Swiss Institute of Bioinformatics

Best Practices for Investigating Gene Expression Differences by Biological Sex

Julien Roux, Vidhya Jagannathan

October 27th, 2025

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«Without careful methodology, the pursuit of sex difference research, despite a mandate from funding agencies, will result in a literature of contradiction»

Janet Rich-Edwards

Today's schedule

09:15 – 10:15 Introduction
10:15 – 10:30 Coffee break
10:30 – 12:00 Biological sex in experimental design (Frédéric Schütz)
12:00 – 13:00 Lunch break
13:00 – 15:00 Hands-on session in subgroups
15:00 – 15:30 Coffee break
15:30 – 17:00 Finishing hands-on, wrap up and discussions

- **Questions welcomed anytime**
- **Exam** (0.25 ECTS credits)

Introducing ourselves

Vidhya Jagannathan (she/her)

- MSc: Biology and Diploma in Bioinformatics, M.K.U, India
- PhD: Applied EM Algorithm to TFBS, University of Lausanne, Swiss Institute of Bioinformatics
- Postdoc: hormonal influence in breast cancer tissues using machine learning algorithms
- Now: Senior scientist, Vetsuisse faculty, University of Bern
  - Member, EU-SABV Consortium – exploring sex as a biological variable

Universität Bern | Universität Zürich
vetsuisse-fakultät
UNIVERSITÄT BERN
SIB

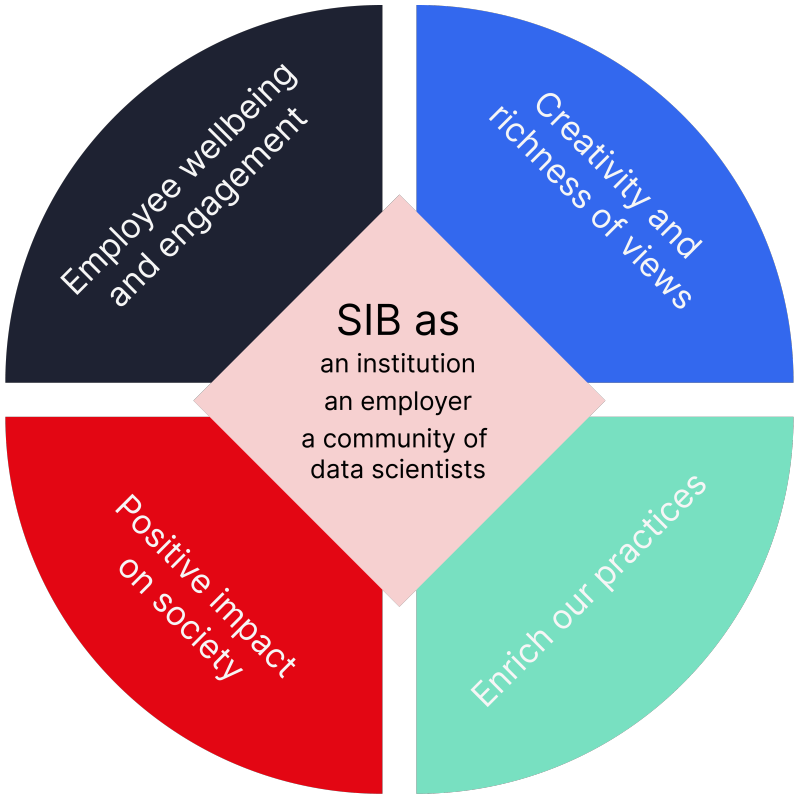
Introducing ourselves

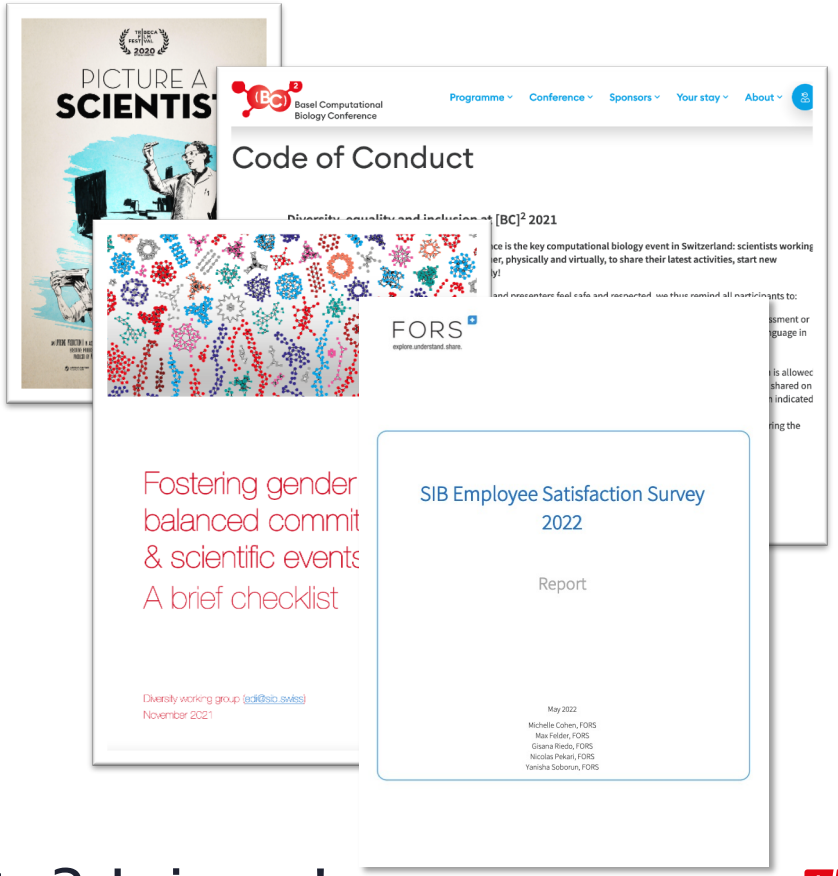
Julien Roux (he/him)

- MSc: Bioinformatics and Modeling, National Institute of Applied Sciences (INSA), Lyon, France
- PhD: Evolutionary genomics, University of Lausanne, Swiss Institute of Bioinformatics
- Postdoc: Evolutionary genomics, University of Chicago
- Now: Senior scientist, Bioinformatics Core Facility, Department of Biomedicine, University of Basel

DBM Basel Department of Biomedicine | Universität Basel | UKBB kompetent & menschlich | Universitätsspital Basel | SIB

The SIB Diversity focus group





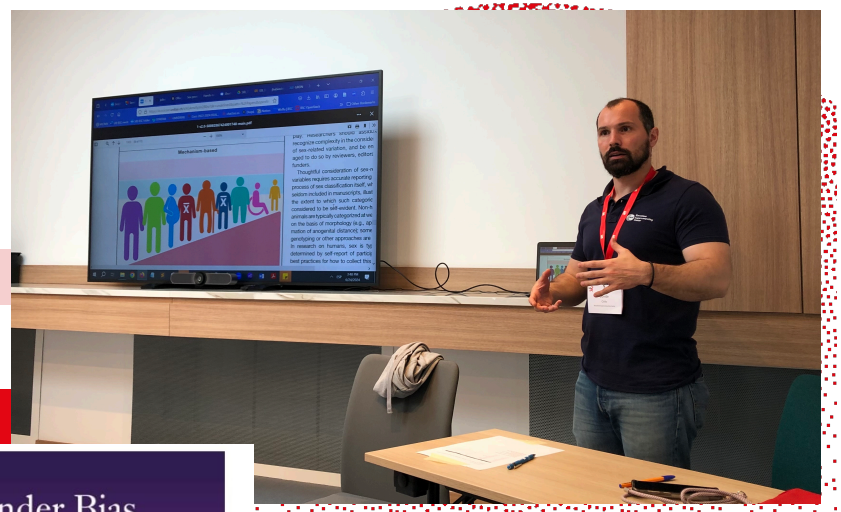
Want to contribute? Join us!

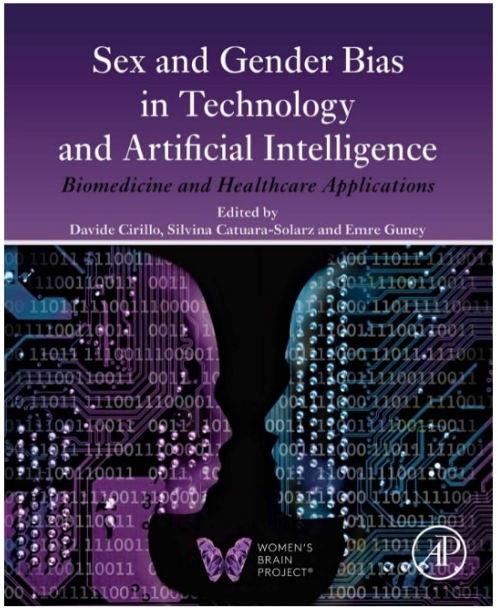
Swiss Institute of Bioinformatics

SIB DAYS – 24 JUNE 2024 – BIEL

Incorporating biologi
variable in the design
of biomedical researc

Workshop from the SIB Diversity focu





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Introducing ourselves

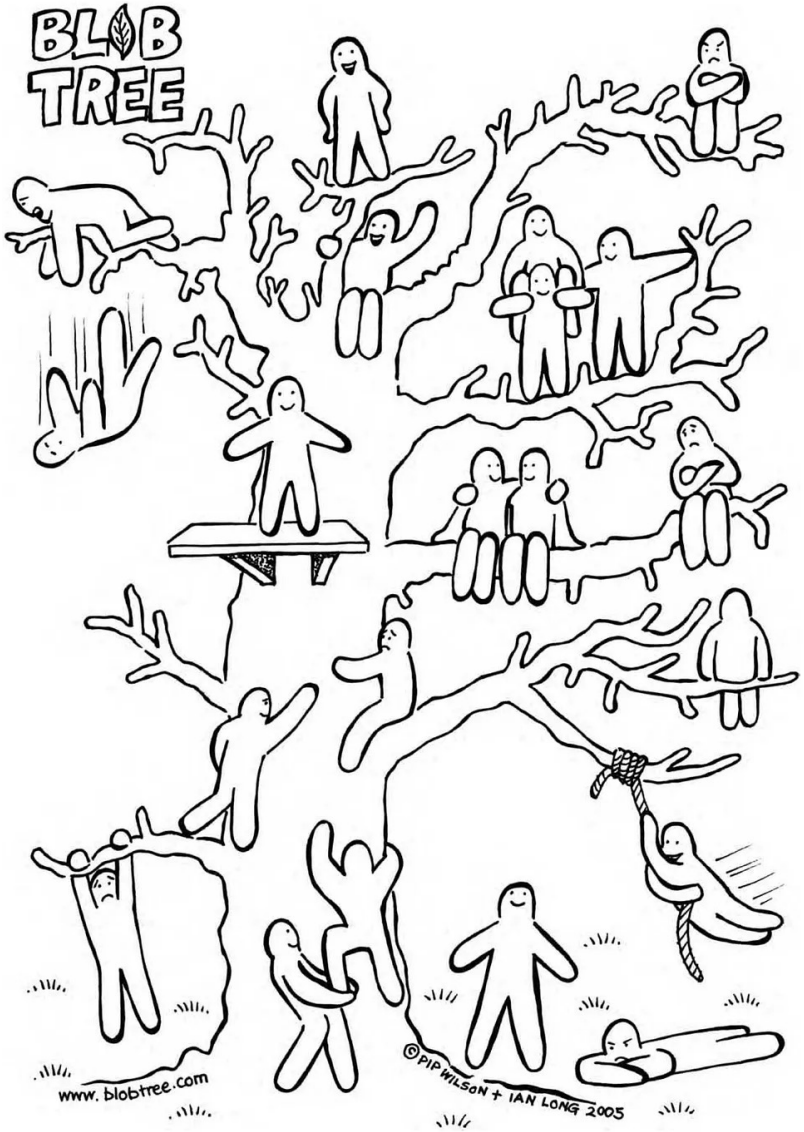
Frédéric Schütz

- Biostatistics platform
- Center for Integrative Genomics
- University of Lausanne

- SIB group leader
- Chair of the Training Advisory Committee

Introducing yourselves

- Your research project(s)
- Expectations from the course?
- How this course could be relevant?
- Involved in design of experiments?
- Experience with bioinformatics? Transcriptomics data analysis with R and Bioconductor?



