
Immunologic disorder	Positive test for anti–double-stranded DNA, anti-Sm, or antiphospholipid antibodies
Abnormal antinuclear antibodies	Positive antinuclear-antibody test

* Four of the 11 criteria are needed for the formal diagnosis of SLE.



MECHANISMS OF DISEASE

Systemic Lupus Erythematosus

George C. Tsokos, M.D.

éruptions cutanées, nephrites, problèmes cardiaques, arthrite…

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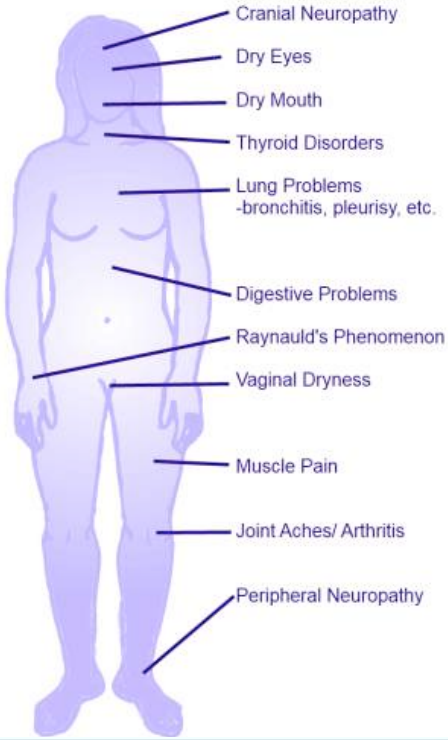
Sjogren's Syndrome

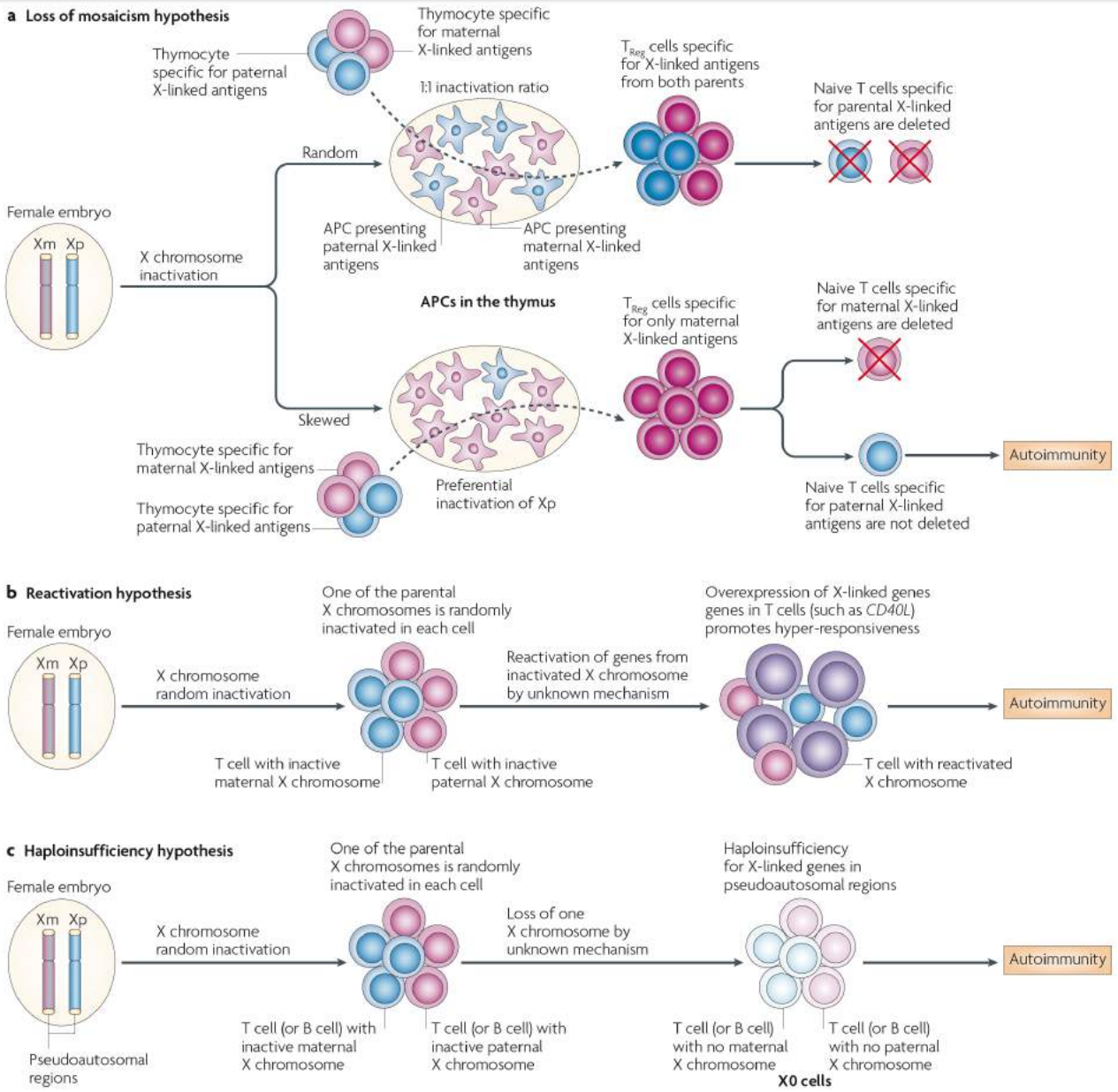
- Henrik Sjögren described and named the disease in 1933, but earlier descriptions existed
- Between 0.2% and 1.2% of the population are affected (primary or secondary form), begins in middle age
- Females affected about ten times more often than males
- The moisture-producing glands of the body are affected => dry mouth and dry eyes.
- Also dry skin, a chronic cough, vaginal dryness, numbness in the arms and legs, feeling tired, muscle and joint pains, and thyroid problems.
- Exact cause not known, but likely to involve a combination of genetics and an environmental trigger such as exposure to a virus or bacteria.
- Environmental factors, such as glandular viral infection, could prompt epithelial cells to activate the HLA-independent innate immune system through toll-like receptors.



Criteria Used to Diagnose Sjogren's Syndrome

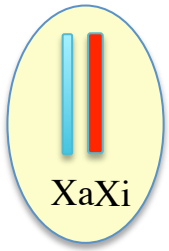
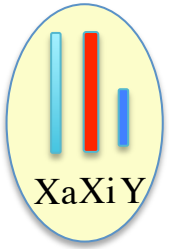
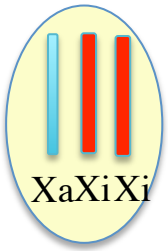
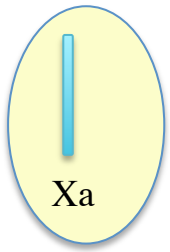
Ocular Symptoms (must have at least one)	• Symptoms of dry eye for at least 3 months • A foreign body sensation in the eyes for 3 or more months • Use of artificial tears 3 or more times per day
Oral Symptoms (must have at least one)	• Symptoms of dry mouth for at least 3 months • Recurrent or persistently swollen salivary glands • Need for liquids to swallow dry foods
Ocular signs (must have at least one)	• Abnormal Schirmer's test • Positive Rose Bengal test *note that these criteria do not include the tear break up time test
Histopathology	• Salivary gland biopsy showing at least 2 lymphocytic foci per 4 mm2
Oral Signs (must have at least one)	• Abnormal parotid sialography • Abnormal salivary scintigraphy • Abnormal sialometry
Autoantibodies	• Anti-Ro (SSA) or anti-La (SSB) or both

Hypotheses for XX-biased Autoimmune Disease



Libert et al, NRI, 2010

X-chromosome number affects lupus & Sjogren's Syndrome susceptibility

Female (XX)	Klinefelter Male (XXY)	Trisomy X female (XXX)	Turner Female (XO)
			
1 in 1000 women (USA)	14-fold higher than males	2.5-fold higher than females	Low risk

- The number of X chromosomes that an individual has will influence their susceptibility for developing the autoimmune disorder Lupus or Sjogrens sydrome.
- 85% of lupus patients are female
- Individuals with Klinefelter's syndrome, who have 2 X-chromosomes and Y, have a 14-fold higher risk than males.
- Triple X females, with 3 copies of an X, have higher risk to develop these autoimmune disorders compared to XX females.
- Turner's syndrome females, who have just 1 X-chromosome, do ***not*** develop lupus.
- Individuals that have more than 1 X-chromosome are predicted to have more expression of genes from the X-chromosome.
- Because the X-chromosome has so many immunity related genes, females are predicted to have higher expression of these genes?

