

## THURSDAY (all times in PT)

<b>8:30 – 9:15</b>	Alie	HWE/LD	Hardy Weinberg Equilibrium, and Linkage Disequilibrium
<b>BREAK</b>			
<b>9:30-10:15</b>	Alie	Population Structure	Ancestry and Principal Component Analysis
<b>BREAK</b>			
<b>10:30-11:15</b>	Sara	Study Designs	Types of genetic epidemiology studies, imputation
<b>LUNCH BREAK</b>			
<b>11:45 – 12:30</b>	Alie	Association studies	Conducting association studies and calculating odds ratios
<b>BREAK</b>			
<b>12:45-1:30</b>	Sara	GWAS	Genome wide association studies
<b>BREAK</b>			
<b>1:45 – 2:30</b>	Sara/Alie	Office Hours	Stop by to ask questions from the day, or schedule time to discuss your own project.

# **SISG 2022:**

## **Module 11**

### **Genetic Epidemiology**

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# **Analysis of Association Studies**



## Genetics Podcasts of Note

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- > **Freakonomics interview with 23andMe founder:**
  - <https://freakonomics.com/podcast/23andme/>
- > **Future of Everything interview with Carlos Bustamonte:**
  - <https://scopeblog.stanford.edu/2019/04/18/the-future-of-genomics-a-podcast-featuring-stanford-geneticists/>

# Learning objectives

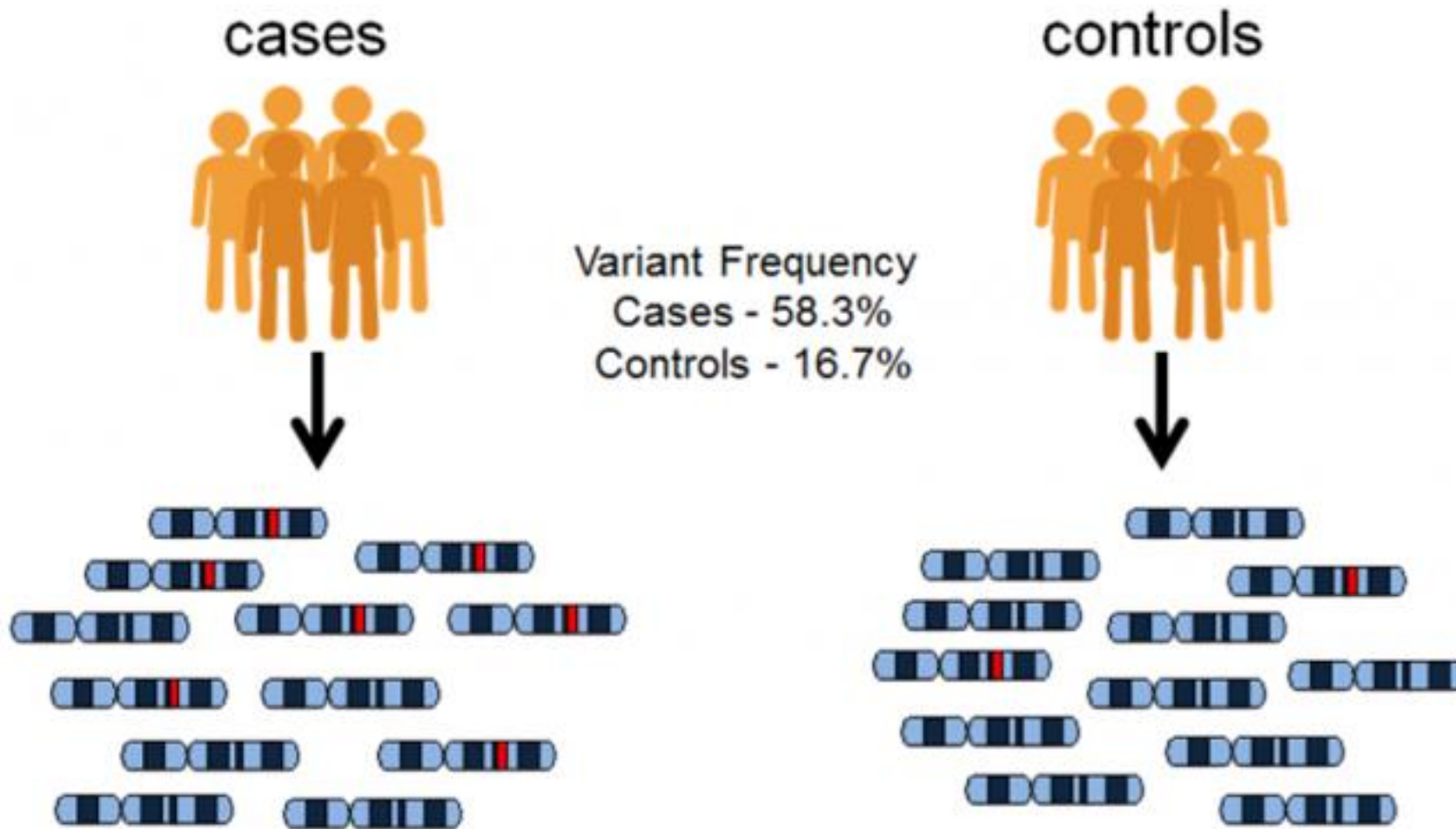
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- > Calculate and interpret odds ratios in case/control genetic association studies.
- > Interpret quantitative trait association studies.

