# Lecture 4 – Review of Probability and Statistics

STAT/BIOF/GSAT 540: Statistical Methods for High Dimensional Biology

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#### Preview of next 6 lectures

- 2020/01/15 Lecture 4: Stats Philosophy, Math/stat background & review
- 2020/01/20 Lecture 5: Statistical Inference two group comparisons
- 2020/01/22 Lecture 6: Statistical Inference linear regression and ANOVA
- 2020/01/27 Lecture 7: Statistical Inference linear models (more than two groups, and interaction testing)
- 2020/01/29 Lecture 8: Statistical Inference continuous model + limma
- 2020/02/03 Lecture 9: Statistical Inference multiple testing

### Outline for today

- Intro: Philosophy, goals, and central concepts
- Review: Random Variables, Probability Distributions, Sampling Distribution, Estimation, Inference, CLT, Hypothesis Testing

#### Your goals:

- 1. be familiar with the terminology
- 2. have a clear understanding of the concepts

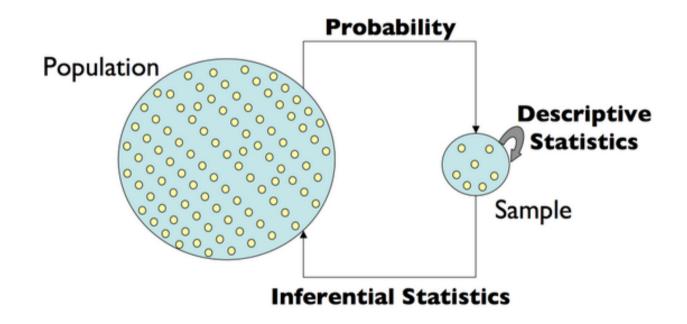
### What is Statistics?

#### **Statistics**

- The field of statistics concerns the science of **collecting**, **analyzing/modeling**, **interpreting** data and **communicating uncertainty** about the results
  - Data science and machine learning have enabled application to 'big data'
- Statistical and computational methods should not be used as generic "recipes" to follow  $\rightarrow$  non-robust science
- We aim for:
  - o rigorous understanding to perform routine statistical analysis
  - solid foundation to follow up on specific topics

#### Statistical Inference

A framework for generating conclusions about a population from noisy data from a sample



- Language of **probability** enables us to make *predictions* and discuss *uncertainty*
- Statistical inference enables us to understand the data and make conclusions
- We need both to learn from data

### Review: terminology & basic concepts

- Random variables and their distributions
- Models, parameters, and their estimators
- Central Limit Theorem (CLT)
- Hypothesis Testing

#### Variables

**Variable** (noun): an element, feature, or factor that is liable to vary or change

- In statistical terminology, a **variable** is an unknown quantity that we'd like to study
- Most research questions can be formulated as
  - What's the *relationship* between two or more variables?

#### Random variables

**Random Variable (RV):** A variable whose value results from the measurement of a quantity that is subject to variation (e.g. the *outcome* an experiment)

- Examples: a coin flip, a dice throw, the expression level of gene X
- An RV has a probability distribution

#### Distributions of RVs

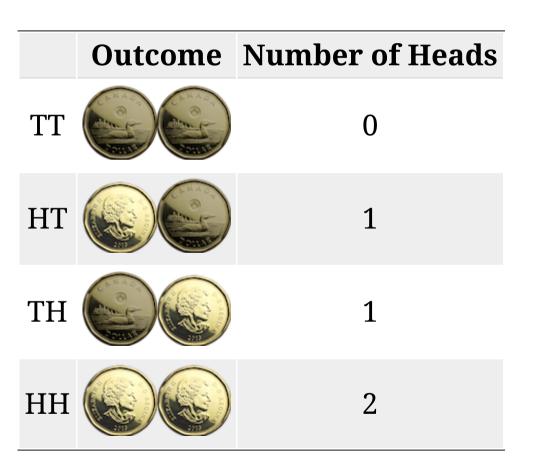
**Probability:** A number assigned to an outcome/event that describes the extent to which it is likely to occur

