

Hypothesis testing: one sample

Is P53 gene expressed at a **lower level** in **cancer** patients than in **healthy** people?

- We are interested if a P53 gene expression is lowered in **population of cancer patients** compared to the **healthy population**.
- We know that mean gene expression in the **healthy population** is $\mu_h = 50$ mRNAs/cell. We are interested in deciding whether or not the mean expression in **cancer population** is lower than in **healthy population**. Let's call hypothesis H_1 . Here H_1 is one-sided
- If we asked: cancer is not equal to healthy H_1 would be a two-sided hypothesis
- Assume we have a sample of **100 cancer patients** with **sample mean $\bar{x} = 48$ mRNAs/cell** and **standard deviation $\sigma = 10$ mRNA/cell**
- Can we use our sample to reject the “business as usual” or null hypothesis H_0 : **cancer = healthy** and select one-sided hypothesis H_1 : **cancer < healthy**

Two types of errors

	decide H_0	decide H_1
true H_0 probability	Correct action $1 - \alpha$	Type I error α
true H_1 probability	Type II error β	Correct action power = $1 - \beta$

$$\alpha = P(\text{type I error}) = P(\text{reject } H_0 \text{ when } H_0 \text{ is true})$$

Sometimes the **type I error probability α**
is called the **significance level**, or the **α -error**

Instructions: get α from your boss or PI (e.g., 5% or 1%)

Prob(H_0 is true given the sample data) $< \alpha$
→ reject H_0 and accept H_1

Prob(H_0 is true given the sample data) $> \alpha$
→ accept H_0 and reject H_1

Type II error is much harder to estimate. Will deal with it later

P-Values of Hypothesis Tests

- **P-value**: what is the probability to get the observed value of sample mean of $\bar{x} = 48$ mRNAs/cell (or even smaller) and $\sigma = 10$ mRNAs/cell in a healthy population with $\mu_h = 50$ mRNAs/cell
- If **P-value is small** – the null hypothesis is likely wrong and thus, the **probability of making a type I error** (incorrectly rejecting the null hypothesis) **is small**
- P-value answers the question: if I reject the null hypothesis H_0 based on the sample, what is the probability that I am making a type I error?

P-Value vs α in Hypothesis Testing

- Problem with using a predefined α : you don't know by how much you exceeded it
- Another approach is to calculate $\text{Prob}(H_0 \text{ is true given the sample data})$ referred to as P-value.
It is the smallest α that would lead to rejection of null hypothesis
- You give your boss the P-value and let him/her decide if it is good enough
- Routinely with big datasets in genomics and systems biology P-values can be $10^{-\text{large number} \sim 10-100}$. This number is used to judge the quality of the hypothesis