# Association studies

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- > **Determine if a particular genetic feature (exposure) co-occurs with a trait (disease) more often than would be expected by chance.**
- > **Binary: Calculate 'odds' of an outcome occurring.**
  - Framed as an 'odds ratio', the odds of an outcome with an exposure (genotype) in relation to the odds of an outcome without the exposure (reference genotype).
- > **Quantitative: calculate change in an outcome for every unit increase of an exposure.**



\*Still probabilistic – with a variant allele, you are MORE LIKELY to develop heart disease, but it is not guaranteed that you will, or that you won't if you have the 'wildtype' allele.

## **“Odds”**

the chances or likelihood of something happening

## **“Odds Ratio”**

the likelihood of something happening in one group in relation to the likelihood of something happening in another group

# Odds ratio

The odds ratio is our measure of association for a case-control study. It tells us whether and how much an exposure increases the likelihood of our outcome of interest. We need to look at two things:

**The estimate** -- the odds ratio itself. How big is the connection between an exposure and an outcome? Are those with an exposure more likely to have the outcome?

**The p-value** -- how certain are we that the odds ratio didn't just happen by chance?



# Association testing in case-control studies

		Disease status		
		Cases	Controls	Total
Genotype	AA/AT	a	b	a+b
	TT	c	d	c+d
Total		a+c	b+d	

# measure of events out of all possible events (Ratio) vs ratio of events to non-events (Odds)

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$$RR = \frac{\text{Risk of event in the Treatment group}}{\text{Risk of event in the Control group}} = \frac{a/(a + b)}{c/(c + d)}$$

$$OR = \frac{\text{Odds of event in Treatment group}}{\text{Odds of event in Control group}} = \frac{a/b}{c/d} :$$

If an outcome occurs 10 out of 100 times, the risk is 10%  
But the odds is 10/90 = 11.1%

# Association testing in case-control studies

		Disease status		
		Cases	Controls	Total
Genotype	AA/AT	a	b	a+b
	TT	c	d	c+d
Total		a+c	b+d	

Calculate Odds Ratio (OR) as the odds of being a case among genotype AA/AT divided by the odds of being a case among genotype TT.

$$\frac{a/b}{c/d} = \frac{ad}{bc}$$

$$OR = \frac{ad}{bc}$$

# Association testing in case-control studies

		Disease status		
		Cases	Controls	Total
Genotype	AA/AT	a	b	a+b
	TT	c	d	c+d
Total		a+c	b+d	

**$H_0$ : OR = 1 (no association)**

**OR > 1 indicates increased odds**

**OR < 1 indicates decreased odds  
(protective)**

# Confidence intervals for odds ratios

		Disease status	
		Cases	Controls
Genotype	AA/AT	a	b
	TT	c	d

$$OR = \frac{a/b}{c/d} = \frac{ad}{bc}$$

$$s.e(\log(OR)) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

\*log transformation  
allows linear algebra

Confidence interval:  $e^{\log(OR) \pm z_{\alpha/2} \times s.e(\log(OR))}$

Lower limit of 95% confidence interval:  $e^{\log(OR) - 1.96 \times s.e}$

Upper limit of 95% confidence interval:  $e^{\log(OR) + 1.96 \times s.e}$

## Odds ratio - rs2233385 and neuropsychiatric adverse events

The odds of having a neuropsychiatric reaction to tamiflu for people with two variant rs2233385 alleles are 30x the odds of having a neuropsychiatric reaction for people without any copies of that variant.

Formula:

The odds of the outcome among people with the exposure are odds ratio times the odds of the outcome among people without the exposure.

## Zoom breakout Q1:

Calculate— odds ratio and 95% confidence interval

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$$OR = \frac{ad}{bc}$$

$$s.e(\log(OR)) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

# Why do we even use odds and odds ratios???

The odds ratio allows us to calculate the associations between an exposure and an outcome without needing the frequency of the exposure in the general population

(very useful to rare exposures, such as rare diseases).

(we'd have to sample A LOT of people to get a true population picture and even pick up one or two cases of the disease)

The  $\log(\text{odds})$  allows us to transform this weird variable into a linear form, which is easier for us to fit to models, adjust for covariates, and interpret the output.