- Must satisfy certain rules (e.g. be between 0 and 1)
- Represents the (long-term) *frequency* of an event

**Probability distribution:** A mathematical function that maps outcomes/events to probabilities

### Example experiment: Two coin tosses

- Experiment: Toss two coins
- Sample space: set of all possible outcomes  $S = \{TT, TH, HT, HH\}$
- Random Variable of interest: number of heads



### Assigning probability to outcomes

- Let:
  - $\circ \omega =$  an outcome
  - $\circ~X(\omega)=$  number of heads in  $\omega$
- Each possible outcome is associated with a probability
- **Event:** A set of outcomes that satisfy some condition
- Each realization of the RV corresponds to an **event** (e.g.  $X(\omega)=1$  corresponds to the outcomes TH and HT)

	$\omega$	$X(\omega)$	Probability
TT		0	0.25
НТ		1	0.25
TH		1	0.25
НН		2	0.25

### Assigning probability to events

The probability distribution of the Random Variable X tells us how likely each event (number of heads) is to occur in the experiment

Event	x	P(X = x)
	0	0.25
, in the second	1	0.50
	2	0.25

Note on notation: P(X=x) can also be written as  $P_X(x)$ 

### Two types of random variables

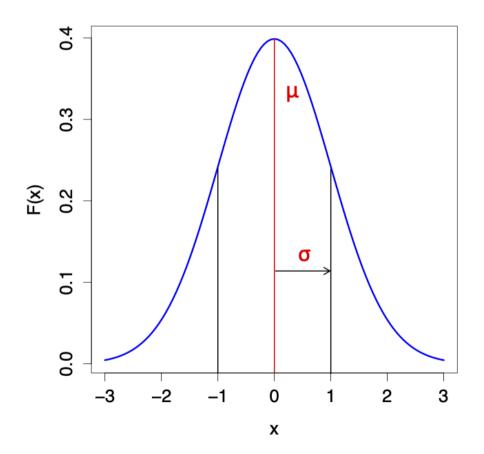
- A discrete RV has a countable number of possible values
  - o e.g. throwing dice, genotype measured on a SNP chip
- A **continuous** RV takes on values in an interval of numbers
  - e.g. expression level of a gene, blood glucose level, height of individuals

# Standard Gaussian (Normal) distribution

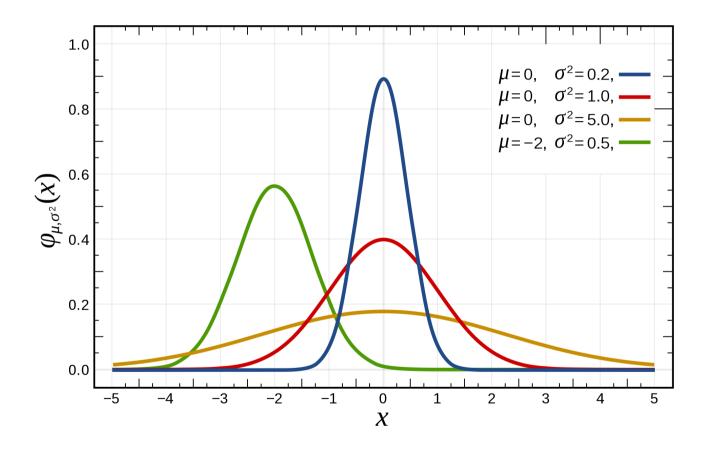
• probability density function:

$$f(x|\mu,\sigma^2) = rac{1}{\sqrt{2\pi}\sigma}e^{-rac{(x-\mu)^2}{2\sigma^2}}$$

- Mean  $= \mu$
- Standard Deviation =  $\sigma$
- ullet For convenience, we write  $N(\mu,\sigma^2)$
- ullet When  $\mu=0$  and  $\sigma=1$ , this is the Standard Normal distribution N(0,1)