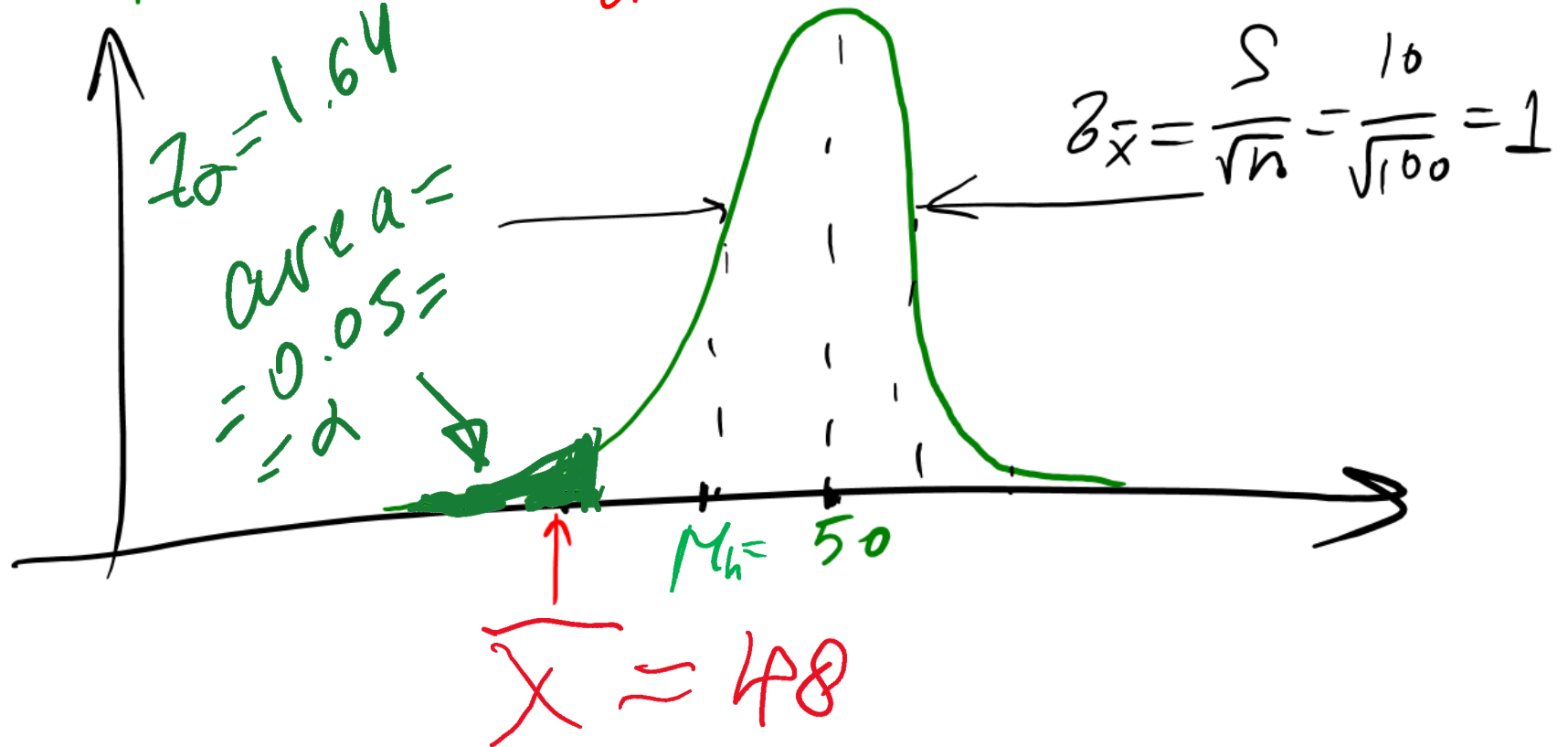
$$\mu_h = 50$$

$$H_0: \mu_c = \mu_h$$

$$n=100, \bar{X}=48, S=10$$

One-sided hypothesis

$$H_1: \mu_c < \mu_h$$



$$P\text{-value} = \text{Prob}(\bar{X}_h < 48 | H_0) =$$

$$\approx 2.5\%$$

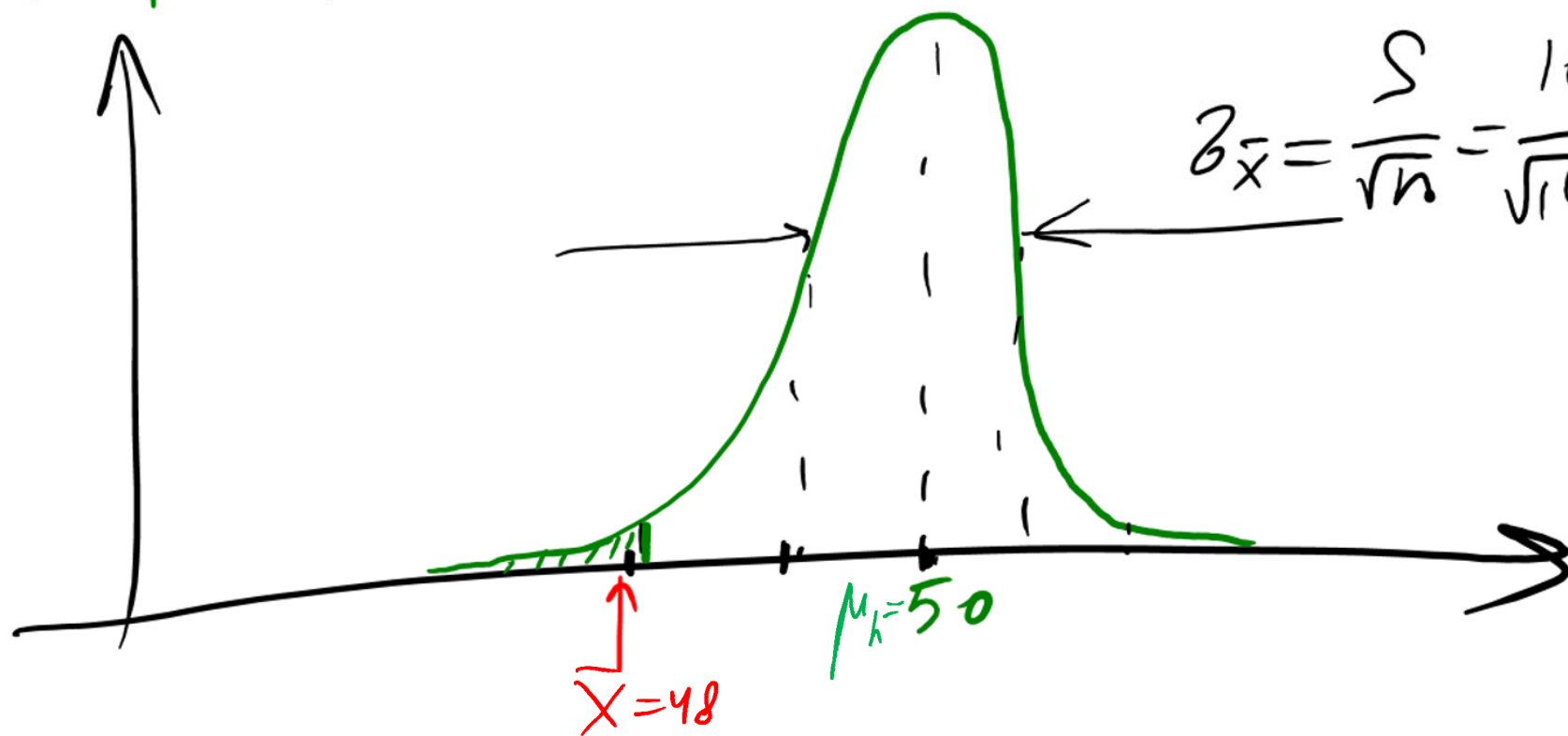
$$\mu_h = 50$$

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$$n=100, \bar{X}=48, S=10$$

$$H_1: \mu_c < \mu_h$$

$$z_{\bar{x}} = \frac{S}{\sqrt{n}} = \frac{10}{\sqrt{100}} = 1$$



$$\mu_h = 50$$

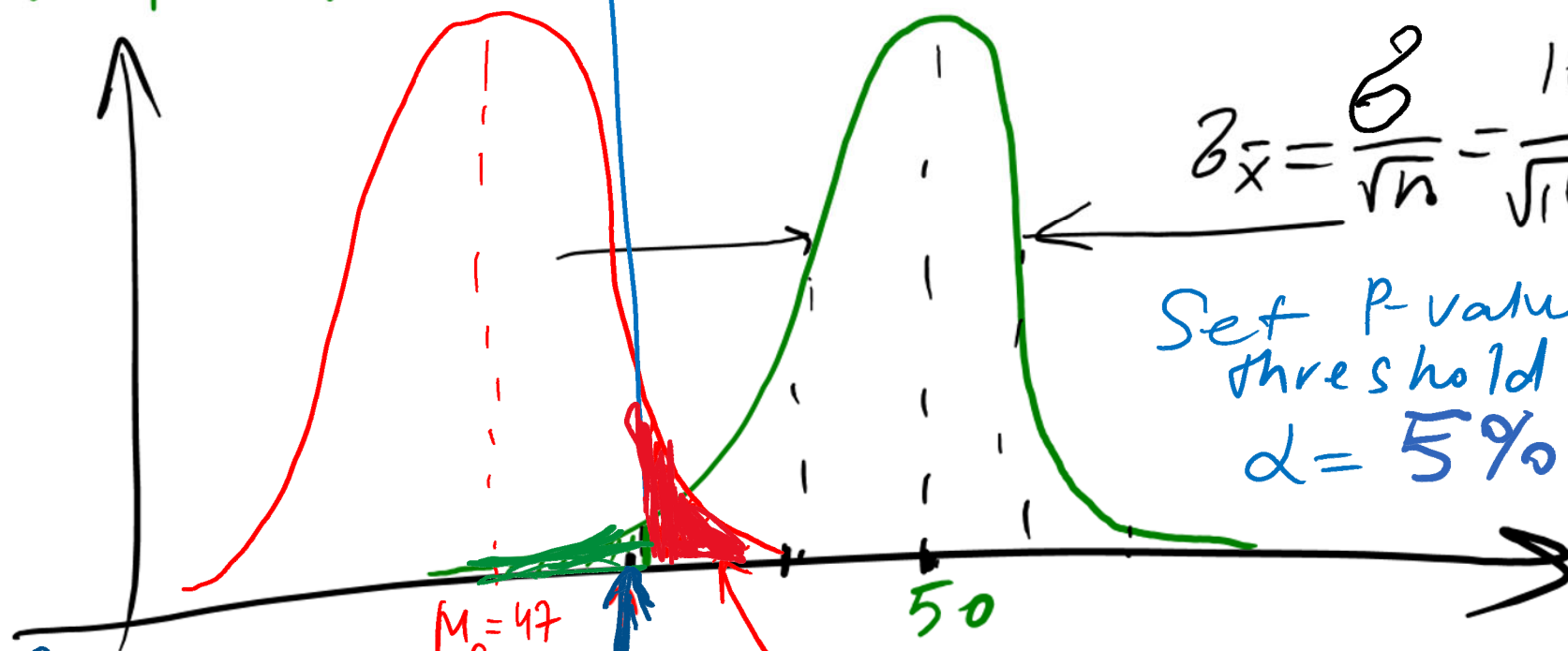
$$H_0: \mu_c = \mu_h$$