# Often use logistic regression for case-control analyses

Allows you to adjust for relevant factors

- Population stratification, age, sex, matching variables etc

$$\ln\left(\frac{p}{1-p}\right) = \alpha + \beta_1 \mathbf{g} + \beta_2 \mathbf{x}_1 + \dots + \beta_{k+1} \mathbf{x}_k \quad (\mathbf{g} \text{ is genotype, } \mathbf{x}_1, \dots, \mathbf{x}_k \text{ are covariates})$$

Coefficients are estimated using maximum likelihood estimation (MLE)

- >  $\ln\left(\frac{p}{1-p}\right) = \log \text{ odds of an outcome}$
- > **Test  $H_0: \beta_1 = 0$**
- > **The odds ratio is  $OR = e^{\beta_1}$**
- >  **$\beta_1 = \text{SNP effect (log(OR))} \rightarrow e^{\beta_1} = OR$**

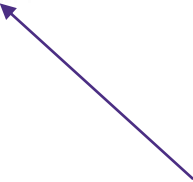
# Logistic regression model and interpretation

Logistic regression works on likelihoods. The likelihood of an outcome given a change in exposure. The dependent variable is the log of the odds.

$$\text{logit}(p) = \log\left(\frac{p(y=1)}{1 - (p=1)}\right) = \beta_0 + \beta_1 \cdot x_{i2}$$

what we are testing: how much does the likelihood of an outcome change when we change our exposure “x”

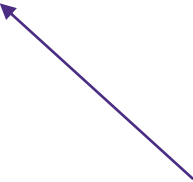
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$$\text{logit}(p) = \log\left(\frac{p(y=1)}{1-p(y=1)}\right) = \beta_0 + \beta_1 \cdot x_{i2}$$


For additive model, “x” is 0 when the allele of interest is not present, 1 when there is one copy, and 2 when there are two copies of the allele

\*depends on suspected mode of inheritance

# Logistic regression model and interpretation

$$\text{logit}(p) = \log\left(\frac{p(y=1)}{1-p(y=1)}\right) = \beta_0 + \beta_1 \cdot x_{i2}$$


Log (odds of reaction when one copy of the allele is present) =  $B_0 + B_1 \cdot 1$

Log (odds of reaction outcome when zero copies of the allele are present) =  $B_0 + B_1 \cdot 0 = B_0$

Log (odds of reaction with one copy of the allele) = Log (odds of reaction outcome with zero copies of the allele) +  $B_1$

Log(odds of reaction with one copy of the allele) - log(odds of reaction outcome with zero copies of the allele) =  $B_1$

Math principle:  $\log(x) - \log(y) = \log(x/y)$

Log(odds of reaction with one copy of the allele/odds of reaction outcome with zero copies of the allele) =  $B_1$

Log(odds ratio of outcome with vs without allele) =  $B_1$

Then, we can take the "e" exponent of each side to get our Odds Ratio =  $e^{B_1}$

# Logistic regression intercept in R

Coefficients:

	Estimate	Std. Error	z value	Pr(> z )	
(Intercept)	-2.944	1.026	-2.870	0.00410	**
l genotype	3.402	1.051	3.236	0.00121	**

(x) This genotype variable is coded 0/1/2. We want to look at whether having this allele changes the odds of an outcome.

This estimate gives us the estimated difference in log odds between having a genotype that is different by one copy of the variant.

# Logistic regression intercept

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This estimate gives us the estimated difference in log odds between having a genotype that is different by one copy of the variant.

**To find the odds ratio of outcome between those with 1 copy of an allele and zero copies of the allele, we calculate  $e^{3.402}$**

# Resources to learn R coding for genetic analysis

- Packet from Sara on the course website
- Dr. Tim Thornton's Course in SISG: R exercises  
<http://faculty.washington.edu/tathornt/SISG2020.html>
- edX: <https://www.edx.org/learn/computer-programming>
- Epidemiologist R Handbook:  
<https://epirhandbook.com/index.html>

# Quantitative outcome genetic association

- Linear regression (instead of logistic)
- Additive coding of SNP (0,1,2) most common

$$Y = \alpha + \beta * SNP + X$$

- $\beta$  = SNP effect (for every SNP, unit increase in outcome)
  - We do not need to use the exponent in quantitative outcomes
- SNP = covariate coded (0,1,2)
- X = additional covariates (e.g. sex, study, age, PCs from population stratification)



# Importance of setting your reference allele

Odds ratio when AA is reference:  $\frac{2}{3} / \frac{1}{3} = \frac{2}{3} * 3 = 2$

**The odds of the outcome are 2x more likely among those with CC genotype compared to among those with the AA genotype.**

Odds ratio when CC is reference.  $\frac{1}{3} / \frac{2}{3} = \frac{1}{3} * \frac{3}{2} = 0.5$

**The odds of the outcome are ½ as likely among those with AA genotype compared to among those with the CC genotype.**

**These are the saying the same thing! But the language matters.**

Always know and be purposeful on your reference

In epidemiology, the reference group always matters.