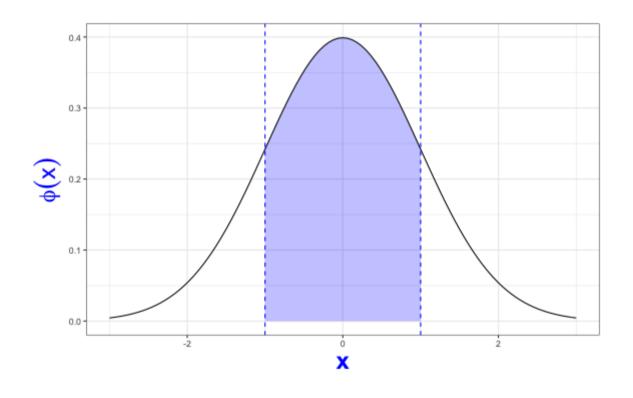


# Gaussian (Normal) distribution



Probability density function: 
$$f(x|\mu,\sigma^2)=\phi(x)=rac{1}{\sqrt{2\pi}\sigma}e^{-rac{(x-\mu)^2}{2\sigma^2}}$$

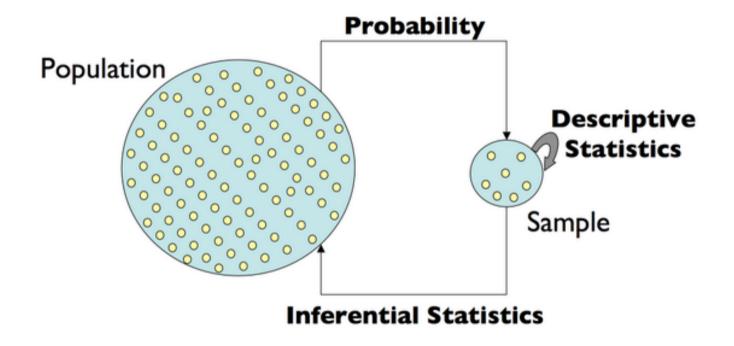
# Density → probability requires integration



#### Statistical Inference

- The **parameter space** is the set of all possible values for the parameter
- One major goal: to "figure out" (i.e. estimate) the parameter values
  - i.e. "fit the model to the data"
- The model is a representation that (we hope) approximates the data and (more importantly) the population that the data were sampled from
- We can then use this model for:
  - hypothesis testing
  - prediction
  - simulation

### Statistical Inference



#### IID

- A requirement (assumption) in many settings is that the data are IID: Independent and Identically Distributed
- Identically Distributed: a set of observations (events) are from the same population (i.e. they have the same underlying probability distribution)
  - e.g. a t-test assumes that under the null, all observations come from the same normal distribution
- Independent: all samples satisfy the condition

$$P(A,B) = P(A)P(B)$$

where A and B are events

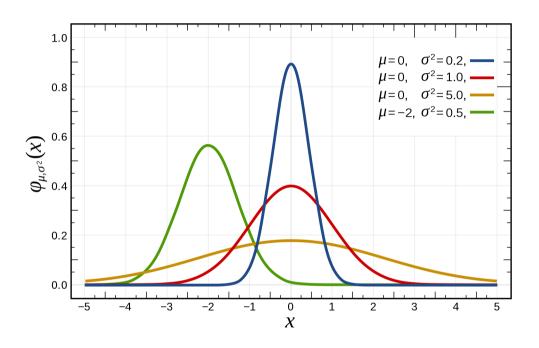
- i.e. the joint probability is the product of the individual event probabilities
- The above statement is for two events, but the same definition applies for any number of events (without loss of generality for any number of events)

### Violations of independence

- Experimental design is in part about trying to avoid unwanted dependence
- Example of design with violation of independence assumption:

Height measurements of individuals sampled from *related* females in a tall family

#### Parameters of the normal distribution



$$f(x|\mu,\sigma^2) = rac{1}{\sqrt{2\pi}\sigma}e^{-rac{(x-\mu)^2}{2\sigma^2}}$$

- Mean  $= \mu$
- Standard Deviation =  $\sigma$
- ullet For convenience, we write  $N(\mu,\sigma^2)$
- Population parameters are unknown