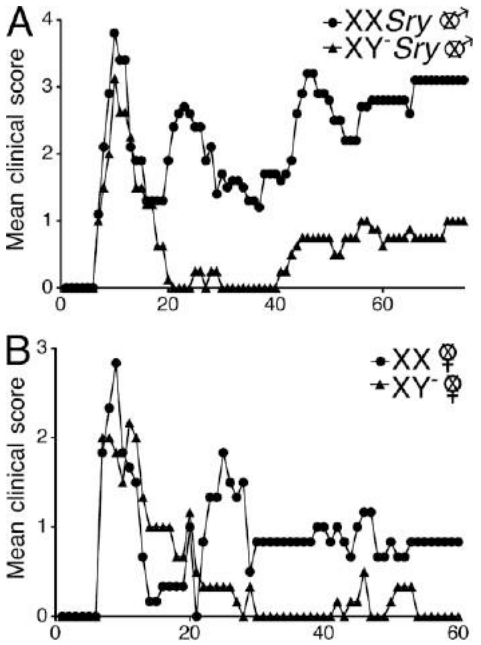
Slae, et al. *Semin in Arth Rhem* 2014
Liu, K et al. *Arthritis Rheumatol*. 2016

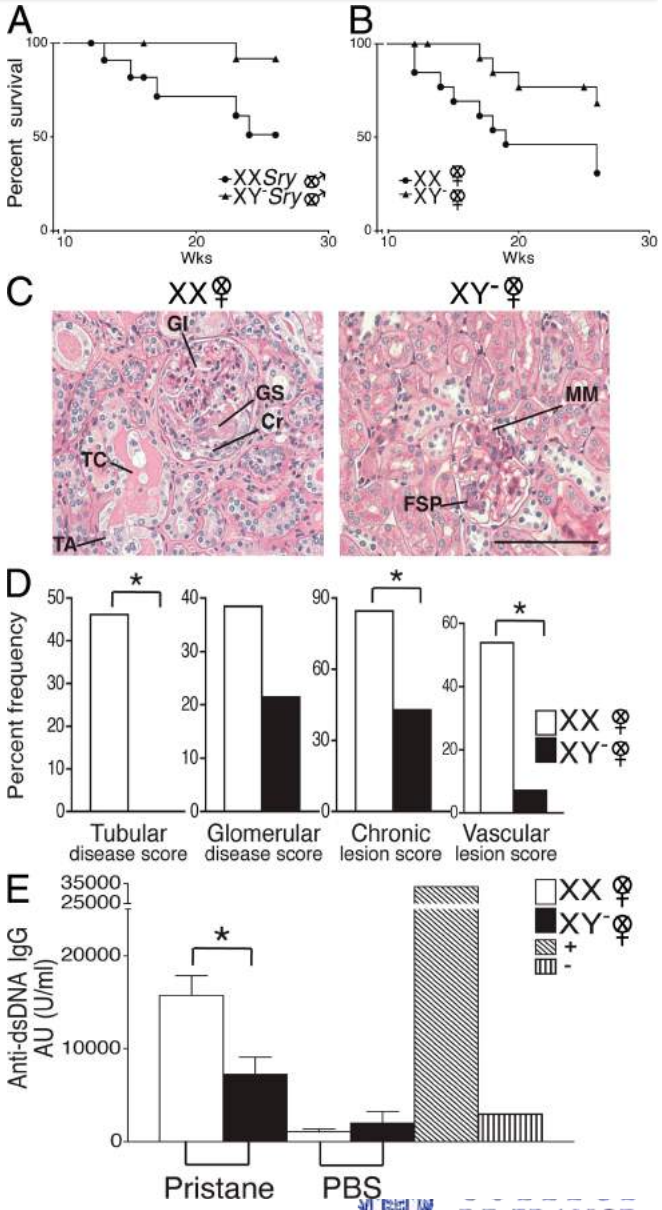
Courtesy of Montserrat Angeura

X-chromosome number affects lupus & Sjogren's Syndrome susceptibility: test this with mouse model

A role for sex chromosome complement in the female bias in autoimmune disease

Deborah L. Smith-Bouvier,[1] Anagha A. Divekar,[3] Manda Sasidhar,[1] Sienmi Du,[1] Seema K. Tiwari-Woodruff,[1] Jennifer K. King,[3] Arthur P. Arnold,[2] Ram Raj Singh,[3,4] and Rhonda R. Voskuhl[1]





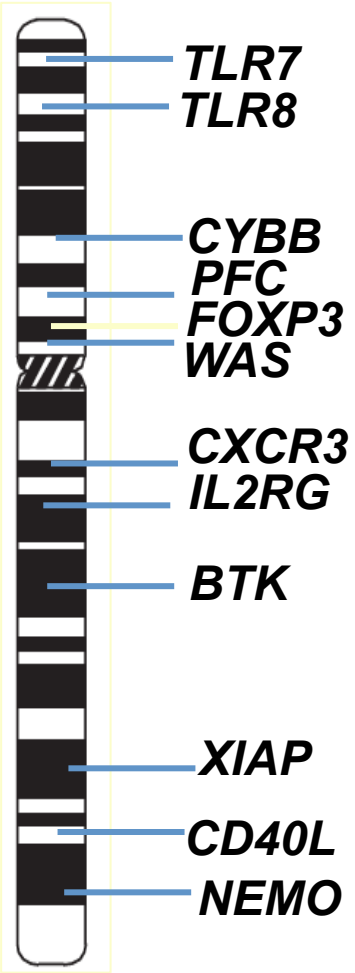
- The XX sex chromosome complement, as compared with the XY−, confers greater disease severity to active EAE.
- Active EAE was induced in castrated XX*Sry* and XY⁻*Sry* male mice with autoantigen PLPctive
- EAE was induced in ovariectomized XX and XY⁻ female mice with autoantigen PLP.
- Clinical disease course was more severe in ovariectomized female XX mice compared with XY⁻ mice.

Slae, et al. *Semin in Arth Rhem* 2014
Liu, K et al. *Arthritis Rheumatol*. 2016

Courtesy of Montserrat Angeura

The X-chromosome has the highest density of immune-related genes

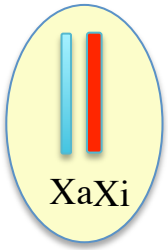
- The X chromosome has the highest density of immune-related genes among all the chromosomes.
- Therefore critical that these genes are regulated properly, especially in cells of the immune system?
- In the autoimmune disorder lupus higher expression levels have been observed for some of these genes
- This change in expression alters the cells behavior, and in the case of lupus, this change is associated with more severe disease phenotypes.



Courtesy of Montserrat Angeura

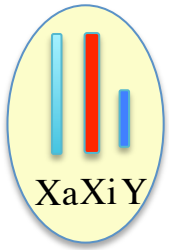
X-chromosome dosage affects SLE & Sjogren's Syndrome susceptibility

Female
(XX)



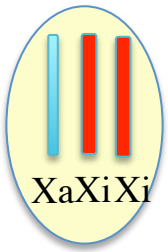
1 in 1000
women (USA)

Klinefelter Male
(XXY)



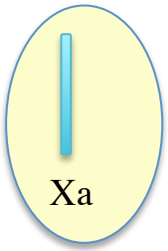
14-fold higher
than males

Trisomy X female
(XXX)

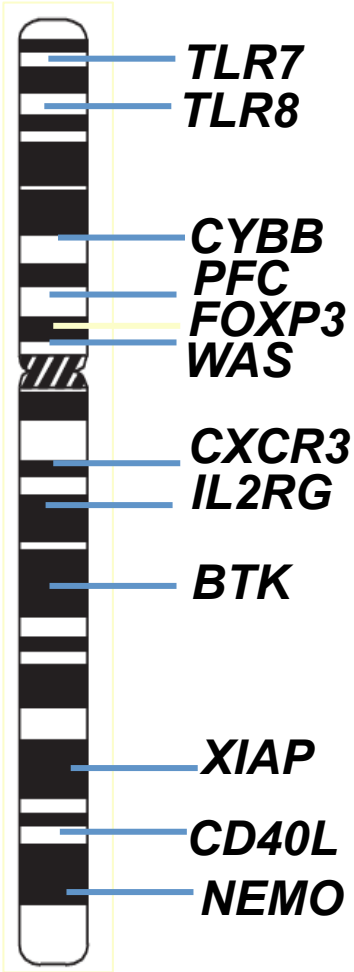


2.5-fold higher
than females

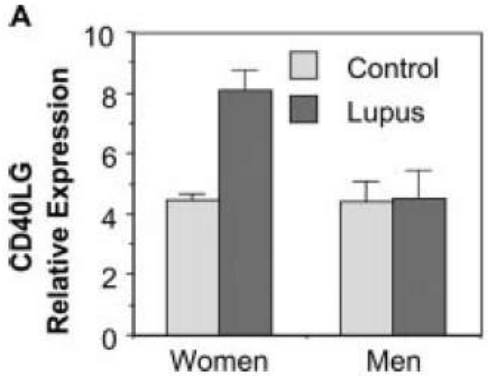
Turner Female
(XO)



Low risk



CD40LG expression in SLE



Lu et al *J Immun*. 2007

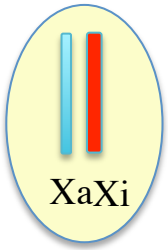
- Female lupus patients have higher levels of CD40LG compared to healthy females, and also male lupus patients.
- At the molecular level, could this increased expression come from the inactive X?