**Exposure** (gene allele reference)

**Outcome** (some outcomes have no “direction”) brown vs black hair

**Population** (other factors are always involved, i.e. age, diet, access to care).

## Zoom breakout #2 and #3

- Interpret the regression output from association studies (one binary and one quantitative trait)

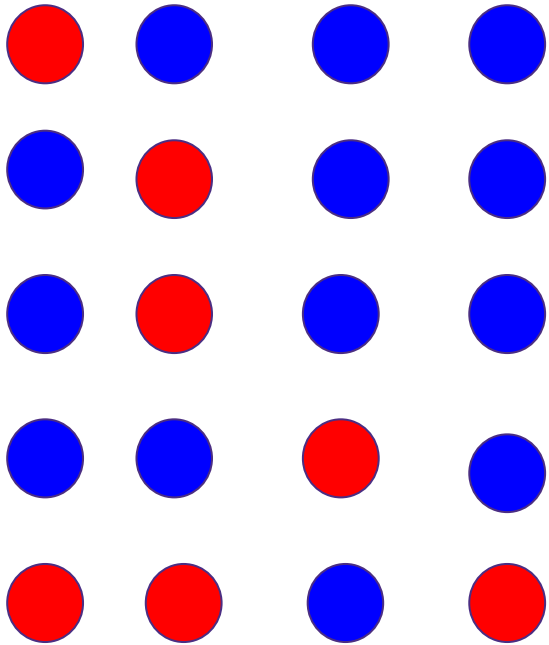
# Summary

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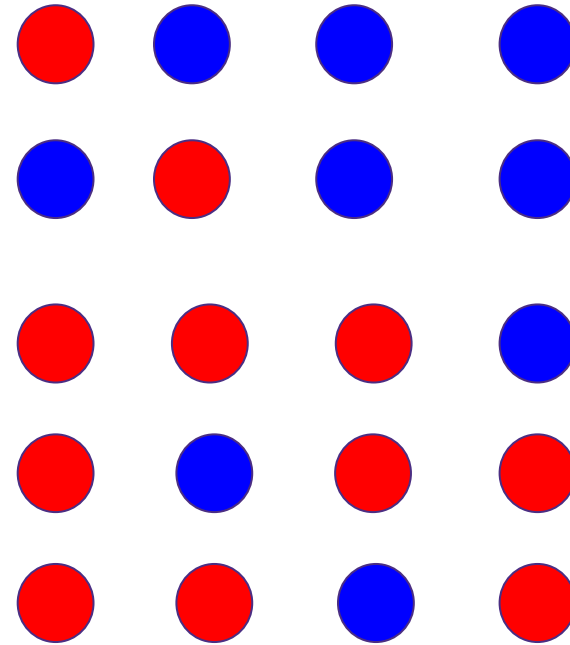
- > **Odds ratios give the odds of an outcome in relation to a reference.**
- > **Linear and logistic regression allow adjustment for other factors.**