#### Parameter estimation

- **Estimator**: A function (or rule) used to estimate a parameter of interest
- Estimate: A particular realization (value) of an estimator

### Estimators for normally distributed data

- If we are given a sample of n observations from a normally distributed population, how do we estimate the parameter values  $\mu$  and  $\sigma$ ?
- Recall  $\mu$  is the mean and  $\sigma$  the standard deviation of the distribution

$$\hat{\mu} = ar{x} = rac{x_1 + x_2 + \ldots + x_n}{n} = rac{1}{n} \sum_{i=1}^n x_i$$

$$\hat{\sigma} = s = \sqrt{rac{\sum_{i=1}^n (x_i - ar{x})^2}{n-1}}$$

### Estimators vs Parameters

	Estimators	Parameters
Summarize	Sample	Population (ground truth)
Value	Computed from data	Unknown
Notation	$\hat{ heta}$	$oldsymbol{ heta}$

#### Normal **Mean**: Estimator vs Parameter

	Estimator	Parameter
Summarizes	Sample/data	Population (ground truth)
Value	$ar{x} = rac{1}{n} \sum_{i=1}^n x_i$	Unknown
Notation	$\hat{\mu}$	$\mu$

### Normal **Standard Deviation**: Estimator vs Parameter

	Estimator	Parameter
Summarizes	Sample/data	Population (ground truth)
Value	$s=\sqrt{rac{\sum_{i=1}^n(x_i-ar{x})^2}{n-1}}$	Unknown
Notation	$\hat{\sigma}$	$\sigma$

### Estimator for normally distributed data

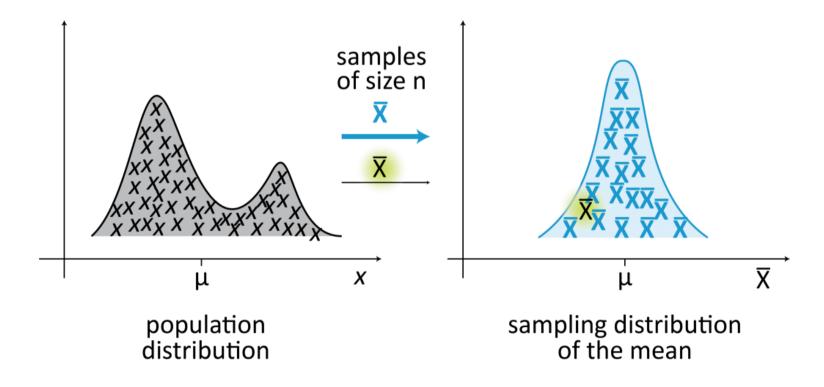
- Let's say we collected a **sample** from a population we assume to be normal
- ullet We estimate the mean  $\hat{\mu}=ar{x}$
- How good is the estimate?
- The answer depends on:
  - sample size
  - variability of the population

### Sampling distribution

- Statistic: any quantity computed from values in a sample
- Any function (or statistic) of a sample (data) is a random variable
- Thus, any statistic (because it is random) has a probability distribution function  $\rightarrow$  specifically, we call this the *sampling distribution*
- Example: the sampling distribution of the mean

## Sampling distribution of the mean

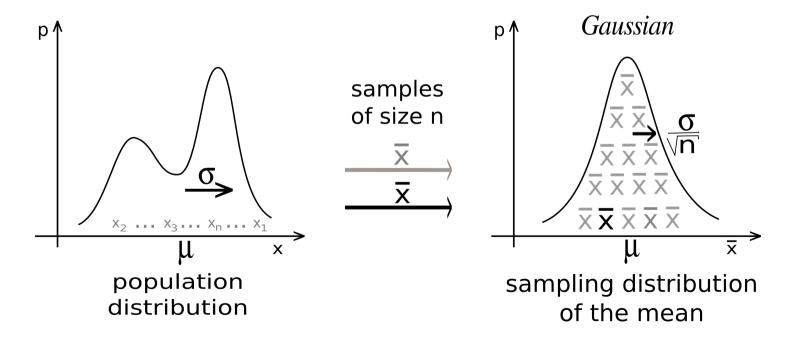
The sample mean  $ar{x}$  is an RV, so it has associated probability or sampling distribution