Slae, et al. *Semin in Arth Rhem* 2014
Liu, K et al. *Arthritis Rheumatol*. 2016

Courtesy of Montserrat Angeura

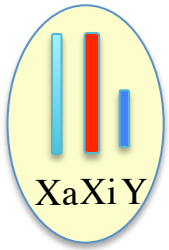
X-chromosome dosage affects SLE & Sjogren's Syndrome susceptibility

Female
(XX)



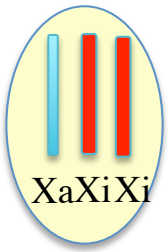
1 in 1000
women (USA)

Klinefelter Male
(XXY)



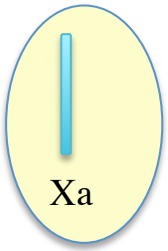
14-fold higher
than males

Trisomy X female
(XXX)

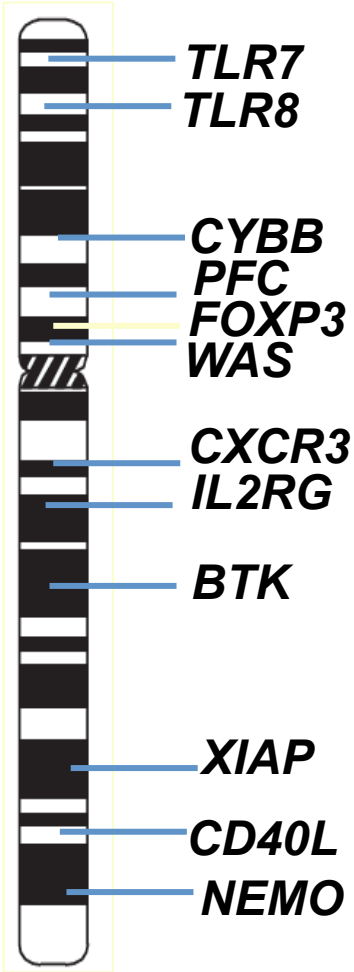


2.5-fold higher
than females

Turner Female
(XO)



Low risk



- BXSB-Yaa mouse model for lupus.
- Mice develop lupus like symptoms, but only the *males*.
- In 2006, discovery that this strain has a translocation of the X-linked TLR7 gene region on the Y chromosome.

⇒ male animals have **double the dosage of TLR7**

⇒ 100% of male animals will develop lupus like symptoms.

BXSB-Yaa lupus model

Tlr7 translocation on Y-chr.

Subramanian et al *PNAS* 2006

Slae, et al. *Semin in Arth Rhem* 2014
Liu, K et al. *Arthritis Rheumatol.* 2016

Courtesy of Montserrat Angeura

Increased expression of the X-linked *TLR7* gene leads to SLE

Autoreactive B Cell Responses to RNA-Related Antigens Due to *TLR7* Gene Duplication

Prapaporn Pisitkun,[1] Jonathan A. Deane,[1] Michael J. Difilippantonio,[2] Tatyana Tarasenko,[1]
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A *Tlr7* translocation accelerates systemic autoimmunity in murine lupus

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Control of Toll-like Receptor 7 Expression Is Essential to Restrict Autoimmunity and Dendritic Cell Proliferation

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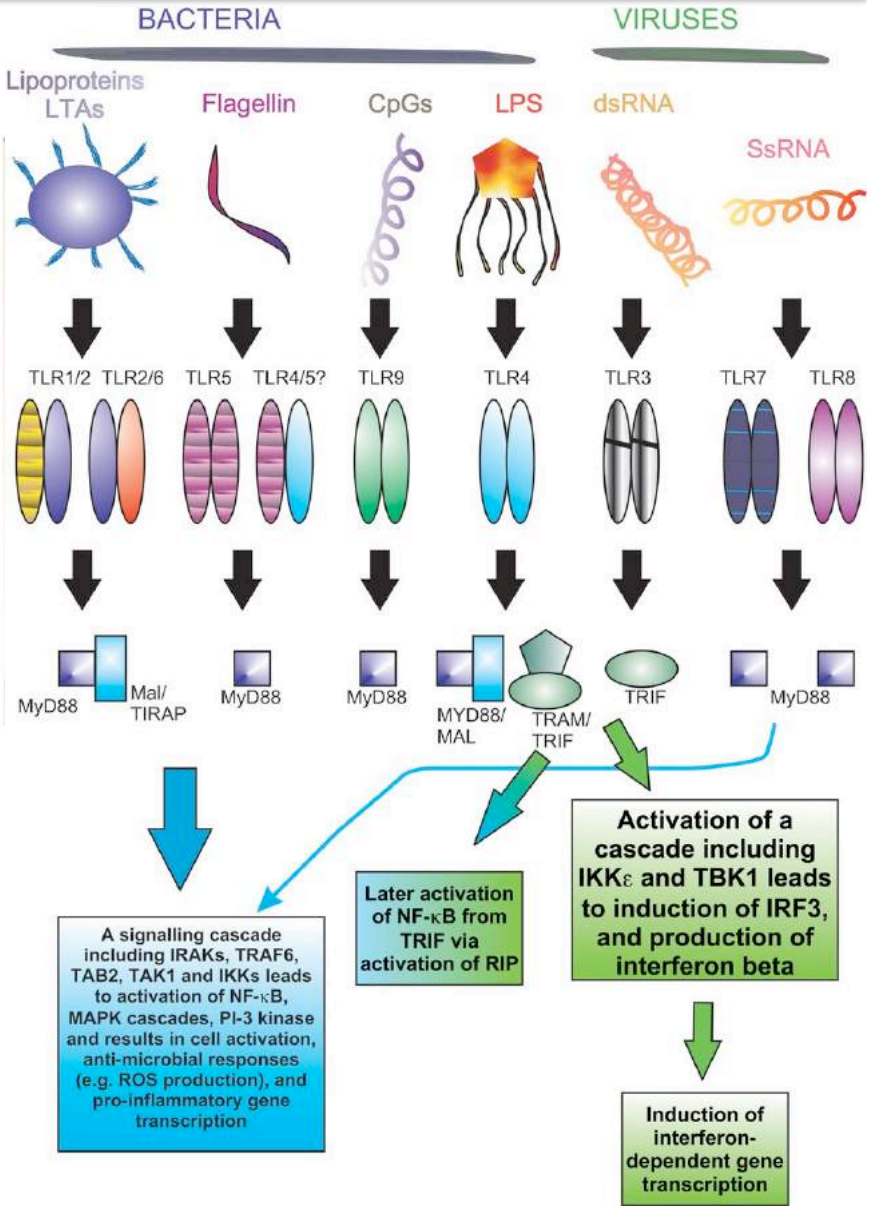
Toll-like Receptors (TLRs)

- Toll-like receptors (TLRs) are Immune receptors expressed on the membranes of leukocytes (sentinel cells such as dendritic cells, macrophages, natural killer cells, cells of the adaptive immunity (T and B lymphocytes)
- Also present on non-immune cells (epithelial and endothelial cells, and fibroblasts)
- Named due to similarity to *toll* gene identified in Drosophila in 1985 by C. Nüsslein-Volhard.
- They recognize structurally conserved molecules, from microbes (proteins, peptides, nucleic acids…).
- If microbes breach physical barriers such as skin, they are recognized by TLRs, which activate immune cell responses.
- Ligands that bind to TLRs = invasive moieties during **infection**, or adjuvant **used in vaccinations**
- Natural ligands include bacterial cell-surface lipopolysaccharides (LPS), lipoproteins, lipopeptides, and lipoarabinomannan; proteins such as flagellin from bacterial flagella; double-stranded RNA of viruses; the unmethylated CpG islands of bacterial and viral DNA; CpG islands found in the promoters of eukaryotic DNA; as well as certain other RNA and DNA molecules.