Code of conduct

SIB abides by the ELIXIR Code of Conduct

- Topic is complex, very oriented and cleaving
- Be respectful, be inclusive, be open to discussion
- Disclaimer: we are not specialist, but willing to learn together

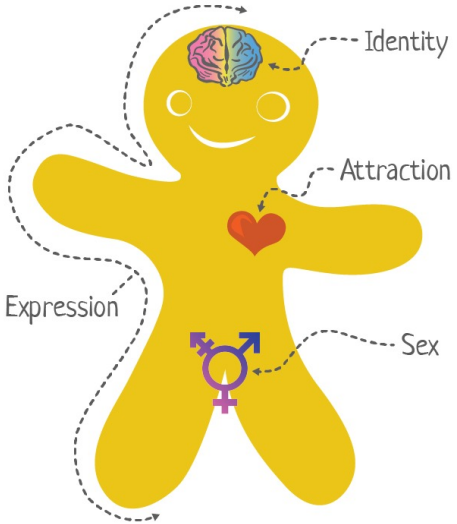
https://elixir-europe.org/events/code-of-conduct
https://api.swiss-academies.ch/site/assets/files/25607/kodex_layout_en_web-1.pdf

What is sex? What is gender?

Sex and gender are often used interchangeably, despite having different meanings

The Genderbread Person v4

by it's pronouncedMETROsexual.com



⊘ means a lack of what's on the right side

Gender Identity
- ⊘ → Woman-ness
- ⊘ → Man-ness

Gender Expression
- ⊘ → Femininity
- ⊘ → Masculinity

Anatomical Sex
- ⊘ → Female-ness
- ⊘ → Male-ness

Identity ≠ Expression ≠ Sex
Gender ≠ Sexual Orientation

Sex Assigned At Birth
☐ Female ☐ Intersex ☐ Male

Sexually Attracted to... and/or (a/o)
- ⊘ → Women a/o Feminine a/o Female People
- ⊘ → Men a/o Masculine a/o Male People

Romantically Attracted to...
- ⊘ → Women a/o Feminine a/o Female People
- ⊘ → Men a/o Masculine a/o Male People

 For a bigger bite, read more at www.genderbread.org

https://www.itspronouncedmetrosexual.com/2018/10/the-genderbread-person-v4/

What is sex? What is gender?

Sex and gender are often used interchangeably, despite having different meanings

Gender is a multidimensional sociocultural construct

Gender

Traditional male dress (Baranya, Hungary, 1920s)

Amazons in battle. Greek relief (4th century BC.
Künsthistorische Museum, Vienna)

Slide courtesy of Davide Cirillo

Gender

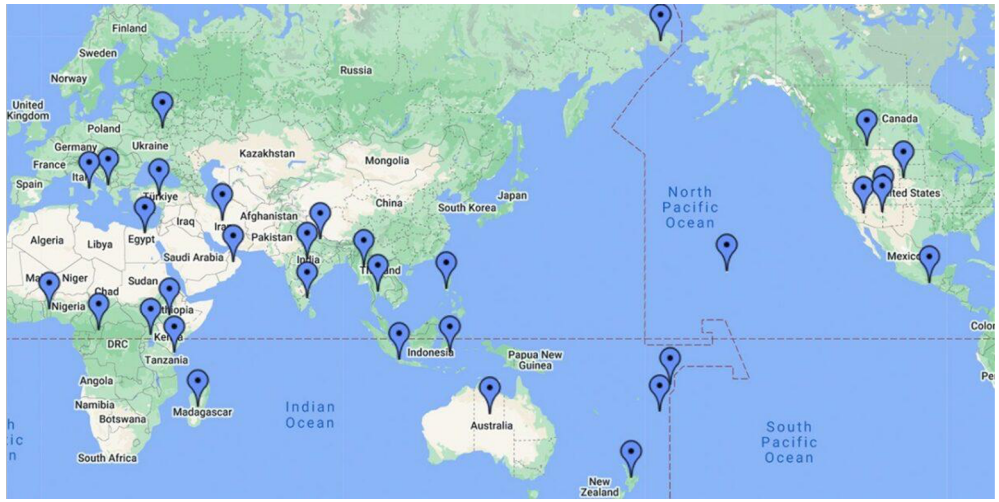
Two-spirit (Indigenous North Americans)

George Catlin, "*Dance to the Berdache*" (1861-1869)

Muxes (Mexico)

Vogue Mexico

Slide courtesy of Davide Cirillo



A map of gender-diverse cultures
https://www.pbs.org/independentlens/content/two-spirits_map-html/

The Gender, Sex, and Sexual Orientation (GSSO) ontology

https://www.ebi.ac.uk/ols4/ontologies/gsso

- gender identity (302)
  - exploring gender identity
  - female gender identity
  - male gender identity
  - no gender identity
  - nonbinary gender identity (294)
    - exclusive gender identity (278)
      - culturally-specific gender identity (274)
      - intersex-specific gender identity (1)
      - neurotype-specific gender identity
    - fluctuating gender identity (2)
    - indescribable gender identity (1)
    - multiple gender identity (3)
    - non-gendered identity (2)
    - overlapping gender identity (1)
    - partial gender identity (2)
  - other gender identity
  - unknown gender identity (1)

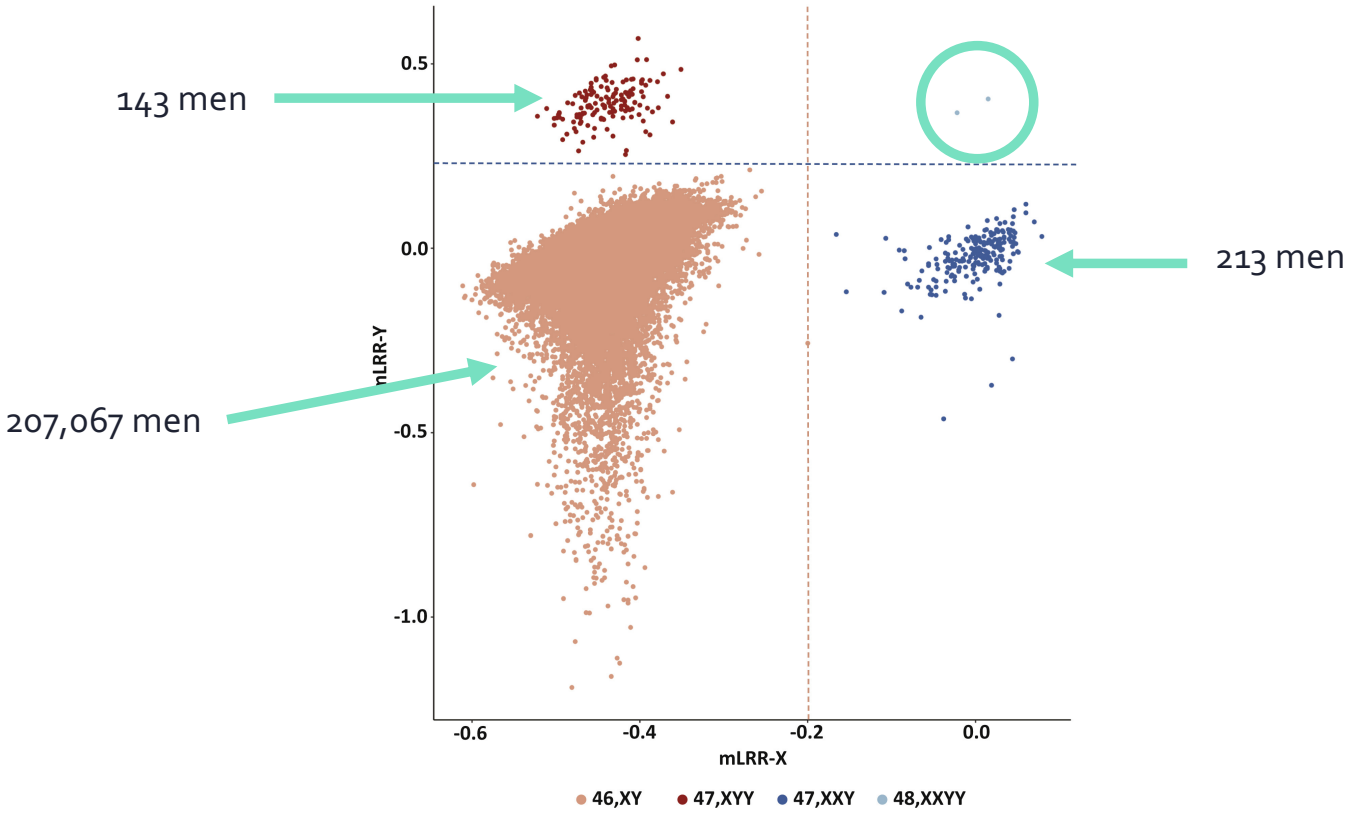
Slide courtesy of Davide Cirillo

Today's focus on biological sex

Why?

- Simpler? (but not simple)
- Quite topical
- Relevant as biologist/bioinformatician
- Relevant as researcher aiming at conducting ethical and responsible research

Sex chromosomes in males in UK Biobank



https://www.sciencedirect.com/science/article/pii/S1098360022007778

Biological sex: definition

Sex refers to a set of **biological attributes** in humans and animals.

It is primarily associated with physical and physiological features including **chromosomes, gene expression, hormone levels and function, and reproductive/sexual anatomy**.

Sex is usually categorised as female or male but **there is variation in the biological attributes** that comprise sex and how those attributes are expressed.

https://cihr-irsc.gc.ca/e/48642.html

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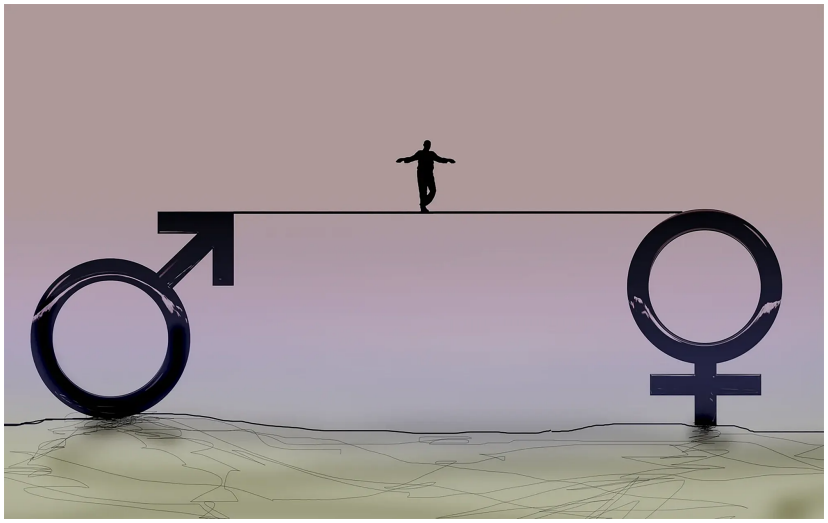
In the news…

- Trump makes 'two sexes' official and scraps DEI policies (links 1 and 2)
  - "Female" means a person belonging, at conception, to the sex that produces the large reproductive cell.
  - "Male" means a person belonging, at conception, to the sex that produces the small reproductive cell.
- Strong reaction from the scientific community
  - Letter from presidents of Society for the Study of Evolution, American Society of Naturalists and Society of Systematic Biologists ("a revised version is in progress", but see link 3)
  - Reaction to the reactions…

- https://www.bbc.com/news/articles/czx84en1yp4o
- https://www.whitehouse.gov/presidential-actions/2025/01/defending-women-from-gender-ideology-extremism-and-restoring-biological-truth-to-the-federal-government/
- https://medium.com/@alysion42/letter-to-the-us-president-and-congress-on-the-scientific-understanding-of-sex-and-gender-992051a60318

Is it so simple?