# Central Limit Theorem (CLT)

By the *Central Limit Theorem (CLT)*, we know that the sampling distribution of the mean is Normal:

ullet with mean  $\mu_{ar{X}}=\mu$  and standard deviation  $\sigma_{ar{X}}=rac{\sigma}{\sqrt{n}}$ 





#### Standard deviation vs Standard error



• The sampling distribution of the mean (by CLT):

$$ar{X} \sim N(\mu, rac{\sigma^2}{n})$$

- The *standard error* of the mean is  $\frac{\sigma}{\sqrt{n}}$
- The standard deviation of X is  $\sigma$

#### Estimation of parameters of the sampling distribution of the mean

Just as we estimated  $\mu$  and  $\sigma$  before, we can estimate  $\mu_{ar{X}}$  and  $\sigma_{ar{X}}$ 

$$ullet$$
  $\hat{\mu}_{ar{X}}=\hat{\mu}=ar{x}$ 

• 
$$\hat{\sigma}_{ar{X}}=rac{\hat{\sigma}}{\sqrt{n}}=rac{s}{\sqrt{n}}$$

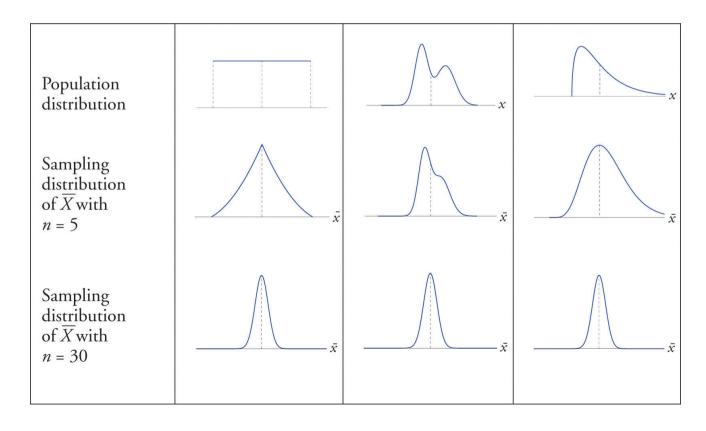
#### Standard error of the mean

$$\hat{\sigma}_{ar{X}} = rac{\hat{\sigma}}{\sqrt{n}} = rac{s}{\sqrt{n}}$$

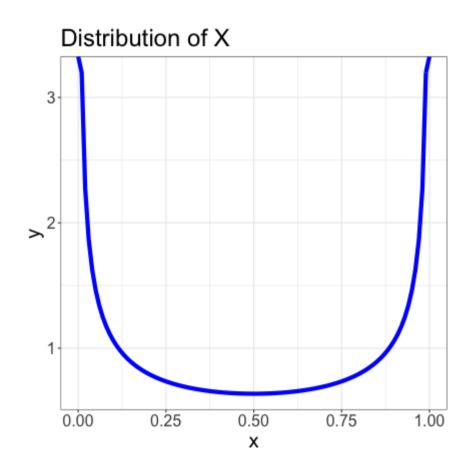
- ullet The standard error (SE) of the mean reflects uncertainty about the value of the population mean  $\mu$
- The CLT assumes a 'large enough' sample:
  - when the sample size is ~30 or more, the normal distribution is a good approximation for the sampling distribution of the mean
  - $\circ$  for smaller samples, the SE  $\frac{s}{\sqrt{n}}$  is an underestimate

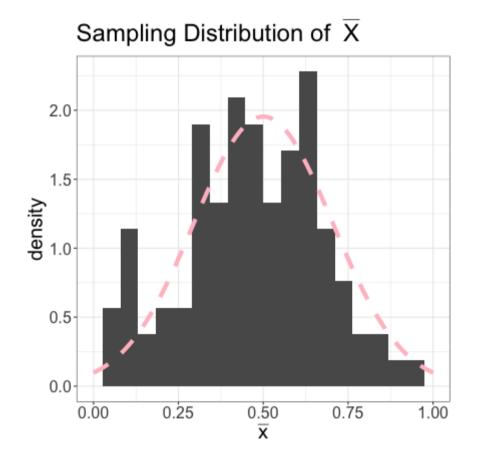
# CLT applies to any population (regardless of distribution)