E. Heard, February 26h, 2018

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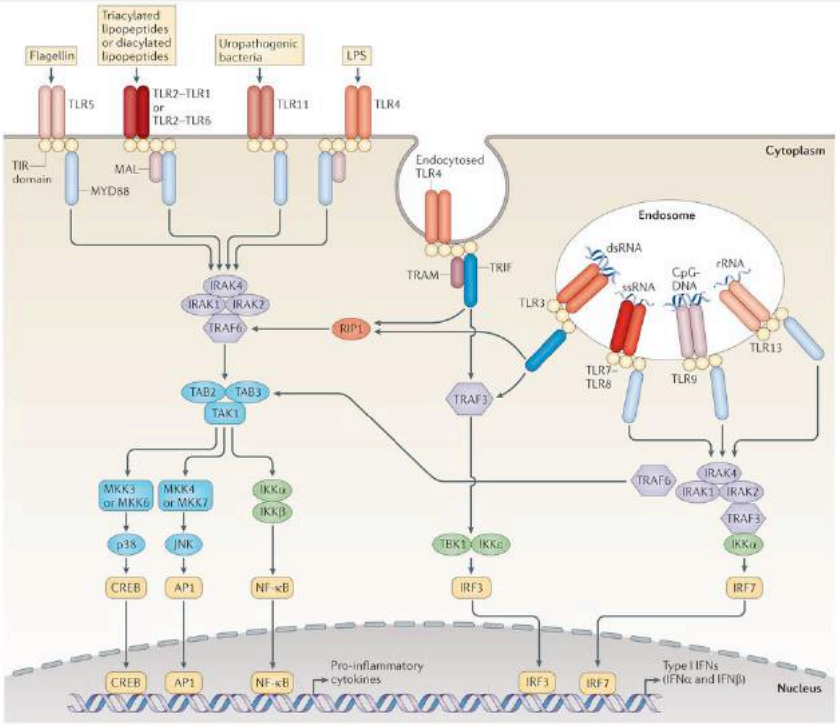
- Ligands that bind to TLRs = invasive moieties during **infection**, or adjuvant **used in vaccinations**
- The stereotypic inflammatory responses normally provoked by TLR activation led to proposal that **endogenous activators** of TLRs might participate in **autoimmune diseases**.
- TLRs may bind "**host"** ligand s including **self DNA or RNA** (endogenous ligands are usually produced as a result of non-physiological cell death – and are normally degraded by nucleases, but can form a complex with endogenous proteins, become resistant to these nucleases and gain access to endosomal TLRs as TLR7 or TLR9).



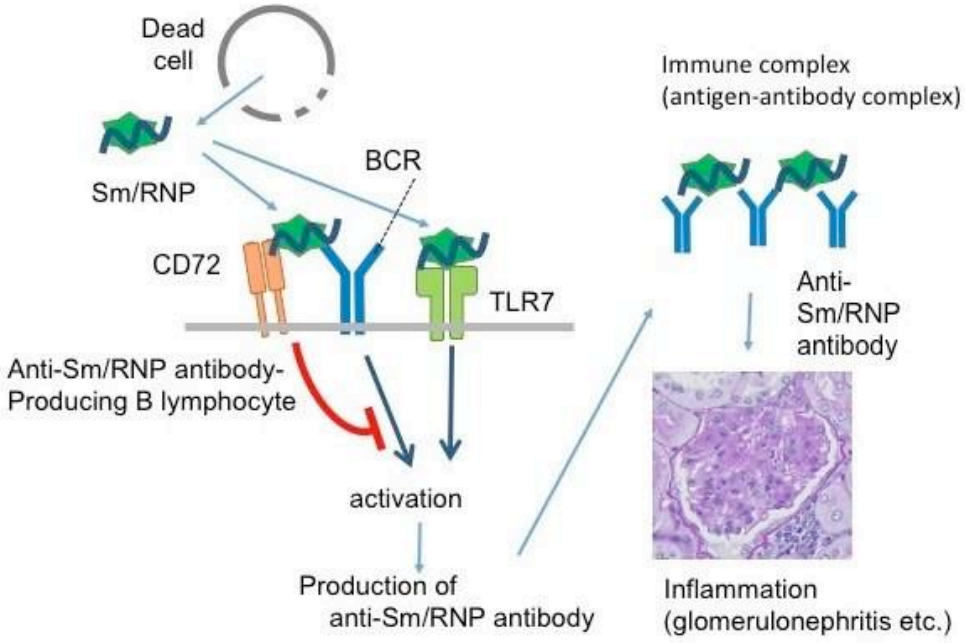
O'Neill et al. Nature Reviews Immunology (2013)

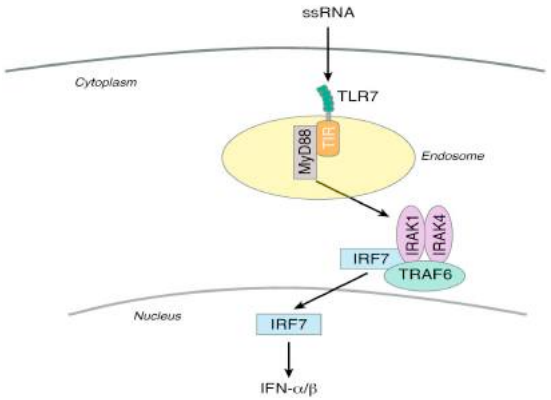
- Ligand binding triggers the key molecular events that ultimately lead to innate immune responses AND development of antigen-specific acquired immunity.
- Upon activation, TLRs recruit adapter proteins (proteins that mediate other protein-protein interactions) within the cytosol of the immune cell in order to propagate the antigen-induced signal transduction pathway.
- Toll-like receptors provide important links between innate and adaptive immunity via dendritic cells.

Toll-like Receptor 7 (TLR7)

- TLR3, TLR7, TLR8 and TLR9 recognize nucleic acid derived from viruses as well as endogenous nucleic acids in context of pathogenic events. Activation of these receptor leads to production of inflammatory cytokines as well as type I interferons (interferon type I) to help fight viral infection.
- Mainly associated with recognition of ss RNA (bacterial and viral) – also reactivated endogenous retroviruses (ERVs)
- TLR7 is required for the production of autoantibodies to ribonucleoproteins in SLE and TLR7-dependent B cell responses are TLR7 dose–sensitive

⇒ **TLR7 is involved in the pathogenesis of autoimmune disorders such as Systemic Lupus Erythematosus (SLE) as well as in the regulation of antiviral immunity.**