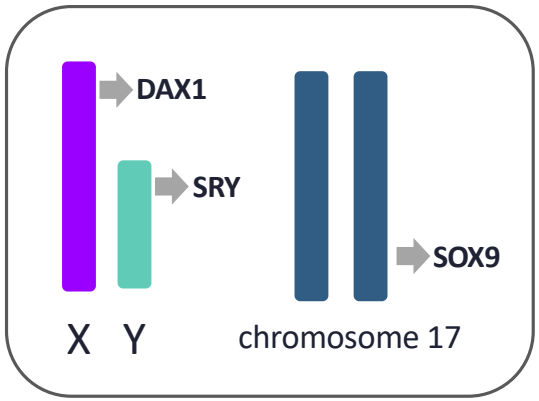
Gary Waters - Getty Images

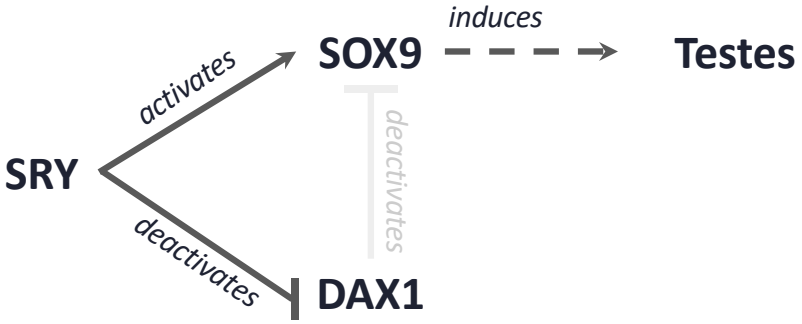
- How to assign sex at birth?
- People with infertility?
- Rare cases of people having both types of reproductive organs and producing both gametes

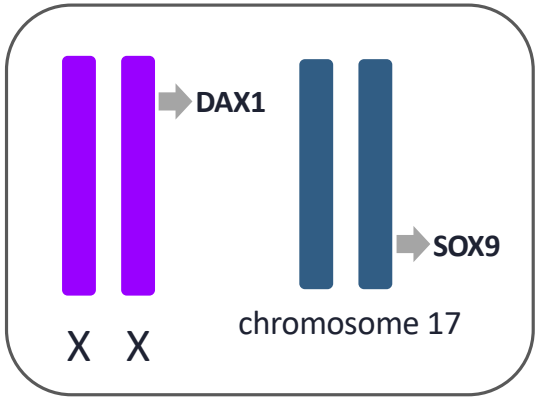
  https://academic.oup.com/bjs/article-abstract/69/5/279/6185759

In the news…

https://www.fis-ski.com/inside-fis/news/2025-26/fis-council-reinforces-commitment-to-athlete-safety
https://worldathletics.org/news/press-releases/sry-gene-test-athletes-female-category







Slide courtesy of Davide Cirillo

Sex screening was used in the past

The IOC used **visual inspections** in the 1960s, but there were concerns they were degrading and invasive, before mandatory **chromosome-based** cheek swab tests were introduced.

There were still issues, however. Spanish hurdler Maria Jose Martinez Patino was barred from competition in 1986 after failing the test, but later examinations showed that while she had XY chromosomes she also had CAIS - meaning her body never gained benefits from the increased testosterone created by her internal testes.

She was reinstated, and the IOC switched to a **SRY gene test**, but amid a number of 'false positives', and fears that female athletes were being **punished for natural variations**, sex verification tests were abolished in the 1990s.

World Athletics insists the new tests are now "extremely accurate and the risk of false negative or positive is extremely unlikely".

https://www.bbc.com/sport/athletics/articles/c9d07jq6vpeo

Is it so simple? (2)

- A male lab technician count contaminate swab samples
- SRY is not *per se* providing an advantage in competition
- Testosterone *is* linked to performance
- Link 2:
  - Between 2000 and 2023, 50-60 athletes *with DSD* competed in finals in athletics
  - "The numbers, which were derived by anti-doping tests that revealed high testosterone levels – and arguably therefore may not capture every case – are significantly higher than many in the sport had expected."
- The test does not tell if SRY is functioning, whether a testis has formed, whether testosterone is produced and, if so, whether it can be used by the body (link 1)
- Genetic testing without medical prescription illegal in some countries
- No genetic counselling to the athletes
- No psychological support if a test is positive

- https://www.mcri.edu.au/news/insights-and-opinions/world-athletics-sry-gene-conversation
- https://www.theguardian.com/sport/2025/sep/19/sex-tests-brought-in-after-data-showed-50-60-dsd-athletes-in-finals-world-athletics-says

Today's focus on biological sex

Why?

- Simpler? (but not simple)
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Relevant as biologist

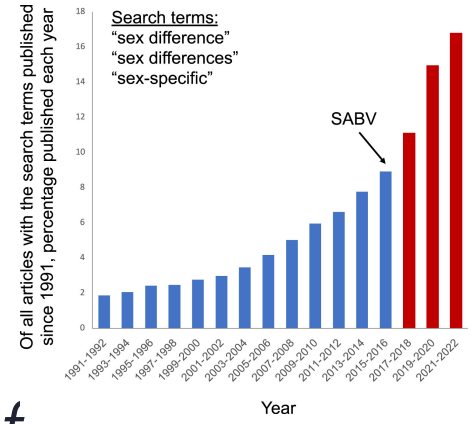
- In animal studies, we usually only talk about biological sex
- Large proportion of mammalian traits influenced by sex
  - https://www.nature.com/articles/ncomms15475
- Sex-differences in tissues
  - https://elifesciences.org/articles/99602
  - https://faseb.onlinelibrary.wiley.com/doi/full/10.1096/fj.14-255000
- Reported sex differences in multiple diseases, e.g.:
  - Sex-specific genetic architecture of human disease https://www.nature.com/articles/nrg2415
  - Neuromuscular disorders https://www.sciencedirect.com/science/article/pii/S0047637423000192
  - Lung disease (COPD) https://www.atsjournals.org/doi/abs/10.1165/rcmb.2024-0226OC
- Age-related somatic mutations, e.g., loss of Y chromosome in red blood cells
  - https://www.nature.com/articles/s43856-025-00966-9
  - https://academic.oup.com/eurheartj/article/46/17/1603/8009275

Funding agencies requirements

NIH's 2014 policy: "NIH expects that **sex as a biological variable** will be factored into research designs, analyses, and reporting in vertebrate animal and human studies"

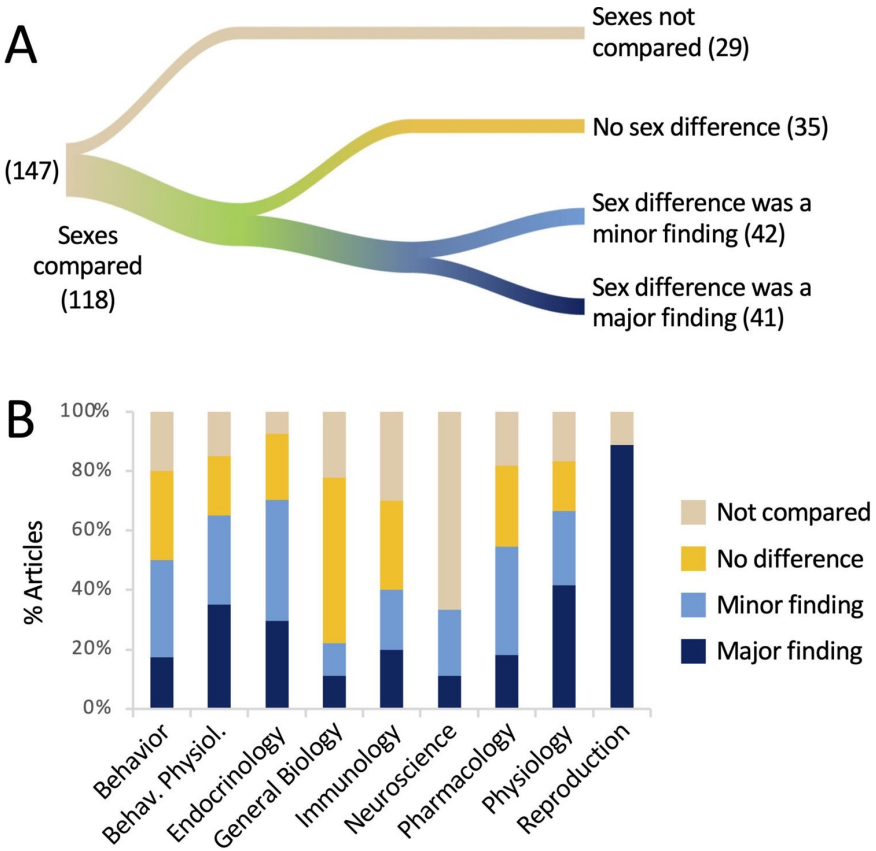
https://orwh.od.nih.gov/sex-as-biological-variable

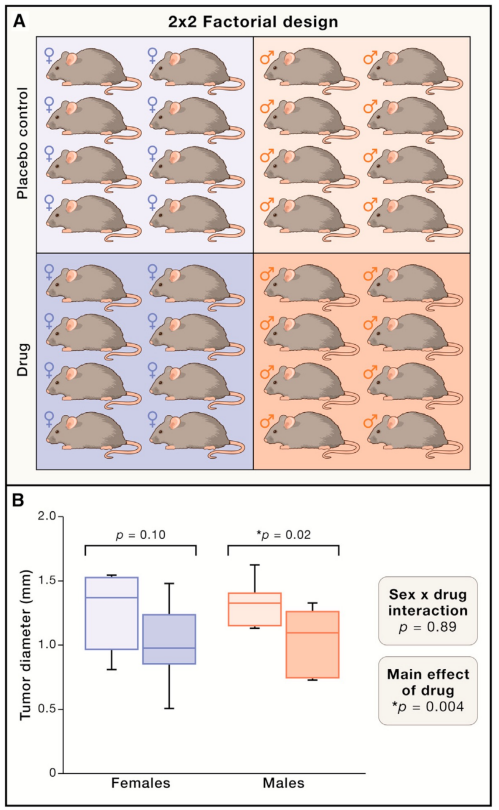
- Increase in mentions of sex-differences in published articles
- But: «*Without careful methodology, the pursuit of sex difference research, despite a mandate from funding agencies, will result in a literature of contradiction*» Janet Rich-Edwards et al. (2018) Endocrine Reviews



https://www.whijournal.com/article/S1049-3867(23)00072-5/fulltext
https://academic.oup.com/edrv/article/39/4/424/4967741