Let  $X_1, X_2, \ldots, X_n$  be a random sample from a population with a non-normal distribution. If the sample size n is sufficiently large, then the sampling distribution of the mean will be approximately normal:  $\bar{X} \sim N(\mu, \frac{\sigma^2}{n})$ 



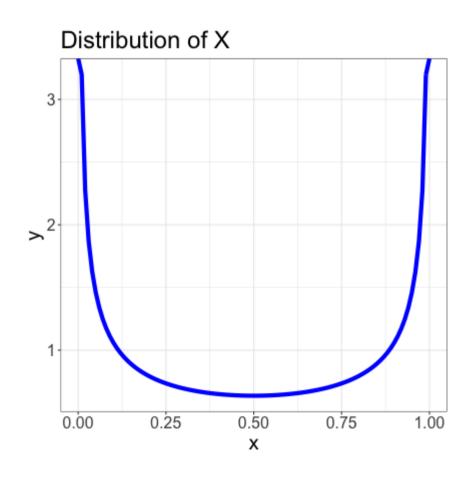
# Illustration (n = 3)





On right: dashed pink line is  $N(\mu,\sigma^2/n)$ 

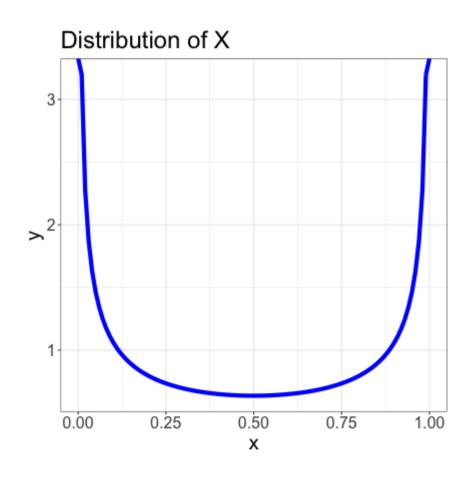
# Illustration (n = 10)



Sampling Distribution of  $\overline{X}$ density 0.50 X 0.25 0.75 0.00 1.00

On right: dashed pink line is  $N(\mu, \sigma^2/n)$ 

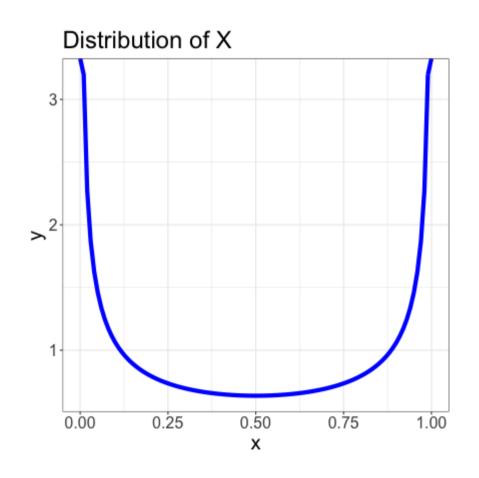
# Illustration (n = 30)