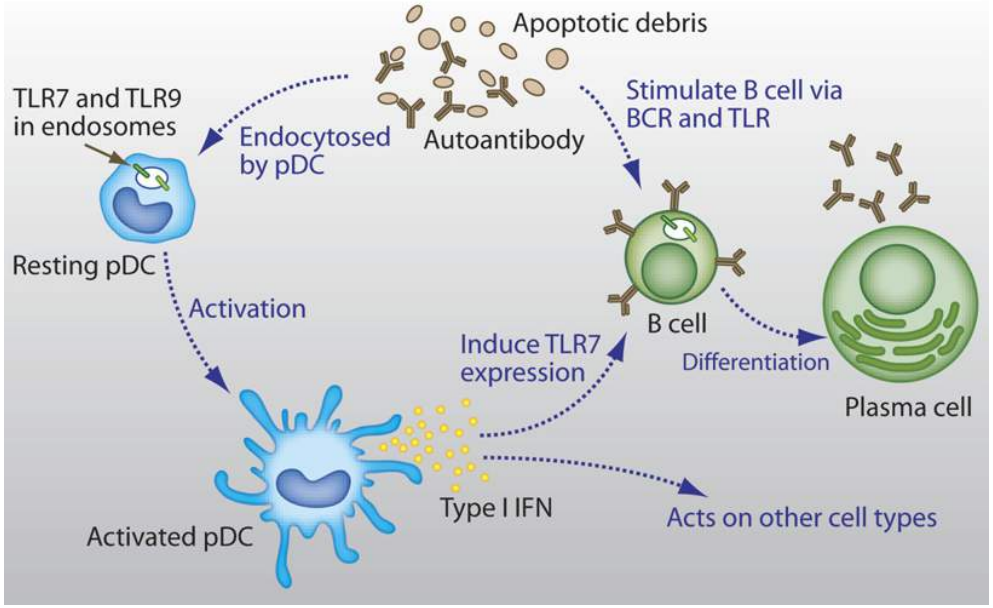




Funk et al Journal of Translational Medicine201412:129

Toll-like Receptor 7 (TLR7) in SLE

- Toll-like receptor 7 (TLR7) detects viral RNA, but can be activated inappropriately by self-RNA => autoimmunity.
- Tlr7 dosage directly related to risk of lupus in mouse model
- TLR7 Activation by Self-RNA Initiates the Type I IFN Cascades, leading to systemic autoimmunity apoptotic blebs, which are enriched in RNA and DNA and their associated proteins, are usually thought to be cleared by macrophages and are not normally endocytosed in large numbers by pDC.
- However, in settings where there is reduced clearance of apoptotic debris, or if pDCs express increased amounts of TLR7, then the amount of endocytosed RNA may suffice to trigger TLR7, inducing secretion of type I IFN.
- Also – in presence of autoantibodies of appropriate specificity, the blebs form immune complexes that are readily taken up by pDC, providing a strong *stimulatory* signal.
- Type I IFN promote autoimmunity in multiple ways, including inducing B cells to express increased TLR7, which may provide a positive-feedback loop exacerbating autoimmunity (Bekeredjian-Ding et al., 2005).
- As a result of the synergy between the B cell receptor and TLRs, B cells specific for RNA- or DNA-associated antigens can get both stimulatory signals and be preferentially stimulated to differentiate into plasma cells.
- Deane et al. (2007) showed that even relatively modest increases in the expression of TLR7 can result in dramatic lymphocyte activation and autoimmunity.



Increased expression of the X-linked *TLR7* gene leads to SLE

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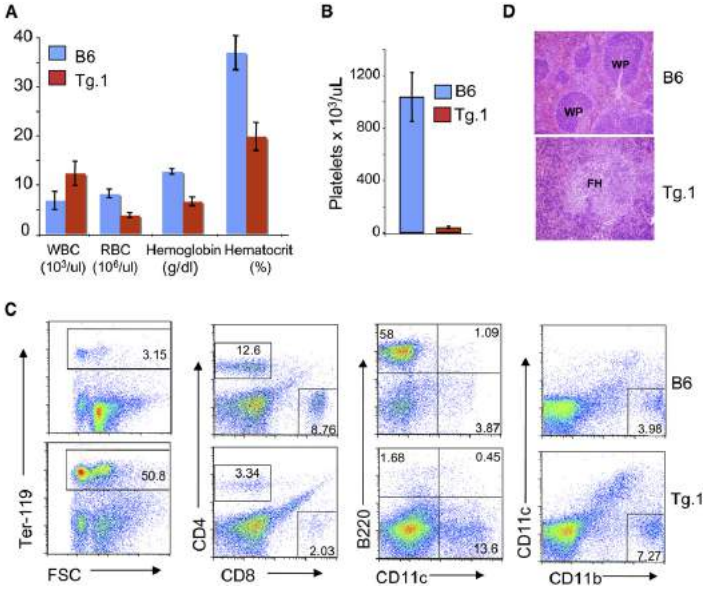
- Spontaneous autoimmunity develops beyond a 2-fold increase in TLR7 expression.
- A *modest* increase in Tlr7 gene dosage promotes autoreactive lymphocytes with RNA specificities and myeloid cell proliferation
- A *substantial* increase in TLR7 expression caused fatal acute inflammatory
- pathology and profound dendritic cell dysregulation.

⇒ **Importance of tightly regulating expression of TLR7 to prevent spontaneous triggering of harmful autoreactive and inflammatory responses.**

TLR7 Overexpression and Anemic Disease



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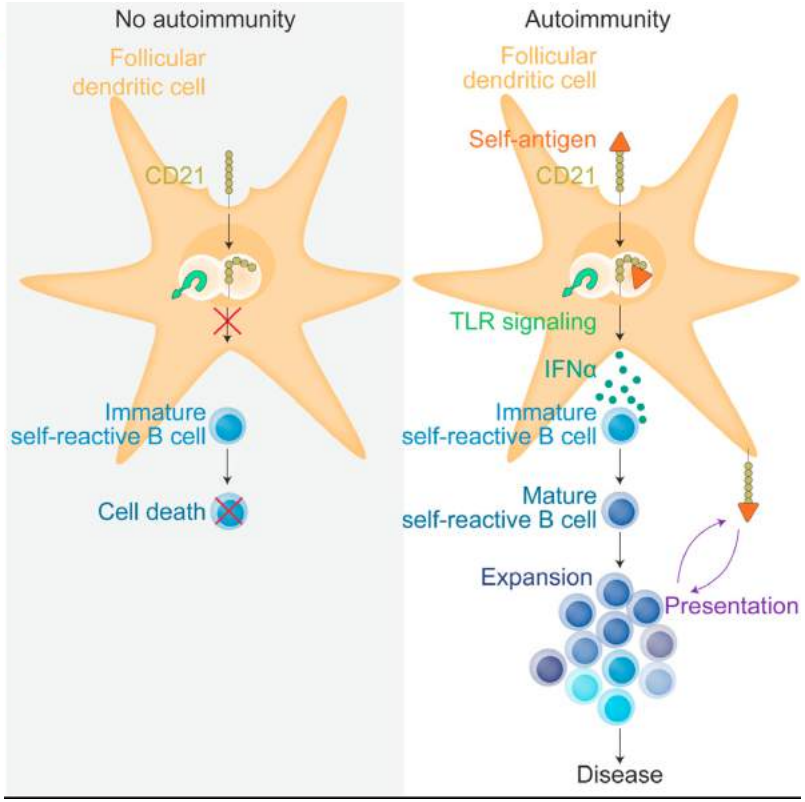
Follicular Dendritic Cells retain Self Antigens, Complement and Auto-Antibody, triggering endosomal TLR7 and interferon secretion

Follicular Dendritic Cell Activation by TLR Ligands Promotes Autoreactive B Cell Responses

Abhishek Das,[1] Balthasar A. Heesters,[1] Allison Bialas,[1] Joseph O'Flynn,[1] Ian R. Rifkin,[3] Jordi Ochando,[4] Nanette Mittereder,[5] Gianluca Carlesso,[5] Ronald Herbst,[6] and Michael C. Carroll[1,2,7,*]

- Internalization of RNP complexes via CD21 triggers TLR7 and IFN-α in mouse and human FDCs
- GC maintenance and α anti-nuclear antibody production are dependent on TLR7 pathway in FDCs
- Loss of B cell tolerance in RNP-specific lupus mice is IFNAR dependent
- FDCs are an essential source of type I IFN in lupus mice

A hallmark of autoimmunity in murine models of lupus is the formation of germinal centers (GCs) in lymphoid tissues where self-reactive B cells expand and differentiate. In the host response to foreign antigens, follicular dendritic cells (FDCs) maintain GCs through the uptake and cycling of complement-opsonized immune complexes. Here, we examined whether FDCs retain self-antigens and the impact of this process in autoantibody secretion in lupus. We found that FDCs took up and retained self-immune complexes composed of ribonucleotide proteins, autoantibody, and complement. This uptake, mediated through CD21, triggered endosomal TLR7 and led to the secretion of interferon (IFN) α via an IRF5-dependent pathway. Blocking of FDC secretion of IFN-α restored B cell tolerance and reduced the amount of GCs and pathogenic autoantibody. Thus, FDCs are a critical source of the IFN-α driving autoimmunity in this lupus model. This pathway is conserved in humans, suggesting that it may be a viable therapeutic target in systemic lupus erythematosus.