The «DISS» error: difference in sex-specific significance





https://elifesciences.org/articles/70817

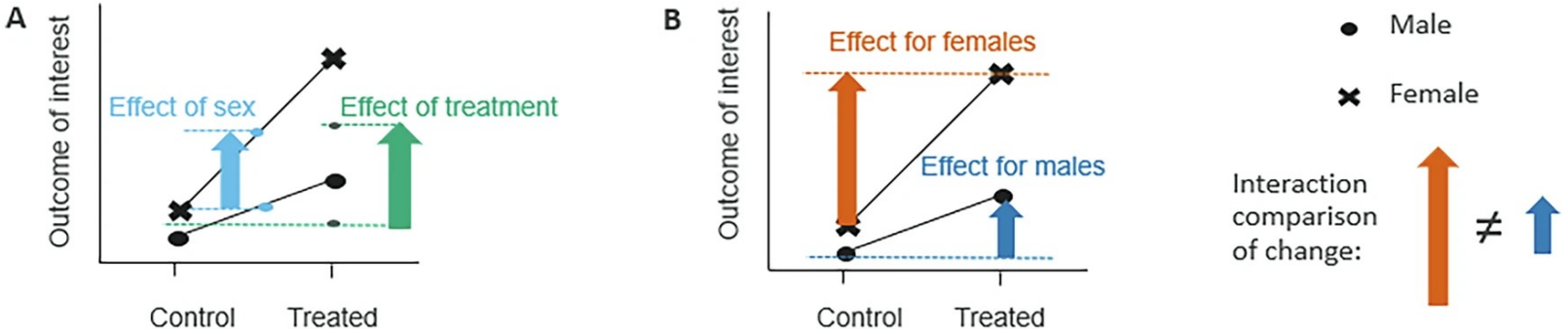
https://www.sciencedirect.com/science/article/pii/S0092867424001740

An evaluation of publicly available resources

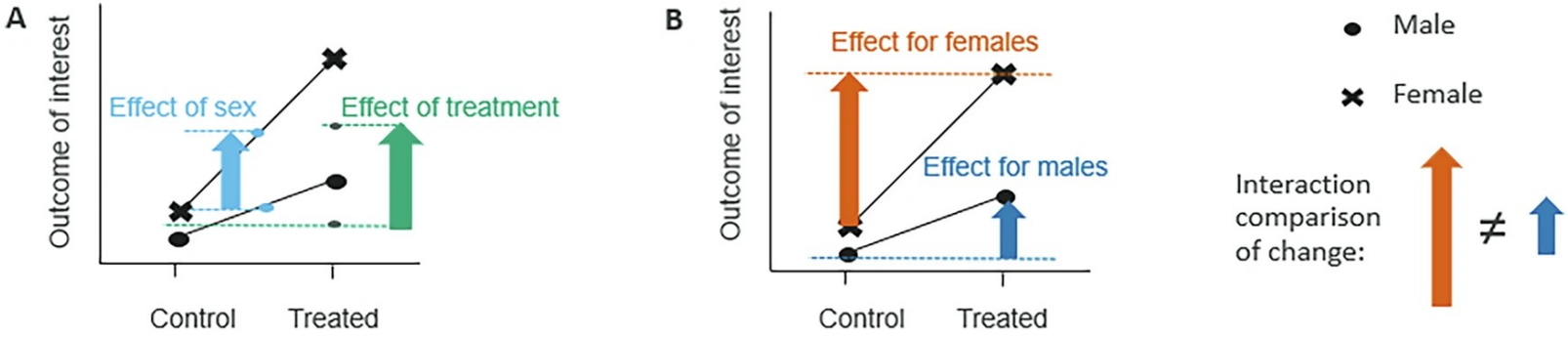
*«We evaluated three sets of **publicly available online training materials** on this topic: (1) Integrating Sex & Gender in Health Research from the Canadian Institutes of Health Research (CIHR); (2) Sex as a Biological Variable: A Primer from the United States National Institutes of Health (NIH); and (3) The Sex and Gender Dimension in Biomedical Research, developed as part of "Leading Innovative measures to reach gender Balance in Research Activities" (LIBRA) from the European Commission.»*

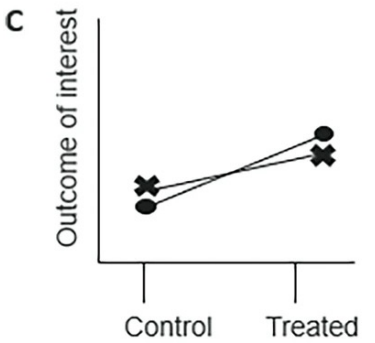
*«All three courses discussed the importance of including males and females to better generalize results, discover potential sex differences, and tailor treatments to men and women. The entangled nature of sex and gender, operationalization of sex, and potential downsides of focusing on sex more than other sources of variation were **minimally discussed**. Notably, all three courses explicitly **endorsed invalid analytical approaches** that produce bias toward false positive discoveries of difference.»*

https://link.springer.com/article/10.1186/s13293-024-00610-6

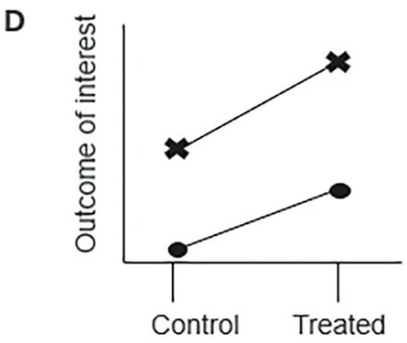


https://www.nature.com/articles/s42003-025-08118-4

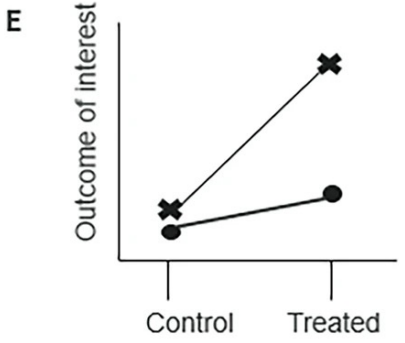




Effect	Significant
Sex	X
Treatment	✓
Interaction	X



Effect	Significant
Sex	✓
Treatment	✓
Interaction	X



Effect	Significant
Sex	✓
Treatment	✓
Interaction	✓

https://www.nature.com/articles/s42003-025-08118-4

As bioinformatician

- When we investigate differences by biological sex, our experiments live or die by their design

- How to best conduct statistical analyses?
  - E.g., do not treat sex as a covariate to be eliminated
- How to avoid common pitfalls of bioinformatics studies?
  - E.g., sexual chromosomes often excluded from analyses

- Deposition in databases
  - "Molecular omics resources should require sex annotation: a call for action"
  - "Addressing sex bias in biological databases worldwide"

https://www.nature.com/articles/s41592-021-01168-6
https://osf.io/preprints/biohackrxiv/n9dkg_v1

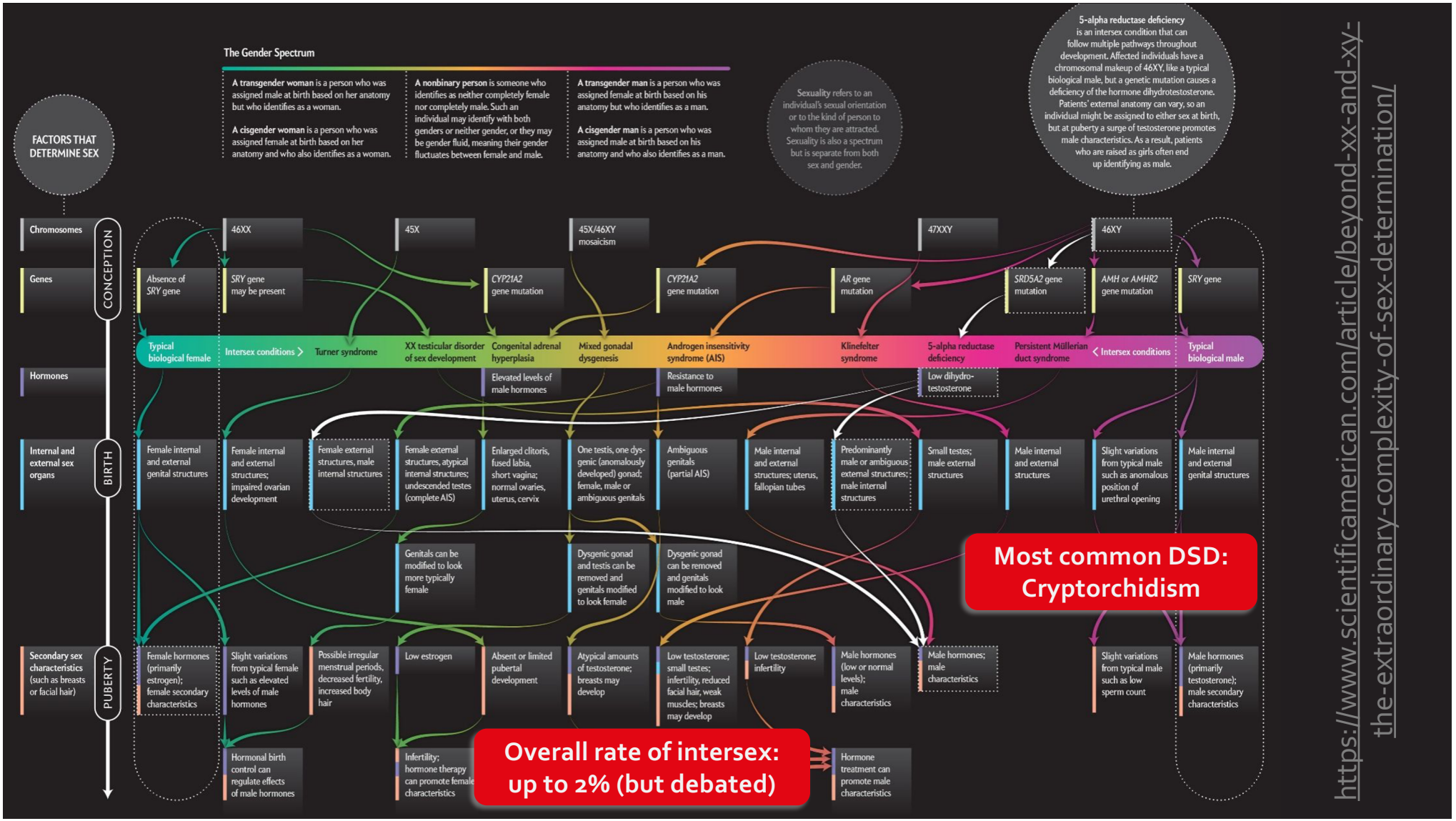
Today's goals

Embrace this complexity! Do not oversimplify

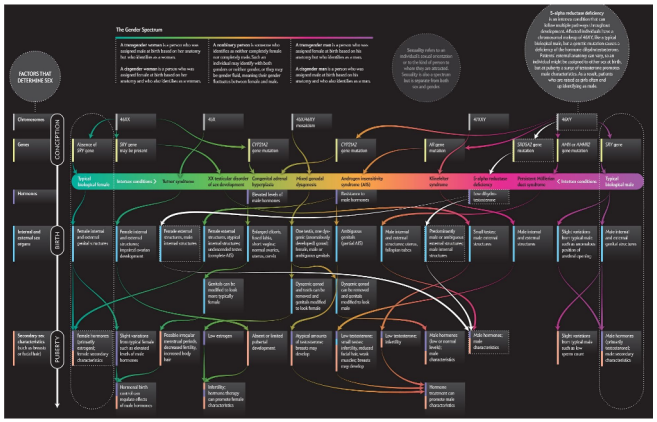
Learn more on how to design, analyze and report accurately

https://www.nature.com/articles/d41586-024-01204-3

Questions?