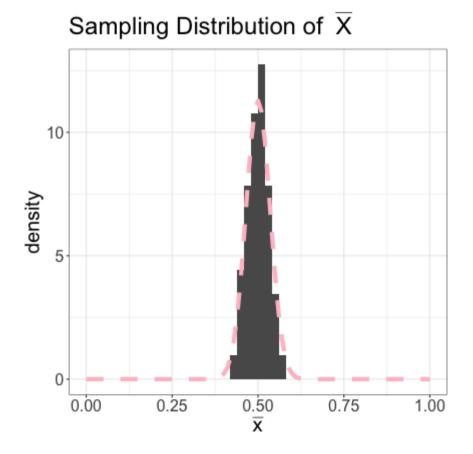


Sampling Distribution of  $\overline{X}$ 6 density 0.25 0.50 0.75 0.00 1.00

On right: dashed pink line is  $N(\mu,\sigma^2/n)$ 

# Illustration (n = 100)





On right: dashed pink line is  $N(\mu,\sigma^2/n)$ 

### **Hypothesis Testing**

- **Hypothesis:** A *testable (falsifiable)* idea for explaining a phenomenon
- Statistical hypothesis: A hypothesis that is testable on the basis of observing a process that is modeled via a set of random variables
- **Hypothesis Testing:** A formal procedure for determining whether to *accept* or *reject* a statistical hypothesis
- Requires comparing two hypotheses:
  - $\circ$   $H_0$ : null hypothesis
  - $\circ H_A$  or  $H_1$ : alternative hypothesis

### Hypothesis Testing: motivating example

• The expression level of gene g is measured in n patients with disease (e.g. cancer), and m healthy (control) individuals:

$$z_1, z_2, \ldots, z_n$$
 and  $y_1, y_2, \ldots, y_m$ 

• Is gene *g* is differentially expressed in cancer vs healthy samples?

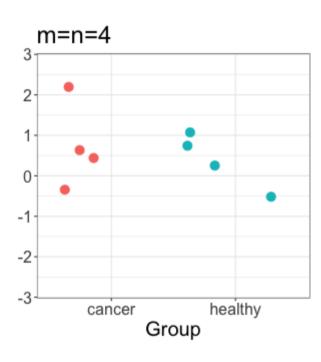
$$egin{array}{ll} \circ & H_0: \mu_Z = \mu_Y \ \circ & H_A: \mu_Z 
eq \mu_Y \end{array}$$

• In this setting, hypothesis testing allows us to determine whether observed differences between groups in our data are *significant* 

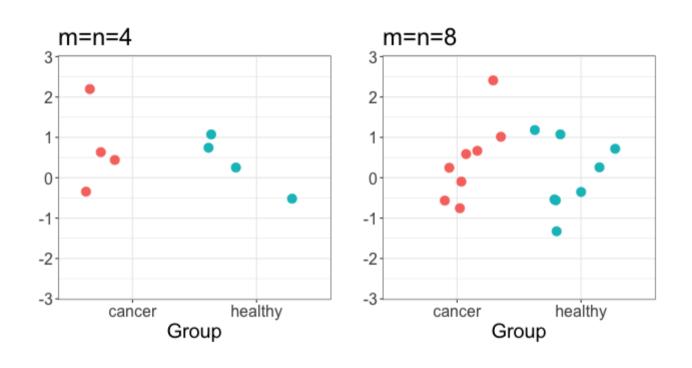
### Steps in Hypothesis Testing

- 1. Formulate your hypothesis as a statistical hypothesis
- 2. Define a test statistic (RV) that corresponds to the question. You typically know the expected distribution of the test statistic *under the null*
- 3. Compute the p-value associated with the observed test statistic under the null distribution  $p(t|H_0)$

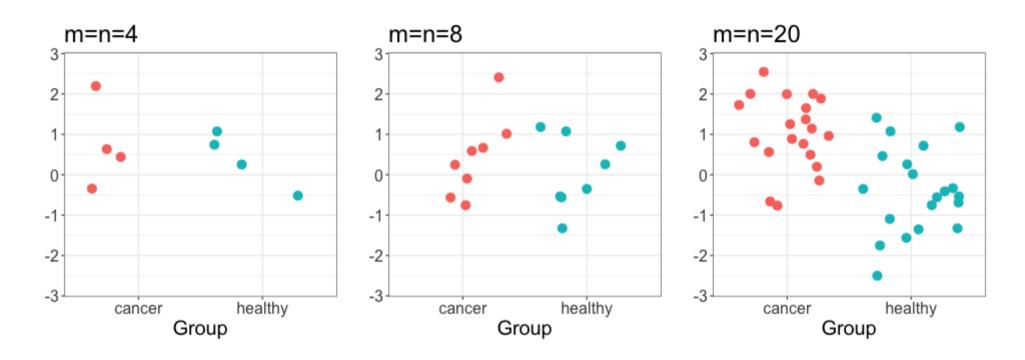
## Motivating example (cancer vs healthy gene expression)