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TLR7 escapes X chromosome inactivation in immune cells

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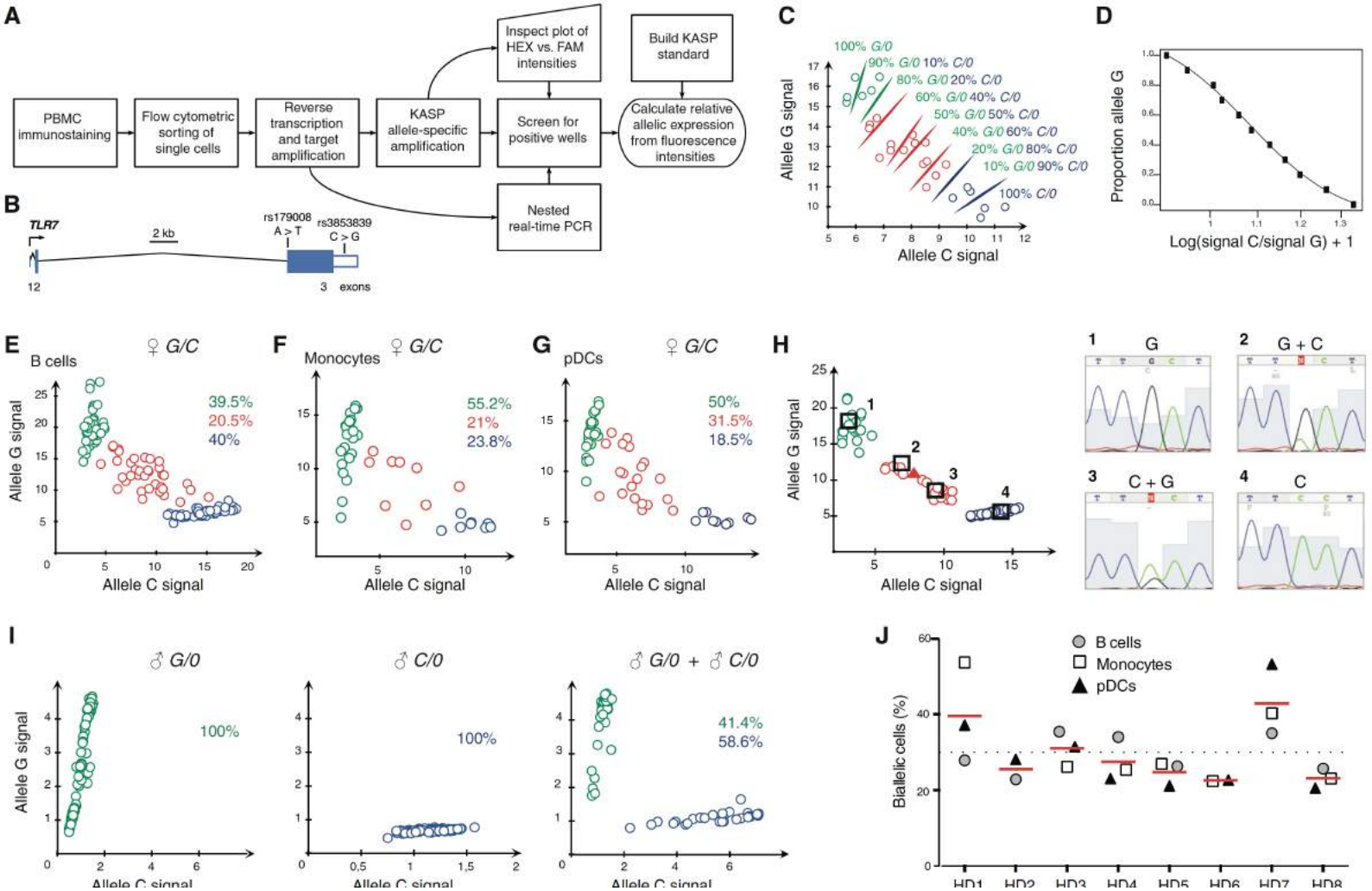
Examine TLR7 allelic expression in primary B lymphocytes, monocytes, and plasmacytoid dendritic cells from 46, XX women, 46, XY and 47, XXY men



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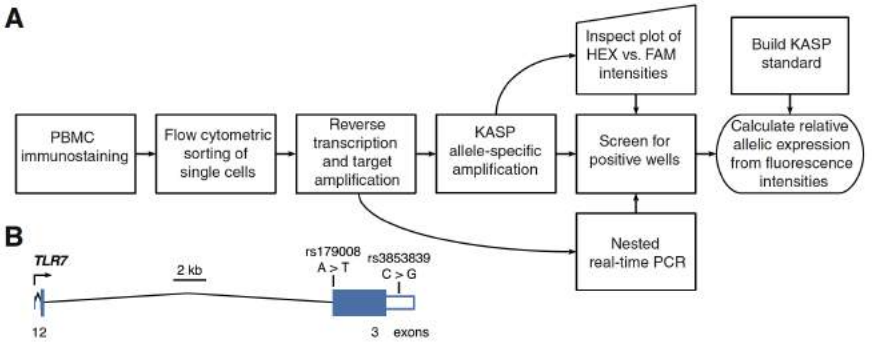

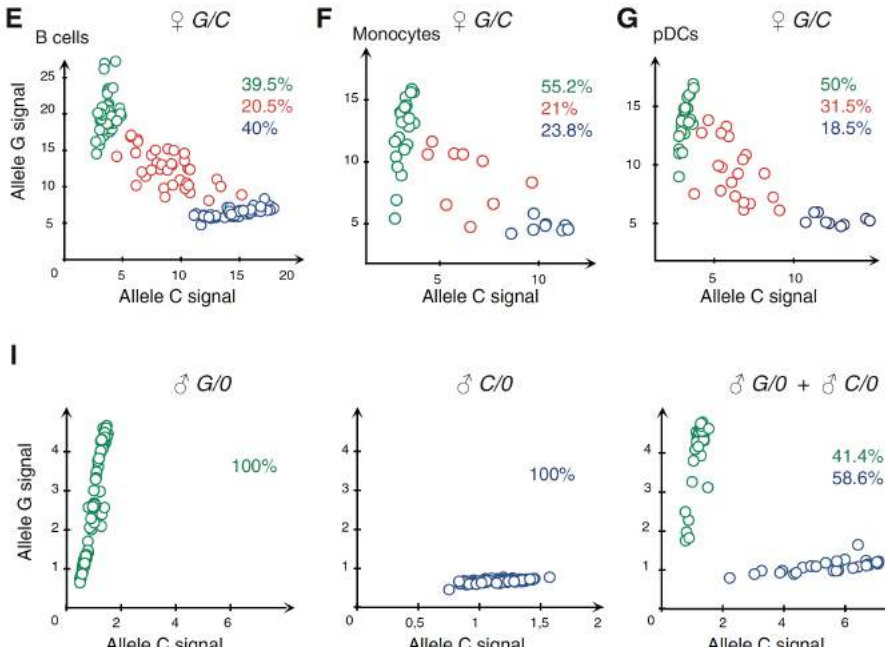

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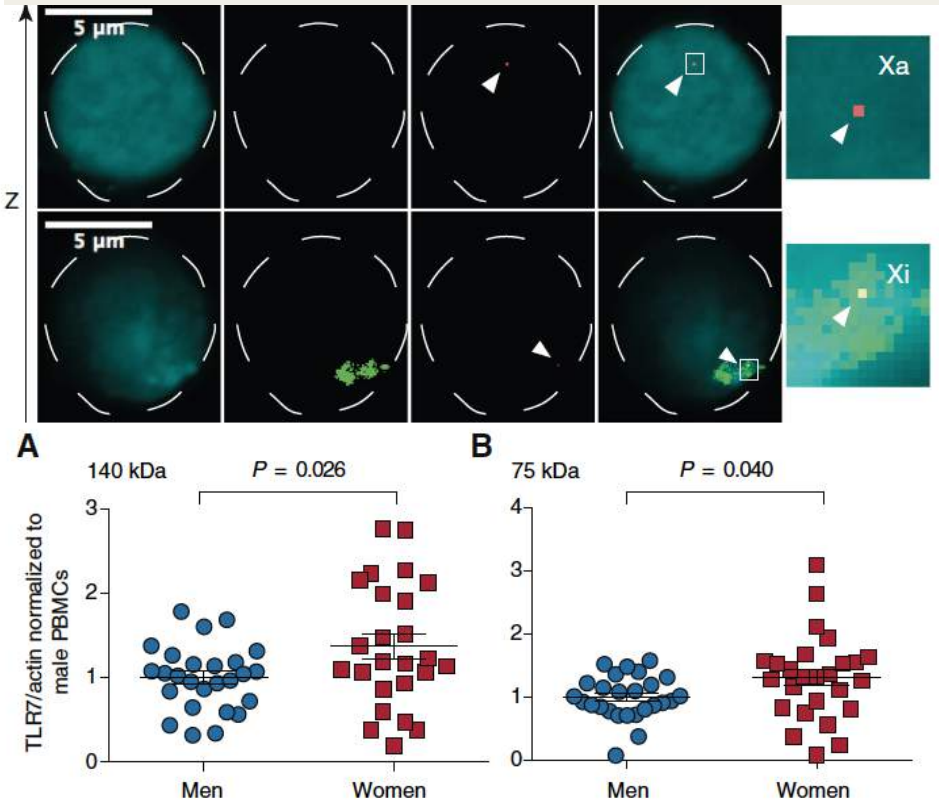

Fig. 4. Enhanced expression of TLR7 protein in PBMCs from women. The TLR7 140-kDa (full-length; **A**) and 75-kDa (proteolytically mature; **B**) forms of the protein were quantitated separately and normalized to β-actin in three Western blot experiments on nonstimulated PBMCs. Cells from 26 males and 26 females were analyzed, and the measurements for female cells were normalized to the mean for male cells in each experiment. The donors were all hemizygous or homozygous for the major alleles of the exonic *TLR7* SNPs, namely, allele A of rs179008 and allele C of rs3853839. *P* values from Student's *t* test.