FACTORS THAT DETERMINE SEX
The Gender Spectrum
A transgender woman is a person who was assigned male at birth based on her anatomy but who identifies as a woman.
A cisgender woman is a person who was assigned female at birth based on her anatomy and who also identifies as a woman.
A nonbinary person is someone who identifies as neither completely female nor completely male. Such an individual may identify with both genders or neither gender, or they may be gender fluid, meaning their gender fluctuates between female and male.
A transgender man is a person who was assigned female at birth based on his anatomy but who identifies as a man.
A cisgender man is a person who was assigned male at birth based on his anatomy and who also identifies as a man.
Sexuality refers to an individual's sexual orientation or to the kind of person to whom they are attracted. Sexuality is also a spectrum but is separate from both sex and gender.
5-alpha reductase deficiency is an intersex condition that can follow multiple pathways throughout development. Affected individuals have a chromosomal makeup of 46XY, like a typical biological male, but a genetic mutation causes a deficiency of the hormone dihydrotestosterone. Patients' external anatomy can vary, so an individual might be assigned to either sex at birth, but at puberty a surge of testosterone promotes male characteristics. As a result, patients who are raised as girls often end up identifying as male.
CONCEPTION
BIRTH
PUBERTY
Chromosomes
Genes
Hormones
Internal and external sex organs
Secondary sex characteristics (such as breasts or facial hair)
46XX
45X
45X/46XY mosaicism
47XXY
46XY
Absence of SRY gene
SRY gene may be present
CYP21A2 gene mutation
CYP21A2 gene mutation
AR gene mutation
SRD5A2 gene mutation
AMH or AMHR2 gene mutation
SRY gene
Typical biological female
Intersex conditions
Turner syndrome
XX testicular disorder of sex development
Congenital adrenal hyperplasia
Mixed gonadal dysgenesis
Androgen insensitivity syndrome (AIS)
Klinefelter syndrome
5-alpha reductase deficiency
Persistent Müllerian duct syndrome
Intersex conditions
Typical biological male
Elevated levels of male hormones
Resistance to male hormones
Low dihydro-testosterone
Female internal and external genital structures
Female internal and external structures; impaired ovarian development
Female external structures, male internal structures
Female external structures, atypical internal structures; undescended testes (complete AIS)
Enlarged clitoris, fused labia, short vagina; normal ovaries, uterus, cervix
One testis, one dysgenic (anomalously developed) gonad; female, male or ambiguous genitals
Ambiguous genitals (partial AIS)
Male internal and external structures; uterus, fallopian tubes
Predominantly male or ambiguous external structures; male internal structures
Small testes; male external structures
Male internal and external structures
Slight variations from typical male such as anomalous position of urethral opening
Male internal and external genital structures
Genitals can be modified to look more typically female
Dysgenic gonad and testis can be removed and genitals modified to look female
Dysgenic gonad can be removed and genitals modified to look male
Most common DSD: Cryptorchidism
Female hormones (primarily estrogen); female secondary characteristics
Slight variations from typical female such as elevated levels of male hormones
Possible irregular menstrual periods, decreased fertility, increased body hair
Low estrogen
Absent or limited pubertal development
Atypical amounts of testosterone; breasts may develop
Low testosterone; small testes; infertility, reduced facial hair, weak muscles; breasts may develop
Low testosterone; infertility
Male hormones (low or normal levels); male characteristics
Male hormones; male characteristics
Slight variations from typical male such as low sperm count
Male hormones (primarily testosterone); male secondary characteristics
Hormonal birth control can regulate effects of male hormones
Infertility; hormone therapy can promote female characteristics
Overall rate of intersex: up to 2% (but debated)
Hormone treatment can promote male characteristics
https://www.scientificamerican.com/article/beyond-xx-and-xy-the-extraordinary-complexity-of-sex-determination/

Team activity (5-10 min)

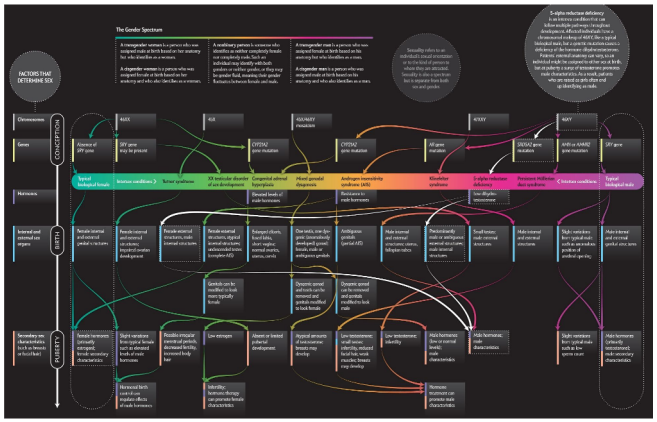


- Breakout rooms, 2 by 2
- Focus on one syndrome/phenotype/case and look for more detailed information.
- Prepare 1 slide to explain to the others
  - Sources: Wikipedia, scientific papers, blog posts, …
  - Mechanism(s)
  - Prevalence
  - …

https://bit.ly/3RAkQao

Team activity (5-10 min)

1. Klinefelter syndrome
2. 45,X/46,XY mosaicism
3. Turner syndrome
4. XX male syndrome
5. AMHR2 mutation
6. CYP21A2 mutation
7. Androgen insensitivity syndrome
8. Denys–Drash syndrome



https://bit.ly/3RAkQao

eLife

FEATURE ARTICLE

SCIENCE FORUM

Best practices to promote rigor and reproducibility i era of sex-inclusive resear

Abstract To enhance inclusivity and rigor, many funding agencies and journals now ma of females as well as males in biomedical studies. These mandates have enhanced generali unprecedented opportunities to discover sex differences. Education in sound methods to subgroup category has lagged behind, however, resulting in a problematic literature in which yses, and interpretations of results are often flawed. Here, we outline best practices for c inclusive mandates, both for studies in which sex differences are a primary focus and for thos not. Our recommendations are organized within the "4 Cs of Studying Sex to Strengthen Scie Collection, Characterization and Communication," a framework developed by the Office of Re Health at the National Institutes of Health in the United States. Following these guidelines sho include females and males in their studies while at the same time upholding high standards of

JANET W RICH-EDWARDS, DONNA L MANEY*

CellPress

Cell
Leading Edge

Perspective

Sex contextualism in laboratory research: Enhancing rigor and precision in the study of sex-related variables

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https://doi.org/10.1016/j.cell.2024.02.008

SUMMARY

and illness requires rigorous and precise approaches to to recognize that sex is not in and of itself a causal mecha- ng a set of categories, usually assigned according to a range n of classification to working with concrete and measurable Whether and how these sex-related variables matter—and ill vary in context-specific ways. Second, when researchers earch designs, rigorous analytical methods are needed to interpretation and reporting of sex-related variation require ch advance health equity for all.

*"When researchers incorporate these sex-related variables into research designs, **rigorous analytical methods** are needed to allow strongly supported conclusions"*

https://elifesciences.org/articles/90623
https://www.cell.com/cell/fulltext/S0092-8674(24)00174-0

SIB

March 2024: **Landmark Cell focus issue centers the voices of sex and gender minorities**

Collection | 22 August 2024

Sex as a biological variable (SABV)

Submission status
Closed

Submission deadline
22 August 2025

Sex as a biological variable (SABV), most simply defined at the chromosomal level, can have impacts ranging from gene expression to drug metabolism, and from organ shape to clinical phenotypes. Yet, historically, most basic biological or biomedical research has been carried out using only one sex, or aggregating both sexes together, for example using animal models or cell lines. This has led to foundational biases and knowledge gaps about when sex does and does not make a difference.

This cross-journal Collection aims to promote research that considers SABV. We encourage submission across all realms of biological research, from molecular and cellular biology to cognitive neuroscience, and across all levels, from in vivo model organisms and humans to in vitro and computational models including studies considering SABV in biomedical and medical research. — show all

Collection content | Participating journals | About the editors | About this collection

Resources and guidelines (1)

- Gendered innovations
  - http://genderedinnovations.stanford.edu/index.html
- MRC Policy on Sex in Experimental Design
  - https://nc3rs.org.uk/sites/default/files/2022-08/MRC%E2%80%99s%20new%20expectations%20on%20the%20inclusion%20of%20sex%20in%20experimental%20design%20%28Dr%20Stella%20Child%2C%20UKRI%20-%20MRC%29_0.pdf
- Working Group on Sex in Experimental Design of Animal Research
  - https://www.ukri.org/wp-content/uploads/2022/03/MRC-090322-SexInExperimentalDesign-SummaryReport.pdf
- Experimental design assistant
  - https://eda.nc3rs.org.uk/experimental-design-animal-characteristics#sex
- The Sex and Gender Perspective in Research Toolkit
  - https://aquas.gencat.cat/web/.content/minisite/aquas/publicacions/2022/toolkit_perspective_sex_gender_research_aquas2022.pdf
- Factsheet Preclinical Research UZH
  - https://www.gendermed.uzh.ch/dam/jcr:e6ead002-e395-4126-9b22-3ee389fd358a/Factsheet_Pr%C3%A4klinische%20Forschung_Jaric_Buch_2024.pdf

Resources and guidelines (2)

- American Thoracic Society
  - https://www.atsjournals.org/doi/10.1164/rccm.202506-1361ST
- Applying the new SABV policy to research and clinical care
  - https://www.sciencedirect.com/science/article/pii/S0031938417302585
- The Sex Inclusive Research Framework to address sex bias in preclinical research proposals
  - https://www.nature.com/articles/s41467-025-58560-5
- Leveraging research into sex differences and steroid hormones to improve brain health
  - https://www.nature.com/articles/s41574-024-01061-0

Questions?

Today's schedule

09:15 – 10:15 Introduction

10:15 – 10:30 Coffee break

10:30 – 12:00 Biological sex in experimental design
(Talk+hands-on by Frédéric Schütz)

12:00 – 13:00 Lunch break

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Hands-on

Hands-on

- Installed R and RStudio before the start of the course?
- Anyone willing to work in subgroups?
- Breakout rooms to help/debug

recount3 study explorer

Under project_home you can subset for GTEx, TCGA or SRA. Note that GTEx v8 and TCGA are split by tissue.

Show 3 entries Search:

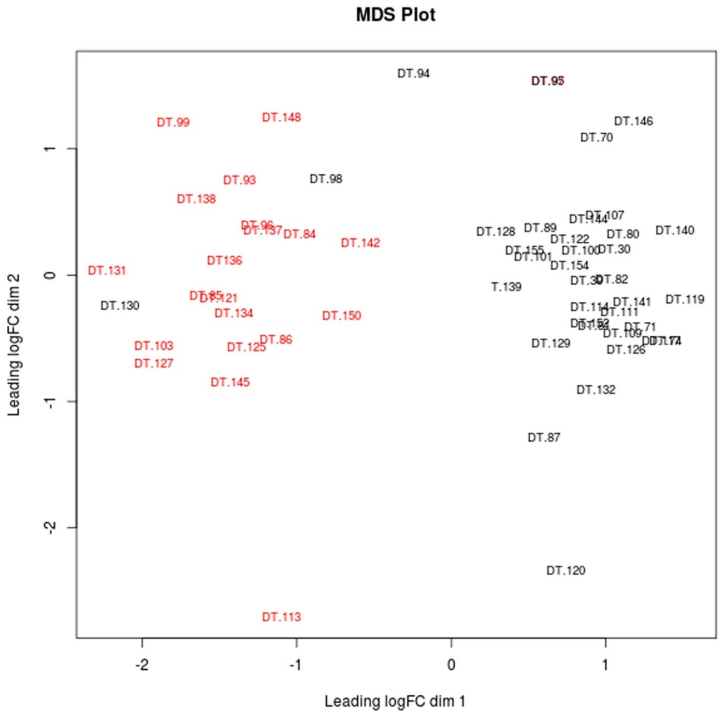
organism	project_home	project	n_samples	study_title	study_abstract
["mo	["data_so	All	8 ... 4{	All	sex
mouse	data_sources/sra	SRP219736	48	Transgenerational self-reconstruction of disrupted chromatin organization after exposure to an environmental stressor in mice	Exposure to environmental stressors is known to increase disease susceptibility in unexposed descendants in the absence of detectable genetic mutations. The mechanisms mediating environmentally-induced transgenerational disease susceptibility are poorly understood. We showed that great-great-grandsons of female mice exposed to tributyltin (TBT) throughout pregnancy and lactation were predisposed to obesity due to altered chromatin organization that subsequently biased DNA methylation and gene expression. Here we analyzed DNA methylomes and transcriptomes from tissues of animals ancestrally exposed to TBT spanning generations, sexes, ontogeny, and cell differentiation state. We found that TBT elicited concerted alterations in the expression of “chromatin organization” genes and inferred that TBT-disrupted chromatin organization might be able to self-reconstruct transgenerationally. We also found that the location of “chromatin organization” and “metabolic” genes is biased similarly in mouse and human genomes, suggesting that exposure to environmental stressors in different species could elicit similar phenotypic effects via self-reconstruction of disrupted chromatin organization. Overall design: We generated RNA-Seq data from mesenchymal stem cells (MSCs) from 8-week old F3 and F4, female and male mice and liver from 33-week old F4 male mice ancestrally exposed to DMSO (0.1%) or TBT (50 nM) during in utero development and lactation. N=5 per treatment for MSCs and N=4 per treatment for liver--> 48 RNA-seq samples in total We generated MBD-Seq data from mesenchymal stem cells (MSCs) from 8-week old F3 and F4, female and male mice and liver from 33-week old F4 male mice ancestrally exposed to DMSO (0.1%) or TBT (50 nM) during in utero development and lactation. N=5 per treatment for MSCs and N=4 per treatment for liver--> 48 MBD-seq samples in total
mouse	data_sources/sra	SRP064968	48	RNA-Seq analysis of 3 month old all Ret-positive (EGFP-labeled) dorsal root ganglia L345 or L6S1	The goal of this set is to build expression data using FACS isolated Ret-positive cells harvested from a Ret-EGFP reporter mouse (3 month old). To enrich for bladder projecting neurons L6S1 DRGs were FACS isolated. L3,4, 5 lumbar levels were also FACS isolated to aid in differential expression of Ret-positive populations from L6S1 and L345 and help enrich bladder Ret-positive neurons. The series further includes these populations from males and females to uncover sex based differences. Overall