# Case-control study - is red associated?

CONTROLS

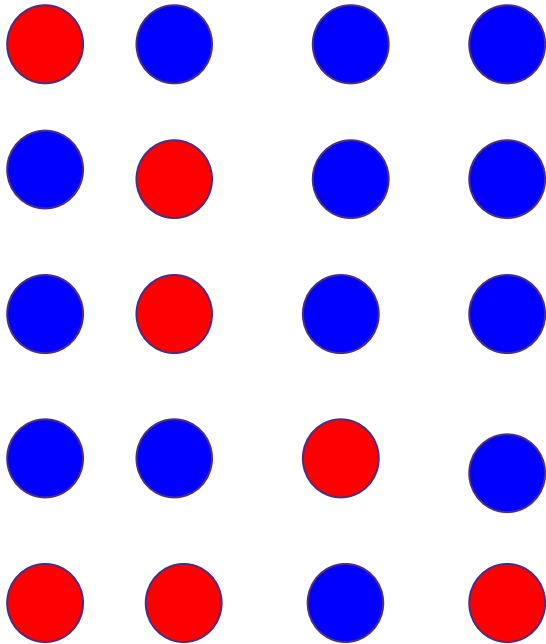


CASES

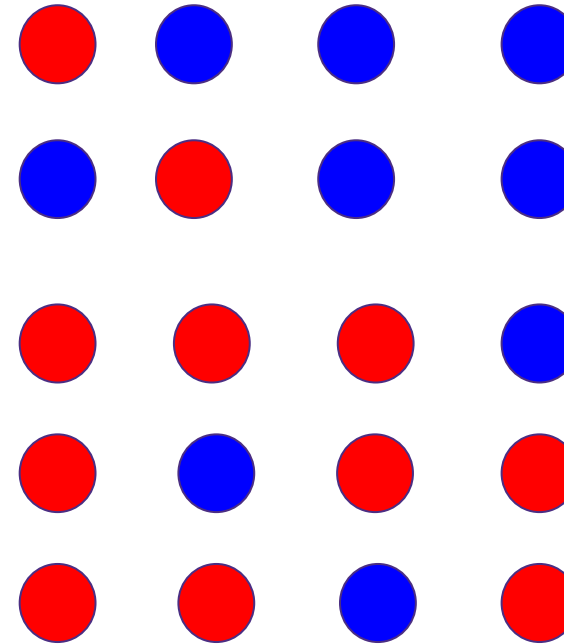


# Case-control study - is red associated?

CONTROLS



CASES



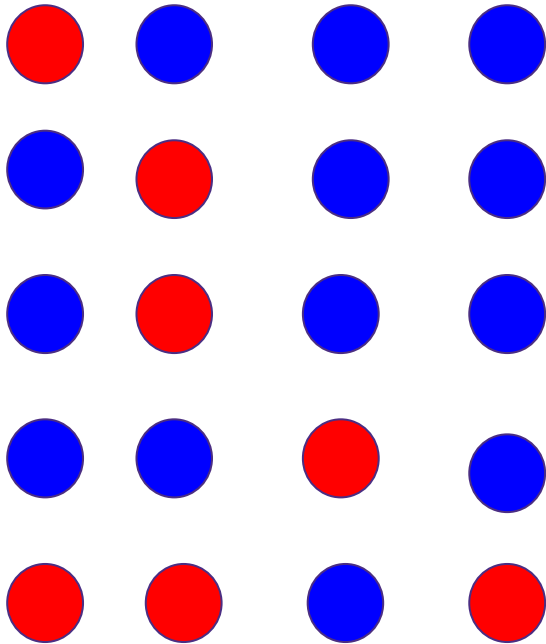
**Calculate odds ratio of being red between cases and controls**

Odds are 7/13 in controls

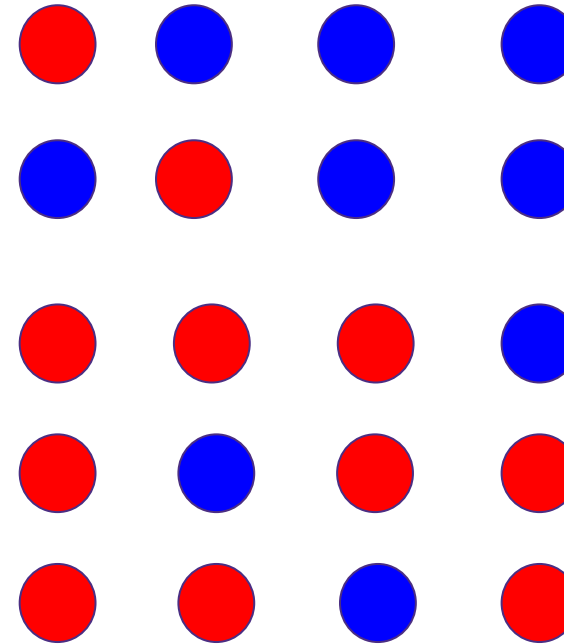
Odds are 11/9 in cases

# Case-control study - is red associated?

CONTROLS



CASES



**Calculate odds ratio of being red between cases and controls**

Odds are 7/13 in controls

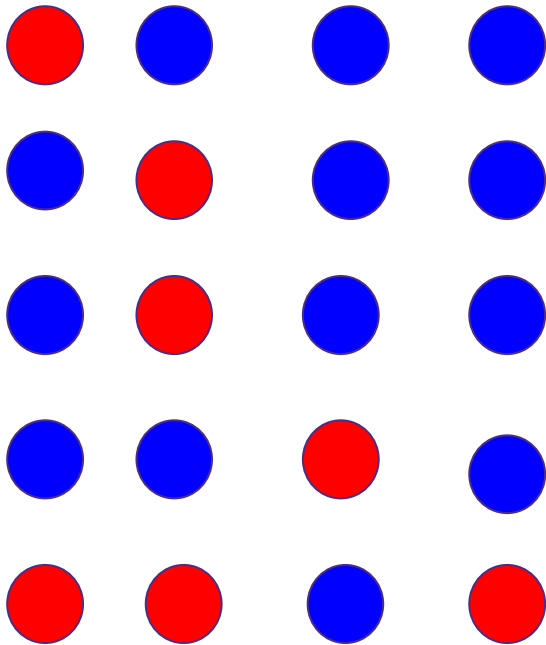
Odds are 11/9 in cases

Odds ratio is  $(11/9) / (7/13) = 2.27!$

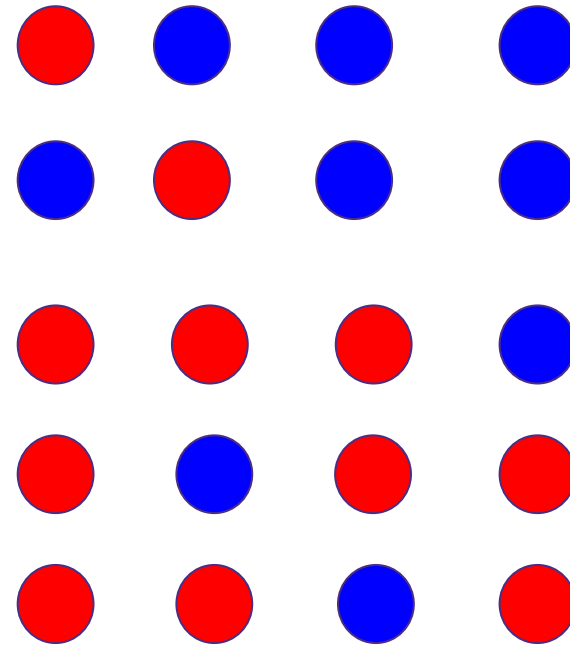
# Case-control study - is red associated?