TLR7 escapes X-Chromosome Inactivation

***TLR7* escapes X chromosome inactivation in immune cells**

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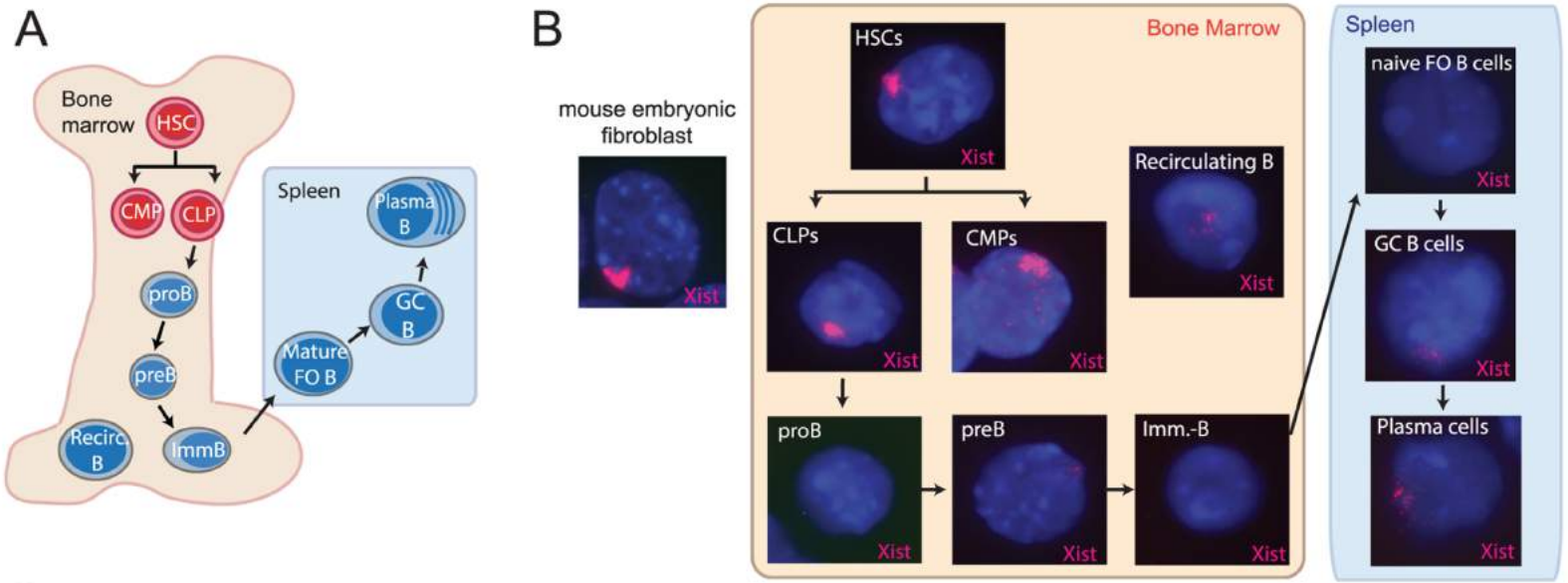
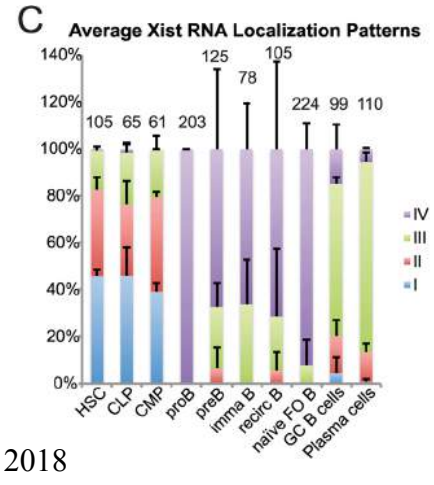
- TLR7 increased in women and XXY men, owing to consistent escape (expressed from Xi) in high % (>30%) primary B lymphocytes, monocytes, and plasmacytoid dendritic cells (pDCs)
- Biallelic B lymphocytes from women show greater TLR7 transcriptional expression than the monoallelic cells, correlated with **higher TLR7 protein** in female vs male leukocytes
- Find enhanced TLR7-driven responses of biallelic female B cells during key stages of effector B cell development.
- Supports the hypothesis that TLR7 overexpression through biallelismis is a candidate contributor to the risk of SLE not only in women and also in 47,XXY men
- See substantial intra-individual stability, however whether variation in escape and TLR7 expression levels occurs during physiological processes, age, genetics, and the environment remains to be examined

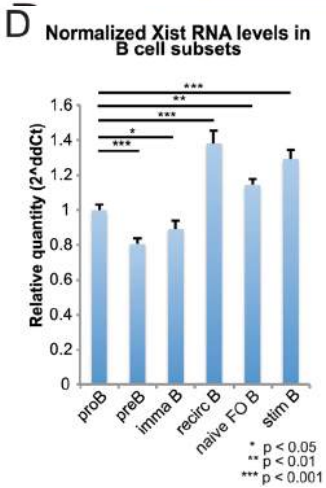
How inactive is the X chromosome in XX immune cells…?

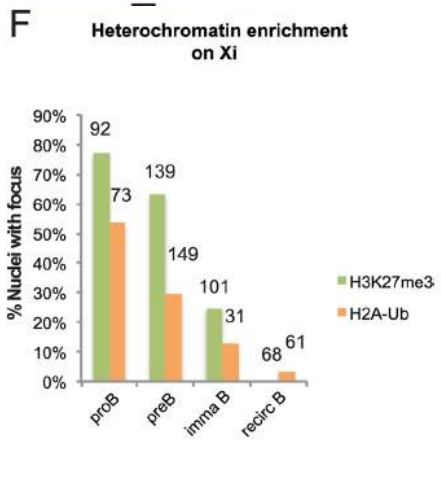
Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells

Unusual maintenance of X chromosome inactivation predisposes female lymphocytes for increased expression from the inactive X









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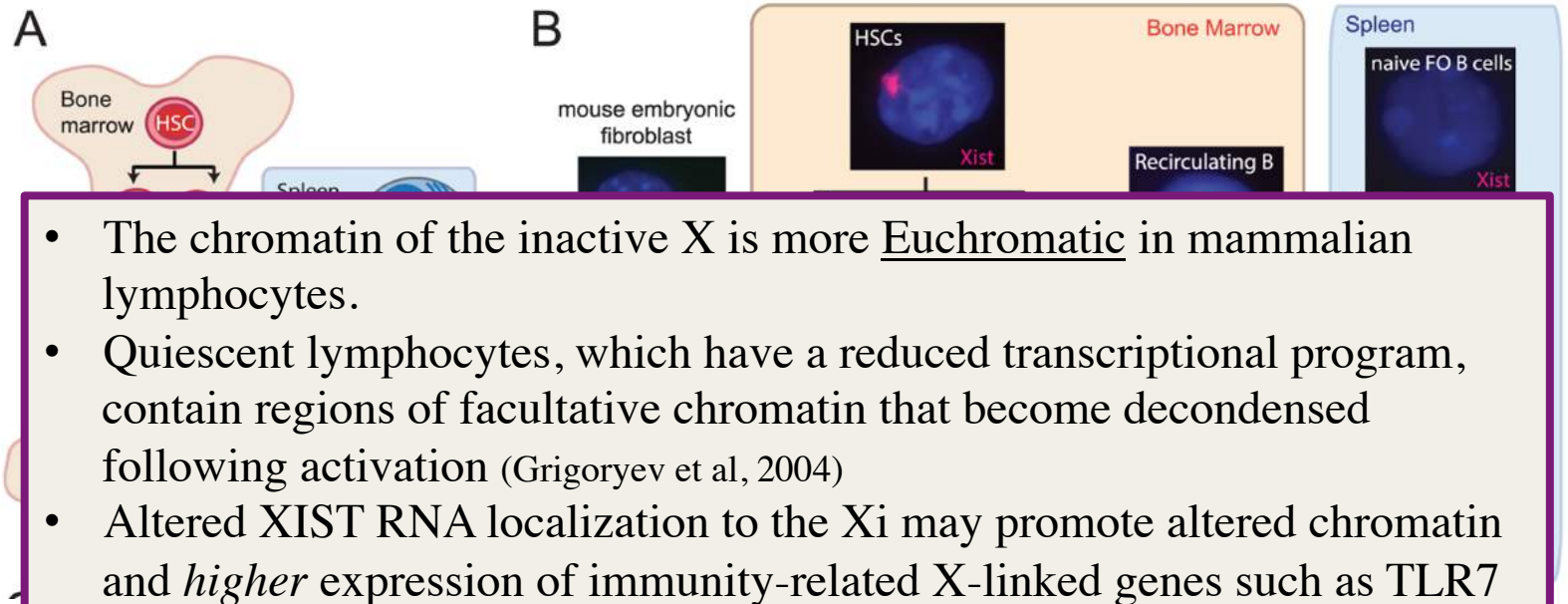