http://rna.recount.bio/docs/index.html

https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02533-6

SIB

Hepatic gene expression variations in response to high-fat diet-induced impaired glucose tolerance using RNAseq analysis in collaborative cross mouse population



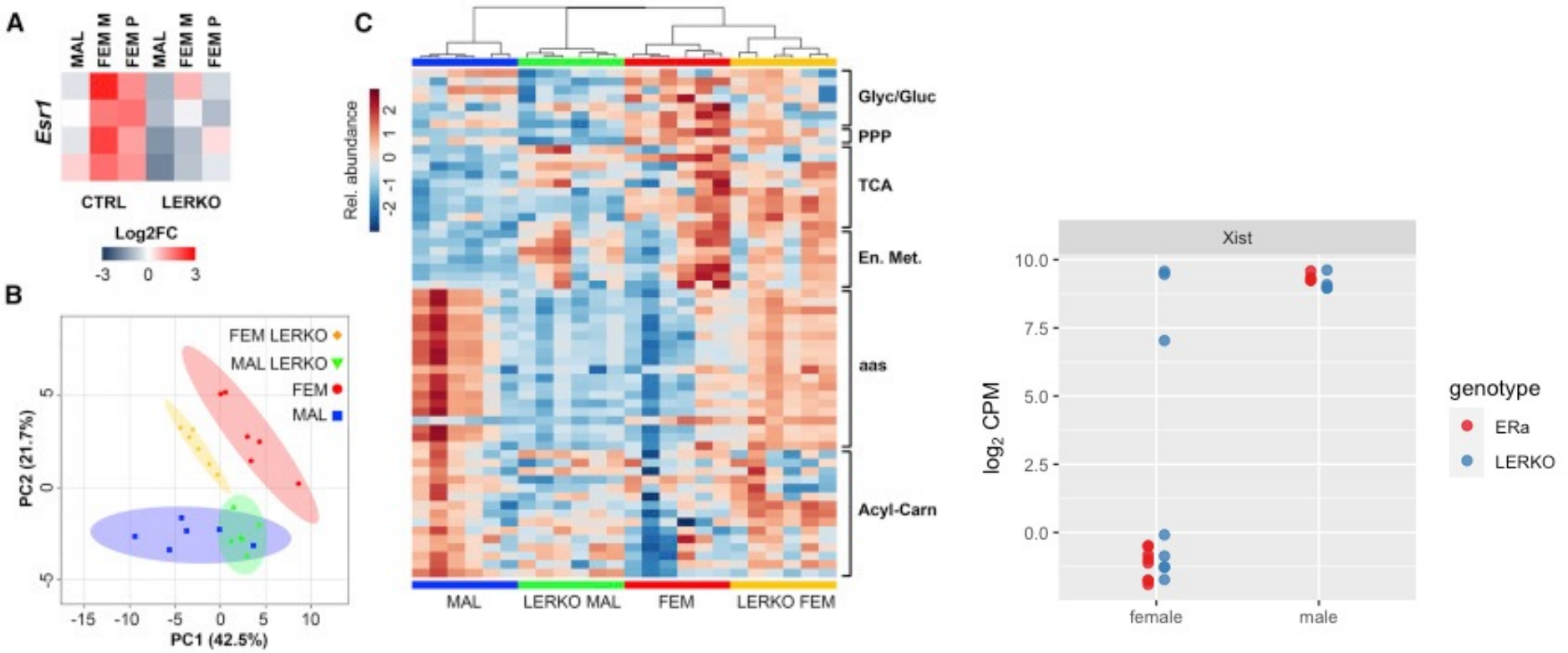
SRP185805/GSE126490

"The Female-Nondiabetic-Normal samples (control group) versus the Female-Nondiabetic-Obese samples (case group) DE genes list consisted of 1382 DE genes."

" Hepatic gene expression differs significantly between obese and non-obese mice, with a significant sex effect, where male and female mice exhibit different responses and coping mechanisms."

https://link.springer.com/article/10.1007/s00335-019-09816-1
https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-020-07173-x

Short-Term Fasting Reveals Amino Acid Metabolism as a Major Sex-Discriminating Factor in the Liver