Cases are 2.27x times as likely to be red as controls

CONTROLS



CASES

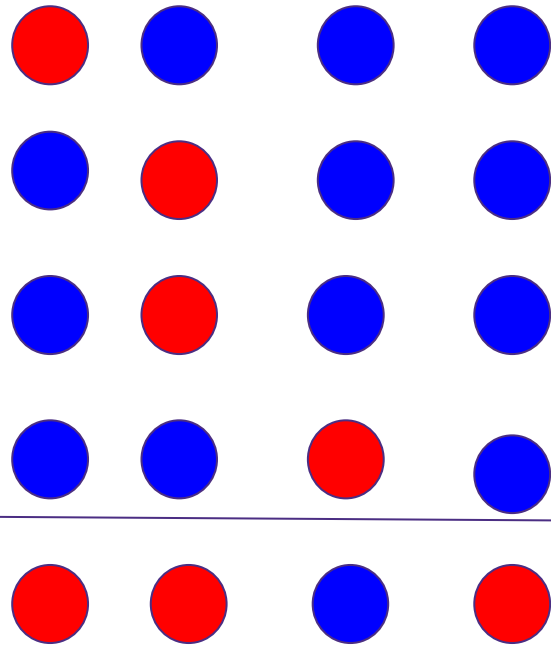




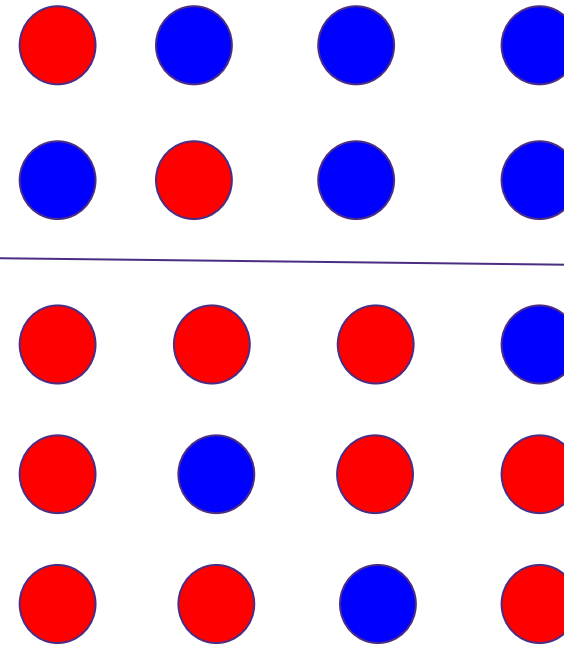
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CONTROLS