- The chromatin of the inactive X is more Euchromatic in mammalian lymphocytes.
- Quiescent lymphocytes, which have a reduced transcriptional program, contain regions of facultative chromatin that become decondensed following activation (Grigoryev et al, 2004)
- Altered XIST RNA localization to the Xi may promote altered chromatin and *higher* expression of immunity-related X-linked genes such as TLR7 and CD40LG (ie higher escape rates) – ***if this escape is not tightly regulated it can lead to autoimmune disease?***

Remarkable dynamics of X-linked gene expression in females due to X inactivation plasticity in some adult lineages…

Sex and gender exert wide-ranging effects on human health

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

Reasons that led to medical research mainly focused on males:

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

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

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

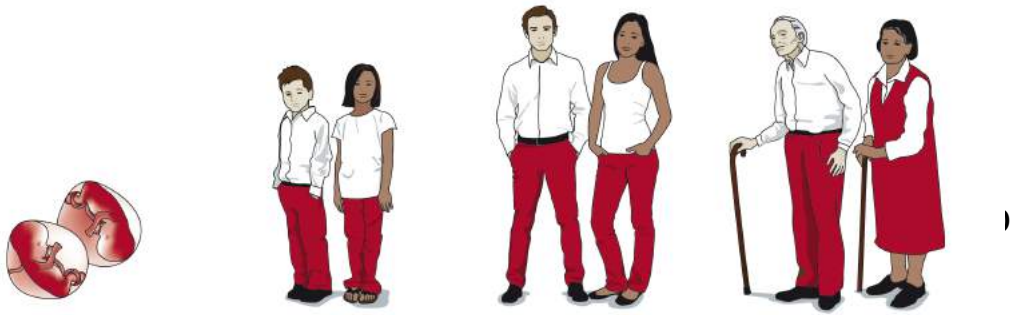
(iv) Clinical trials designed to avoid women in reproductive age groups due to potential risk of pregnancy

Sex Bias in Disease and its Treatment

- Most clinical trials in the past carried out only on men – medication doses were typically adjusted for patient size *and women were simply "small men"*

- Why have females been excluded from drug trials? The 1977 US Food and Drug Administration (FDA) guidelines advised that women of childbearing potential be excluded from drug trials

- Personalized medicine to define targets for more effective prevention and treatment of disease: sex clearly influences innate and adaptive immune responses, in the context of infectious and autoimmune diseases, malignancies, and vaccines.



To achieve effective treatment for all individuals in the era of precision medicine:

Men and women will have to be <u>treated differently,</u>
in order to be protected <u>equally</u>.

E. Heard, February 26h, 2018

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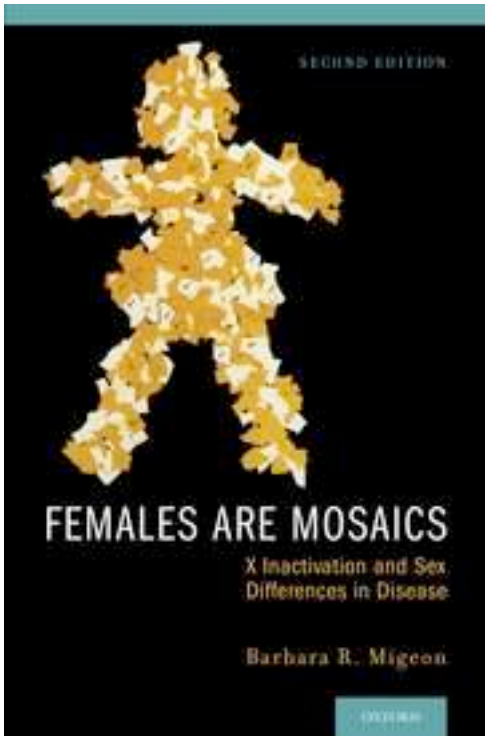
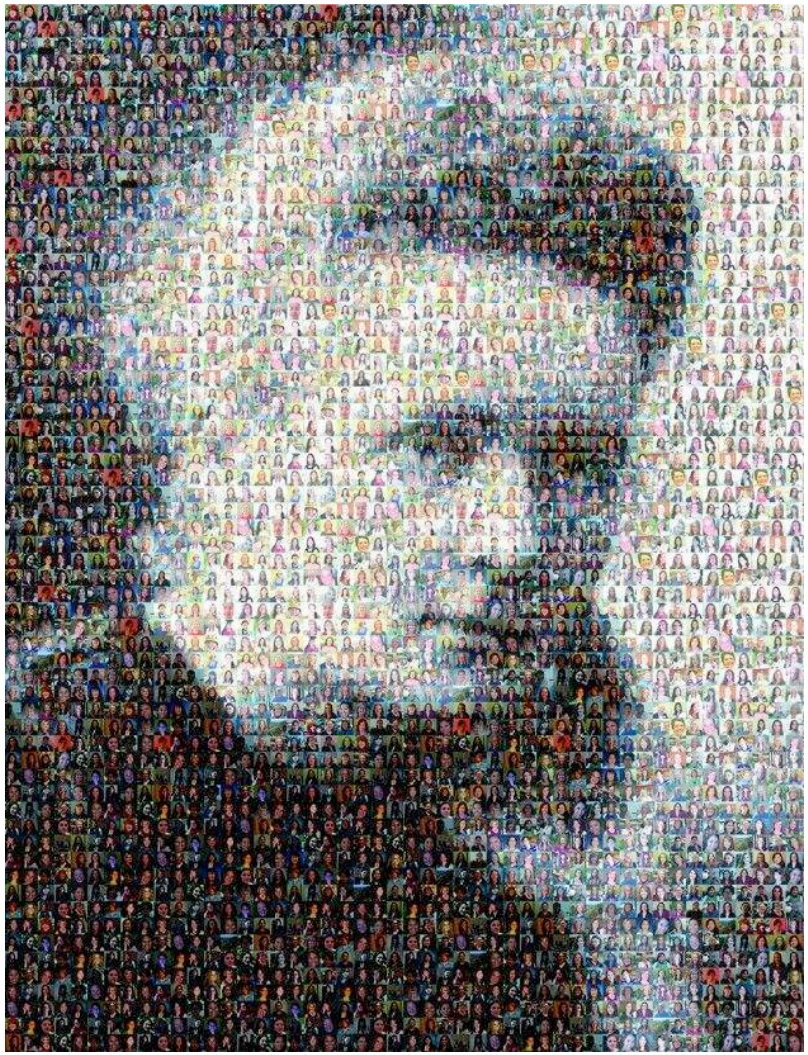
Le chromosome X
paradigme de la génétique et l'épigénetique





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CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

COLLOQUE

14 MAI, 2018

"Le chromosome X - paradigme de la génétique et l'épigénetique"
"The X chromosome: a paradigm of genetics and epigenetics"

Montserrat ANGUERA (USA)
Philip AVNER (Italie)
Christine DISTECHE (USA)
Joost GRIBNAU (Pay Bas)
Gabriel MARAIS (Lyon)
Claire ROUGEULLE (Paris)
James TURNER (UK)
Edda SCHULZ (Allemagne)

Date: Monday 14t May (Lundi 14 mai 2018
Time: 9h-18h
Amphitheatre : Guillaume Budé

E. Heard, February 26h, 2018

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