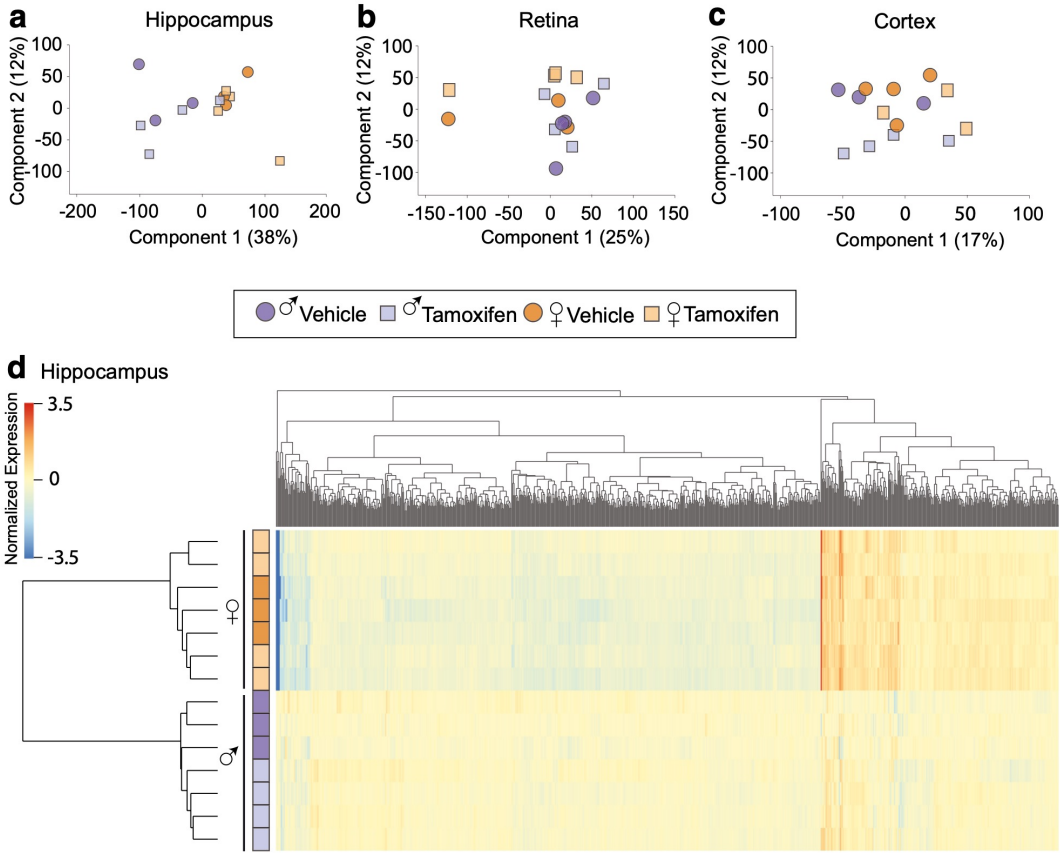
PRJNA395963

https://www.cell.com/cell-metabolism/fulltext/S1550-4131(18)30328-0

Hands-on dataset

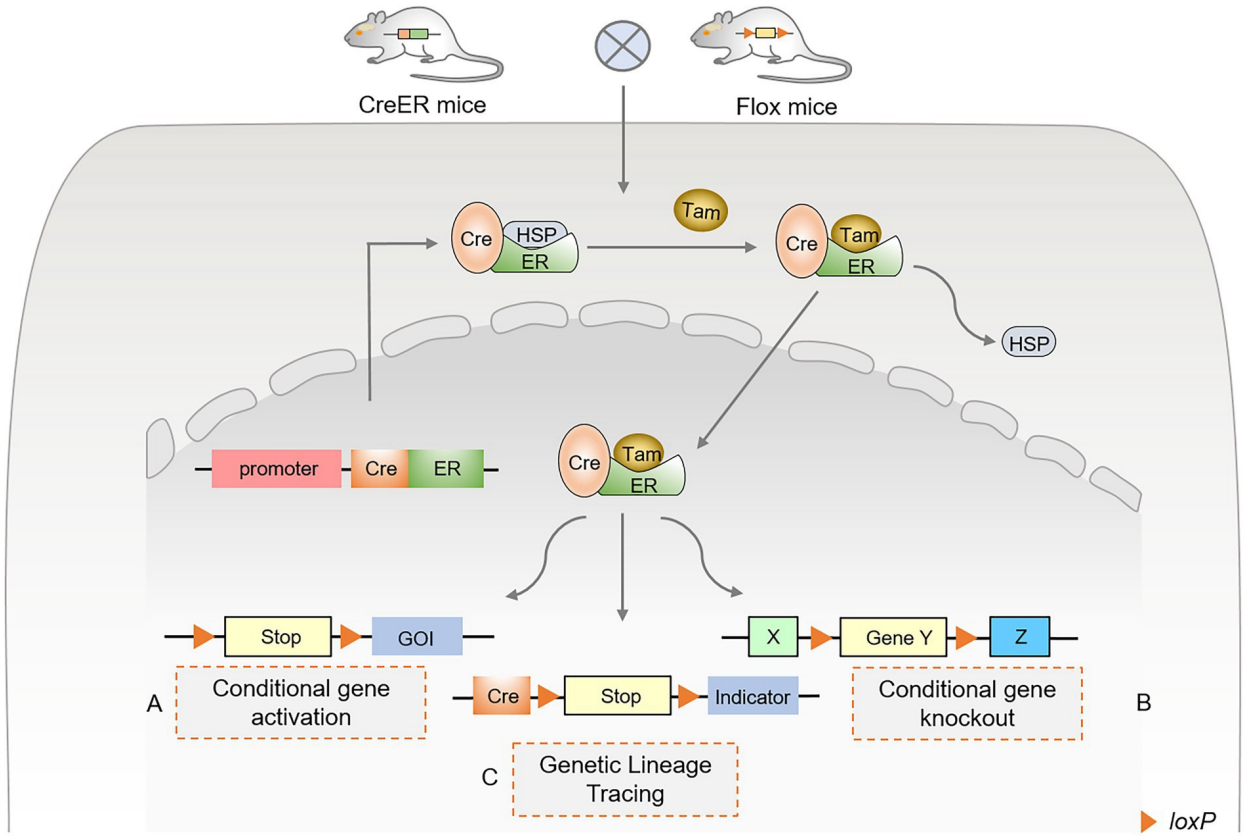
SRP218156

GSE135752



https://link.springer.com/article/10.1007/s11357-019-00090-2

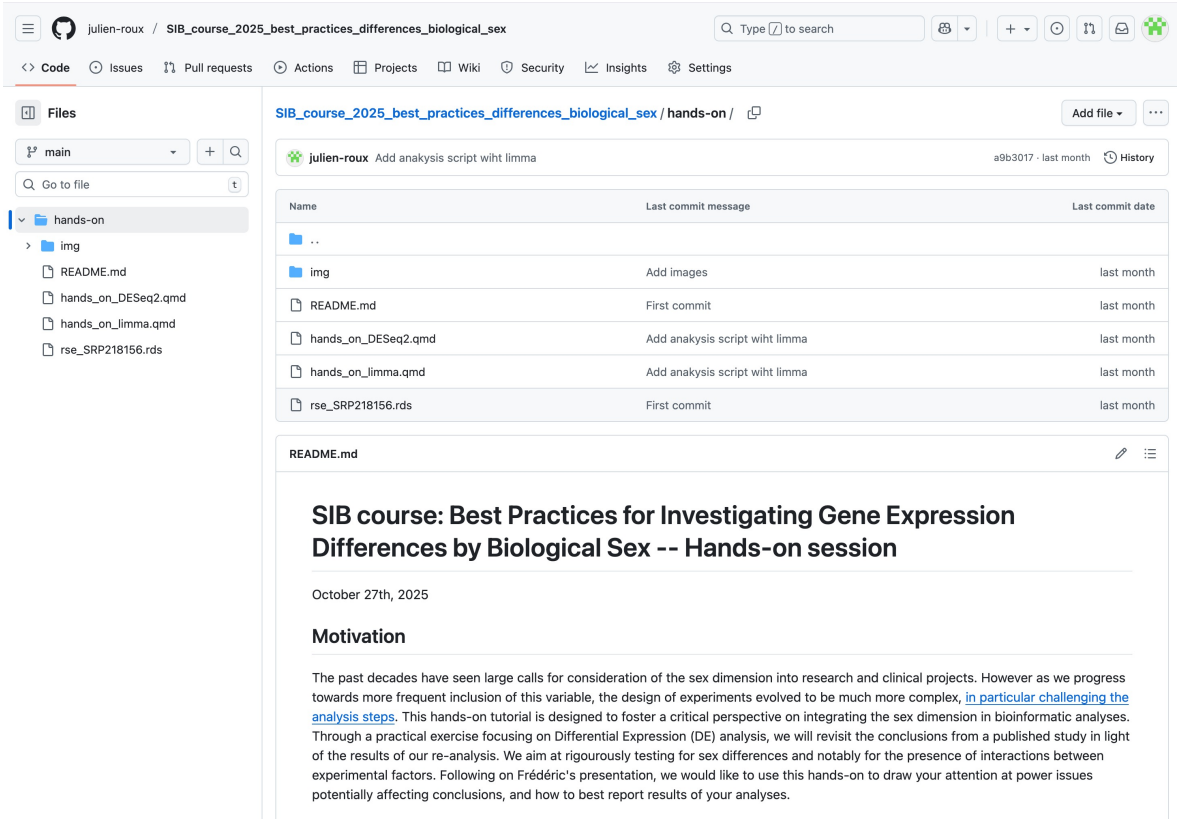
Hands-on



https://www.frontiersin.org/articles/10.3389/fcvm.2023.1085629/full

Hands-on

https://github.com/julien-roux/SIB_course_2025_best_practices_differences_biological_sex



bit.ly/4ngRUAR

Hands-on: studying the long-lasting effects of Tamoxifen treatment on the CNS transcriptome across sexes

SIB course: Best Practices for Investigating Gene Expression Differences by Biological Sex

AUTHOR
Julien Roux, University of Basel, SIB

PUBLISHED
September 18, 2025

Table of contents

Loading data in R

```
## If packages are missing: install.packages('BiocManager')
## BiocManager::install('...')

## Setting up working directory
library(here)

library(tidyverse)
library(ggplot2)

## color palettes
library(RColorBrewer)
myPalette <- c(brewer.pal(9, "Set1"), brewer.pal(8, "Set2"))
```

Hand-on

Analysis strategy

- QC checks
- Design matrix and contrasts
- Other possible strategies? Advantages and drawbacks

Table 2 Summary of the main observations

DESeq	- Conservative with default settings. Becomes more conservative when outliers are introduced.
	- Generally low TPR.
	- Poor FDR control with 2 samples/condition, good FDR control for larger sample sizes, also with outliers.
	- Medium computational time requirement, increases slightly with sample size.
voom / vst	- Good type I error control, becomes more conservative when outliers are introduced.
	- Low power for small sample sizes. Medium TPR for larger sample sizes.
	- Good FDR control except for simulation study β_0^{4000}. Largely unaffected by introduction of outliers.
	- Computationally fast.

http://www.biomedcentral.com/1471-2105/14/91

Hands-on (part 2)

Critical reading of the paper (as a reviewer)

- Motivation
- Materials and methods section: design of study, analysis
- Reporting

What would you improve? How?

https://link.springer.com/article/10.1007/s11357-019-00090-2

Hands-on (part 2)

Methods:

Following sequencing, reads were trimmed, aligned, differential expression statistics and correlation analyses were performed in Strand NGS software package (Agilent). Reads were aligned against the Mm10 build of the mouse genome (2014.11.26). Alignment and filtering criteria included: adapter trimming, fixed 2 bp

of reads are close to background and are highly variable. For statistical analysis of differential expression, a two-way ANOVA with the factors of sex and treatment and a Benjamini-Hochberg multiple testing correction followed by Student-Newman Keuls post hoc test was used. For those transcripts meeting this statistical criterion, a fold change >|1.25| cutoff was used to eliminate those genes which were statistically significant but unlikely to be biologically significant and orthogonally confirmable due to their very small magnitude of change. Visualizations of hierarchical clustering and principal compo-

Results:

hippocampus, 2073 genes by sex and no significant differences with treatment or for an interaction effect were found. Similarly, hierarchical clustering on the hippocampus samples demonstrated that the individual samples were separated by sex with samples from Tam- and Veh-treated mice interspersed by sex (Fig. 3d).

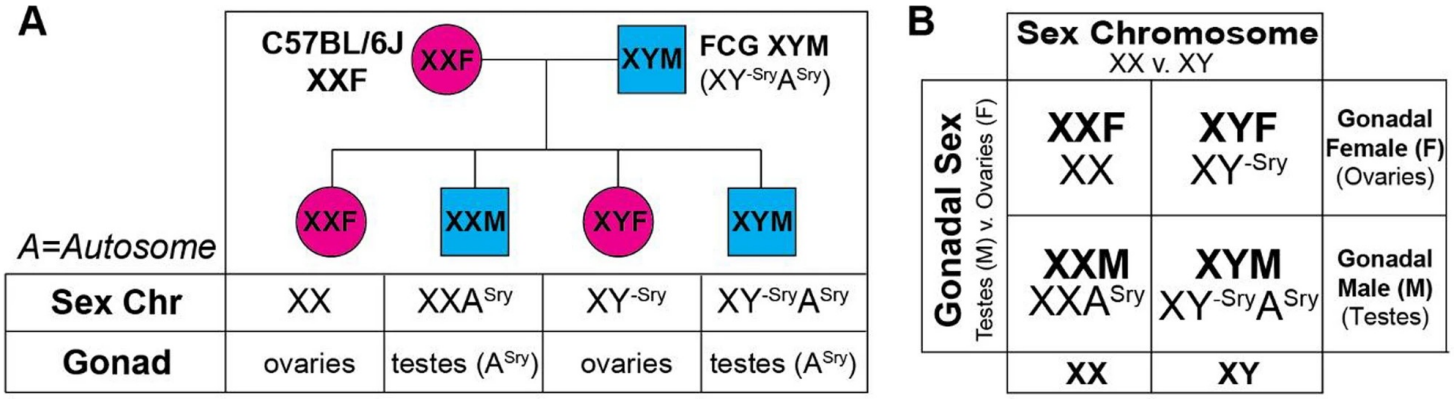
Discussion:

worsen with age and might affect almost every tissue in the body. In contrast, the experiments in this study, started at 3 months of age, and were analyzed at ~4 months of age, which led to no apparent dysfunctional phenotype, and most importantly to our purpose, no adverse effects of Tam on the sex-dependent differences in methylation and transcriptome signatures in the brain.

Chucair-Elliott et al. (2019) Geroscience

Hands-on (advanced)

- Different analysis chocies, e.g., allowing to extract sex/treatment interactions by tissue
- Similar analysis using:
  - Your data?
  - Other studies in recount3
  - Other papers from same group (e.g., Four Core Genotypes mouse model, Ocañas et al. 2022 Mol Neurobio)



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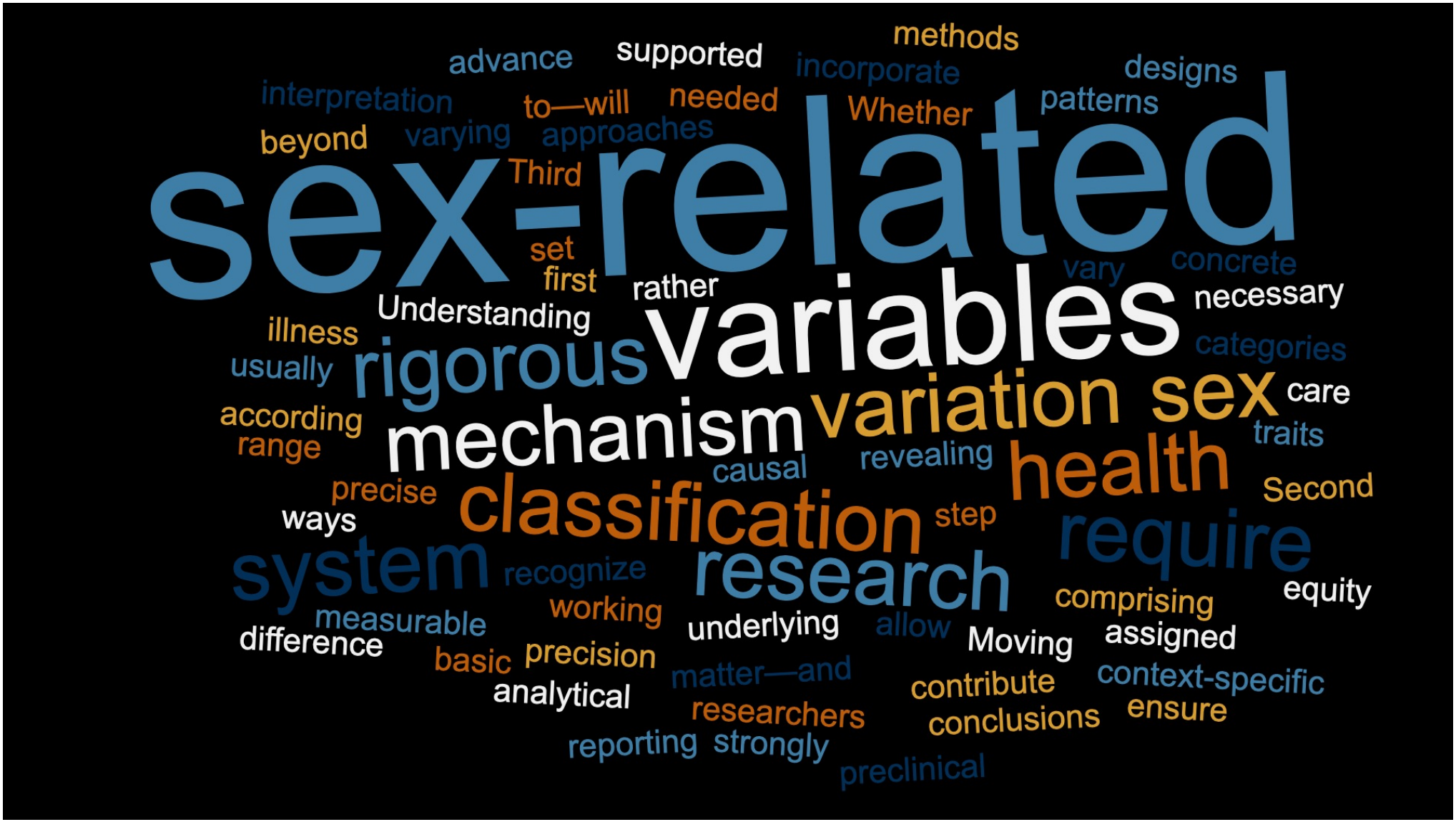
Conclusion