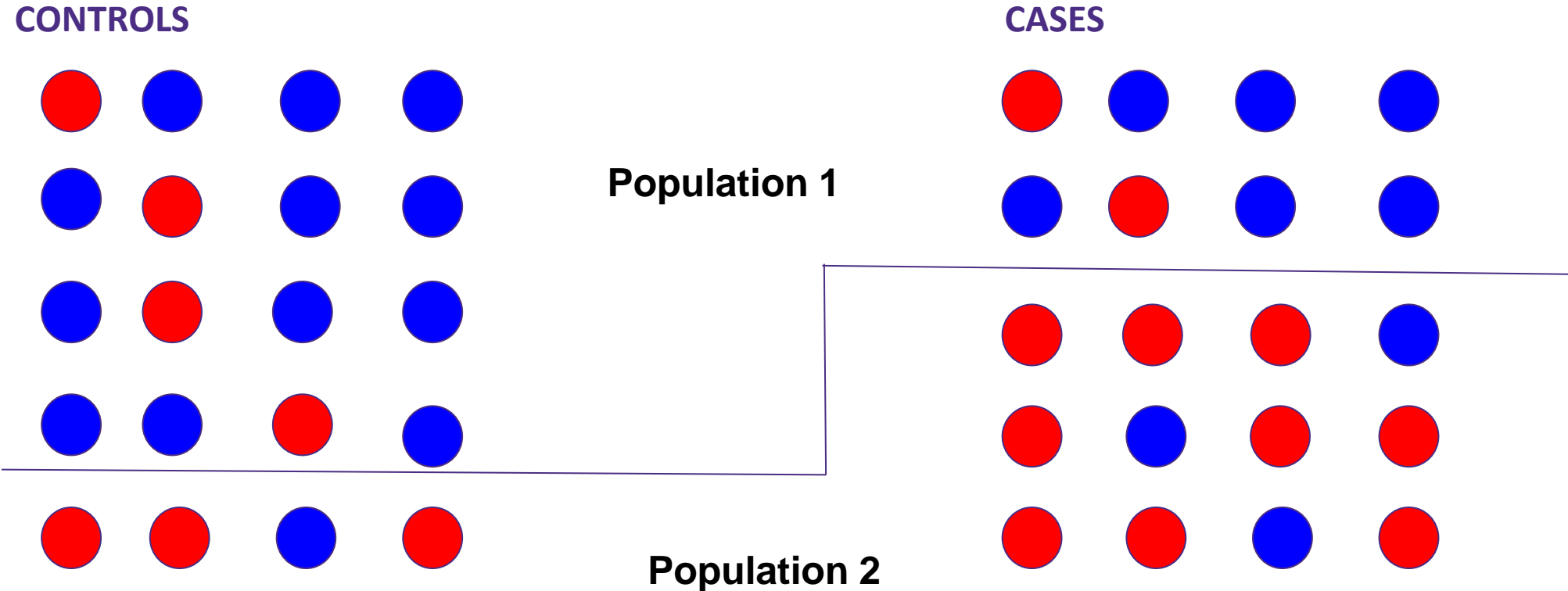
CASES



Population 1

Population 2

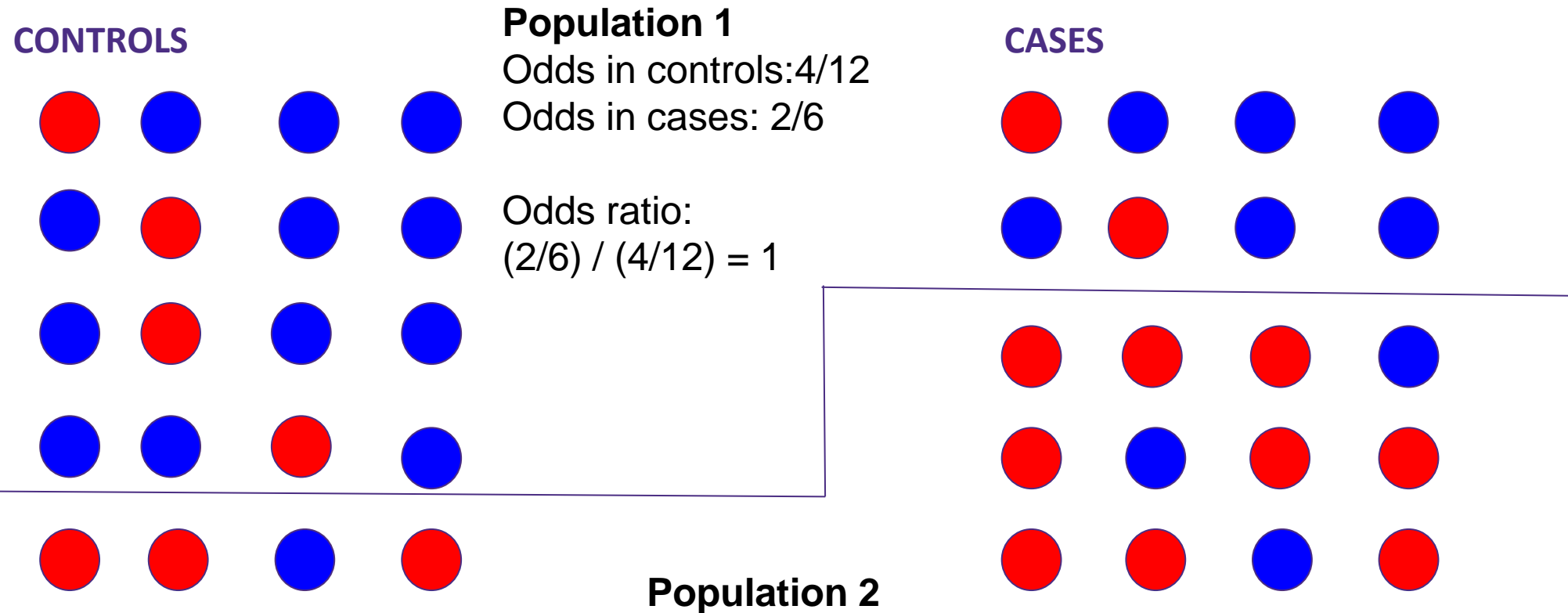
# Case-control study - is red associated?



Now recalculate the odds ratios in population 1 and population 2 separately

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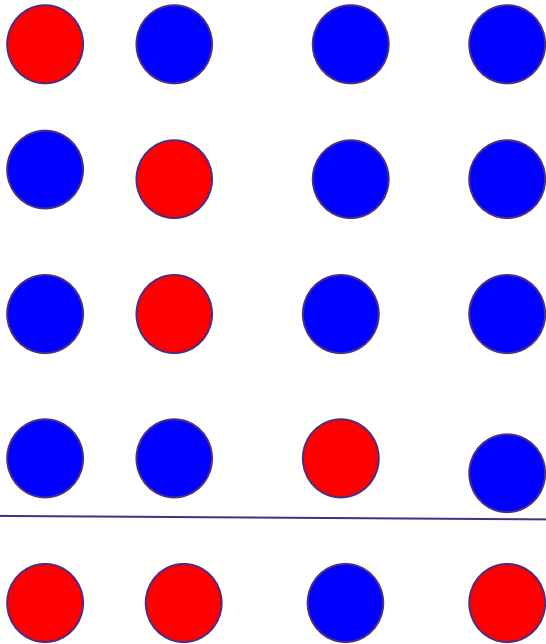
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# Case-control study - is red associated?