## Motivating example (cancer vs healthy gene expression)

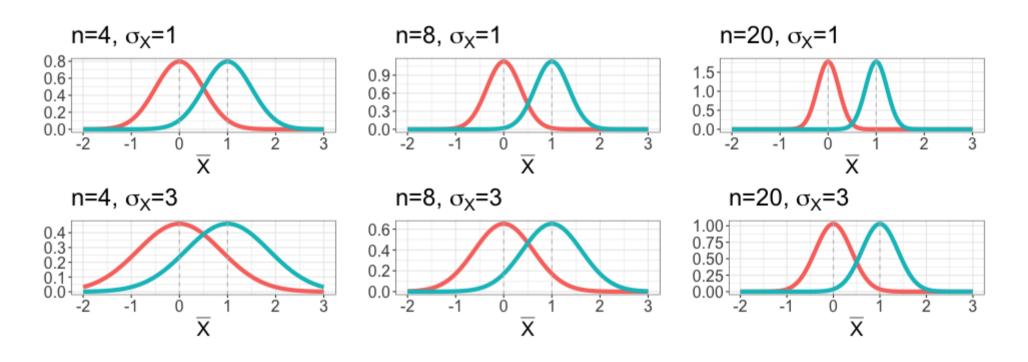


## Motivating example (cancer vs healthy gene expression)



All three samples drawn from iid Normal distributions with equal variance and  $\mu_Z - \mu_Y = 1$ 

#### Is there a **significant** difference between the two means?



- Mean difference needs to be put into context of the *spread* (standard deviation)
- Also depends on the sample size

#### t-statistic / t-test

• Measures difference in means, adjusted for spread/standard deviation:

$$t=rac{ar{z}-ar{y}}{SE_{ar{z}-ar{y}}}$$

for  $z_1, z_2, \ldots, z_n$  expression measurements in healthy samples and  $y_1, y_2, \ldots, y_m$  cancer samples

• Standard error estimate for the difference in means:

$$SE_{ar{z}-ar{y}}=s_p\sqrt{rac{1}{n}+rac{1}{m}} ext{ , where } s_p^2=rac{s_z^2+s_y^2}{(n-1)+(m-1)}$$

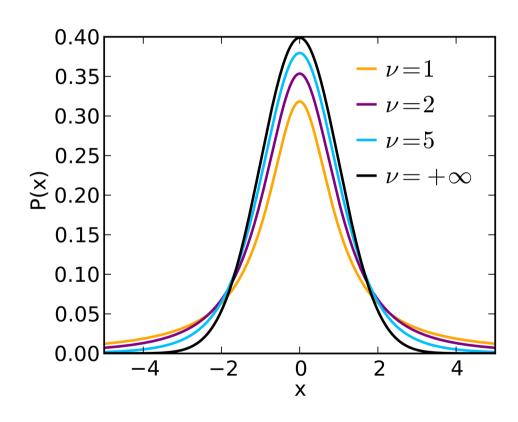
#### t-test

- From the theory, we know the distribution of our test statistic, if we are willing to make some assumptions
- If we assume:
  - Z and Y are normally distributed
  - Z and Y have equal variance

Then our t-statistic follows a t distribution with m+n-2 degrees of freedom

$$t \sim t_{n+m-2}$$

#### t distribution



- t statistic value tells us how extreme our observed data is relative to the null
- obtain p-value by computing area to the left and/or right of the t statistic (one-sided vs twosided)

#### Summary

- Random variables are variables that have an associated probability distribution
- Any statistic of sampled data is an RV, and hence has an associated probability distribution
- The CLT gives us the sampling distribution of the mean
- Hypothesis testing gives us a framework to assess a statistical hypothesis under the null