sex-related
variables
mechanism
rigorous
variation sex
health
classification
research
require
system
methods
advance
supported
incorporate
designs
interpretation
to—will
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Whether
patterns
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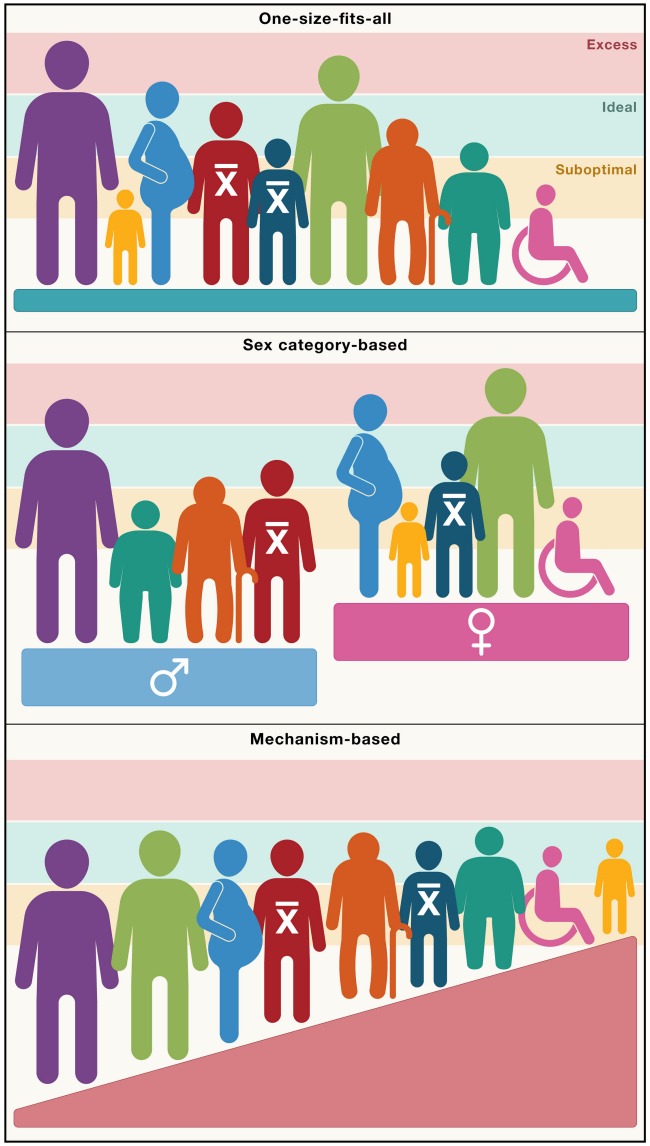


- Rigorous reporting of effect of studies variables
- Testing for interactions and attention to power issues (not significant ≠ absence of effect)
- Honestly report study limitations, beware of hype and distortion (e.g., press releases, communication to public)

Pape et al. (2024) Cell

Back to today's learning outcomes

- Inform colleagues about critical issues and challenges related to sex-based considerations in biological data collection.
- Propose suitable study design options and recognize potential biases that may arise in data collection and interpretation.
- Apply rigorous analytical methods on -omics datasets.
- Interpret and report results transparently and reproducibly in scientific papers.
- Generalize methods to other variables (age, ethnicity, etc) relevant to diversity and inclusion.

Back to you

- What did you learn that is relevant to your research project?
- What was maybe missing?
- Anything else you would like to discuss?
- Feedback, suggestions?

Wrap up

- We'll share with you the slides and hands-on material
- Exam sent in the next few days
- Don't hesitate to get in touch with us if you have ideas or wish to contribute to the SIB Diversity focus group (edi@sib.swiss)

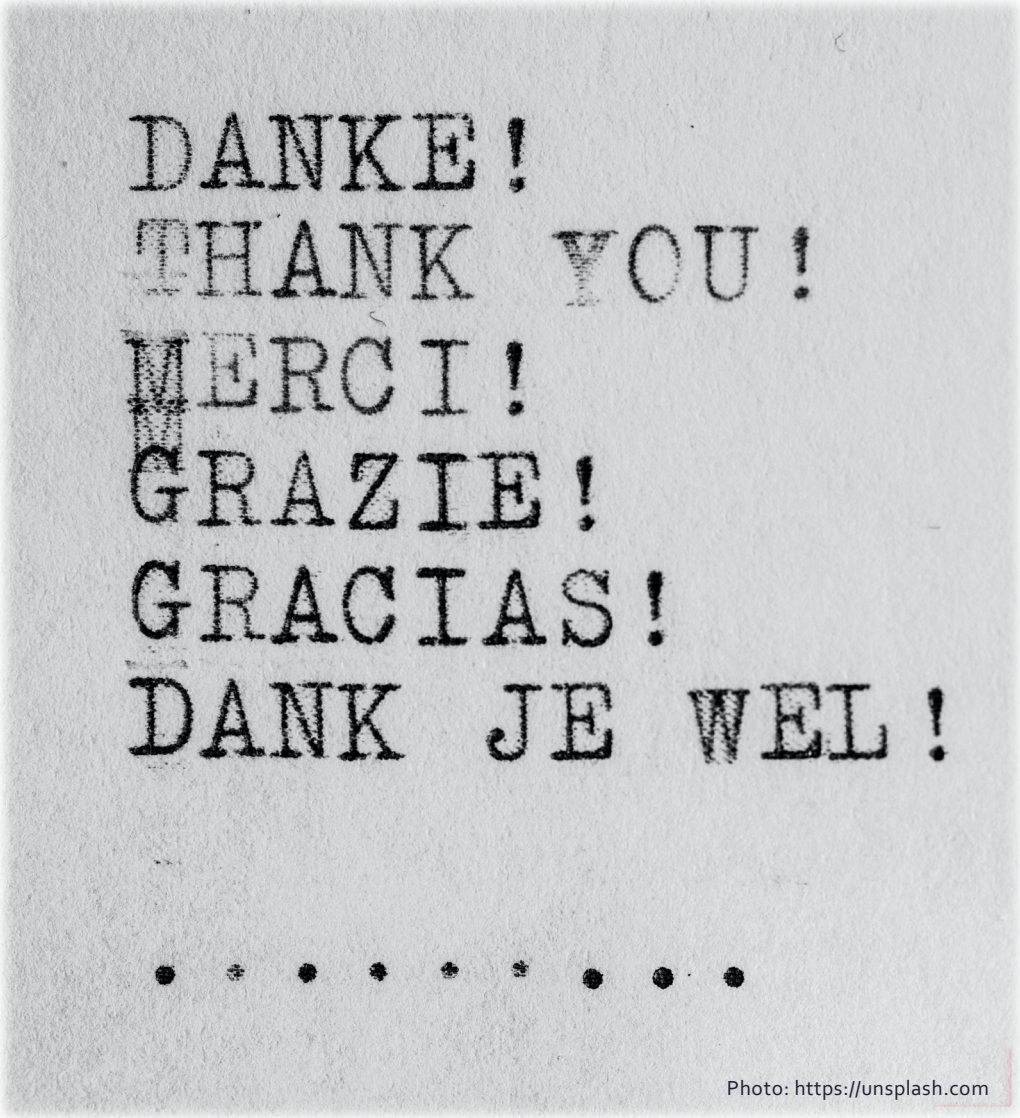
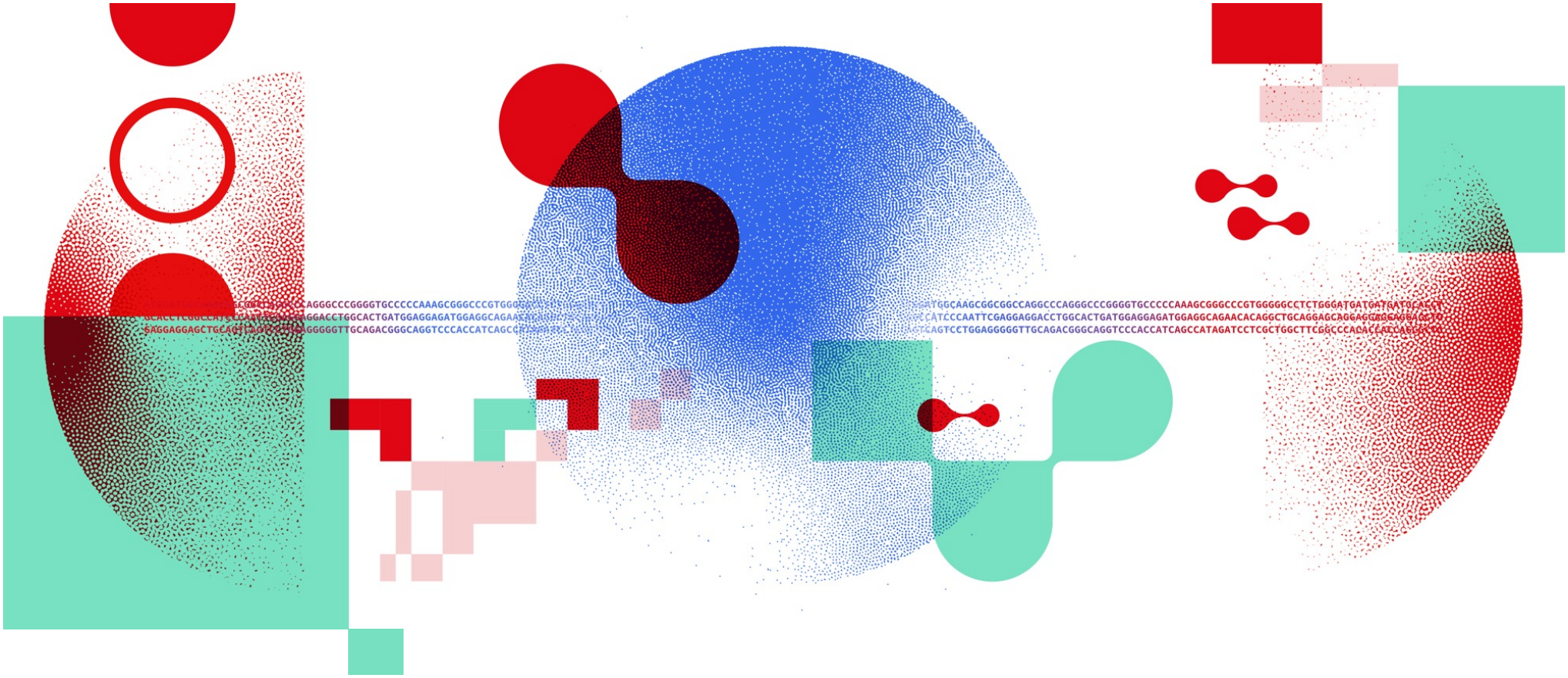


Photo: https://unsplash.com



Thank you!

DATA SCIENTISTS FOR LIFE

sib.swiss