CONTROLS



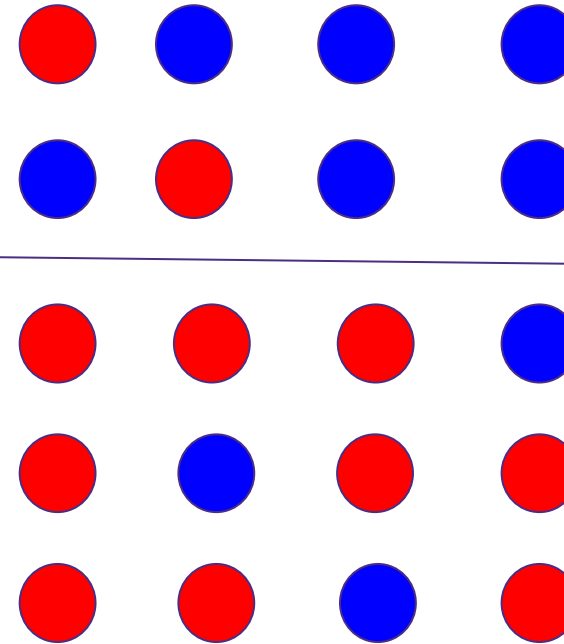
**Population 1**

Odds in controls: 4/12

Odds in cases: 2/6

Odds ratio:  
 $(2/6) / (4/12) = 1$

CASES



**Population 2**

Odds in controls: 3/1

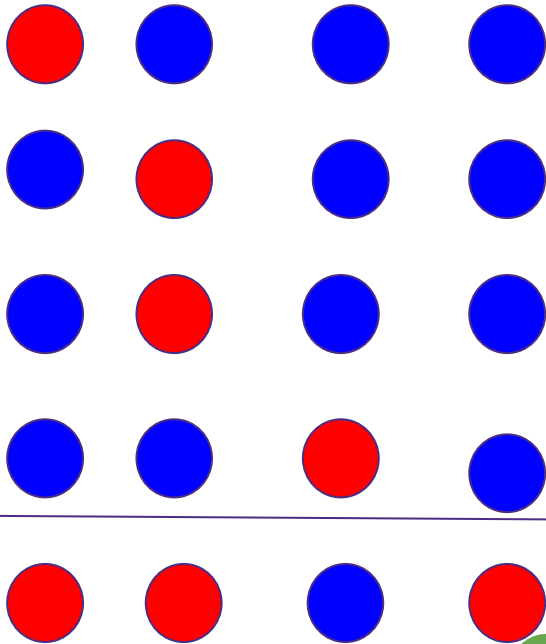
Odds in cases: 9/3

Odds ratio:  
 $(9/3) / (3/1) = 1$

Now recalculate the odds ratios in population 1 and population 2 separately

# Case-control study - is red associated?

CONTROLS

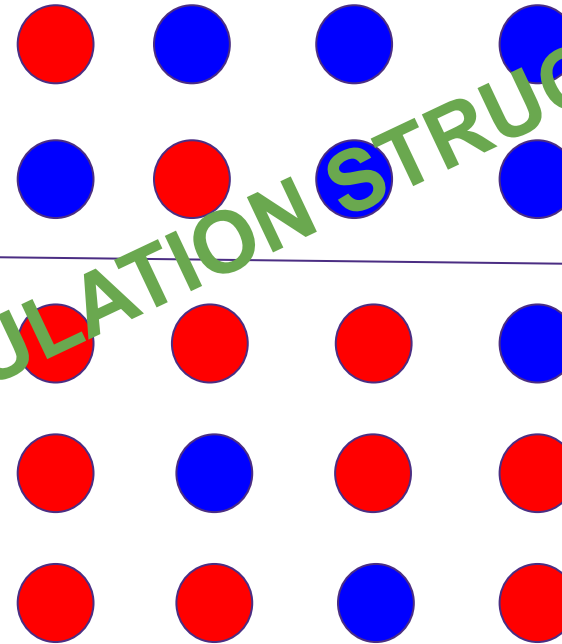


Population 1

Odds in controls: 4/12  
Odds in cases: 2/6

Odds ratio:  
 $(2/6) / (4/12) = 1$

CASES



Population 2

Odds in controls: 3/1  
Odds in cases: 9/3

Odds ratio:  
 $(9/3) / (3/1) = 1$

CONFOUNDING BY POPULATION STRUCTURE