$$n=100, \bar{X}=48, \sigma=10$$

$$H_1: \mu_c < \mu_h$$

$$\sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}} = \frac{10}{\sqrt{100}} = 1$$

Set P-value threshold:
 $\alpha = 5\%$



$$\mu_h - z_{\alpha} \sigma_{\bar{x}} = 50 - 1.64 = 48.36$$

Type II error

$$\beta = P(\text{Accept } H_0 | H_1 \text{ is true}) = \int_{48.36}^{\infty} \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{(x-47)^2}{2}\right) dx =$$

$$\alpha = 1 - \Phi(1.64) = 5\%$$

$$= 1 - \Phi(1.36) = 8.8\%$$

Generalizations

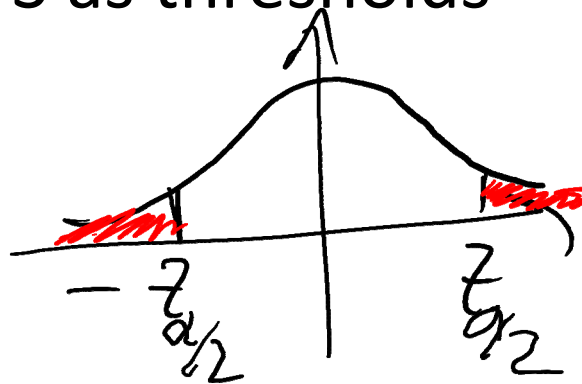
- What if H_1 is a two-sided hypothesis?

- A: P-value is $2(1-\Phi(|Z|))$, where $Z=(\bar{X}-\mu_0)/[S/\sqrt{n}]$

Compare it to: For one sided $\mu_1 > \mu_0$ it is $1-\Phi(Z)$

For one sided $\mu_1 < \mu_0$ it is $\Phi(Z)$

- If α is given, use $\mu_0 \pm z_{\alpha/2} * S$ as thresholds to reject the null hypothesis



- What if the sample size n is small (say $n < 10$):

- A: Use t-distribution with $n-1$ degrees of freedom for 2-sided $P\text{-value} = 2(1-\text{CDF_Tdist}(|T|))$

where $T=(\bar{X}-\mu_0)/[S/\sqrt{n}]$.

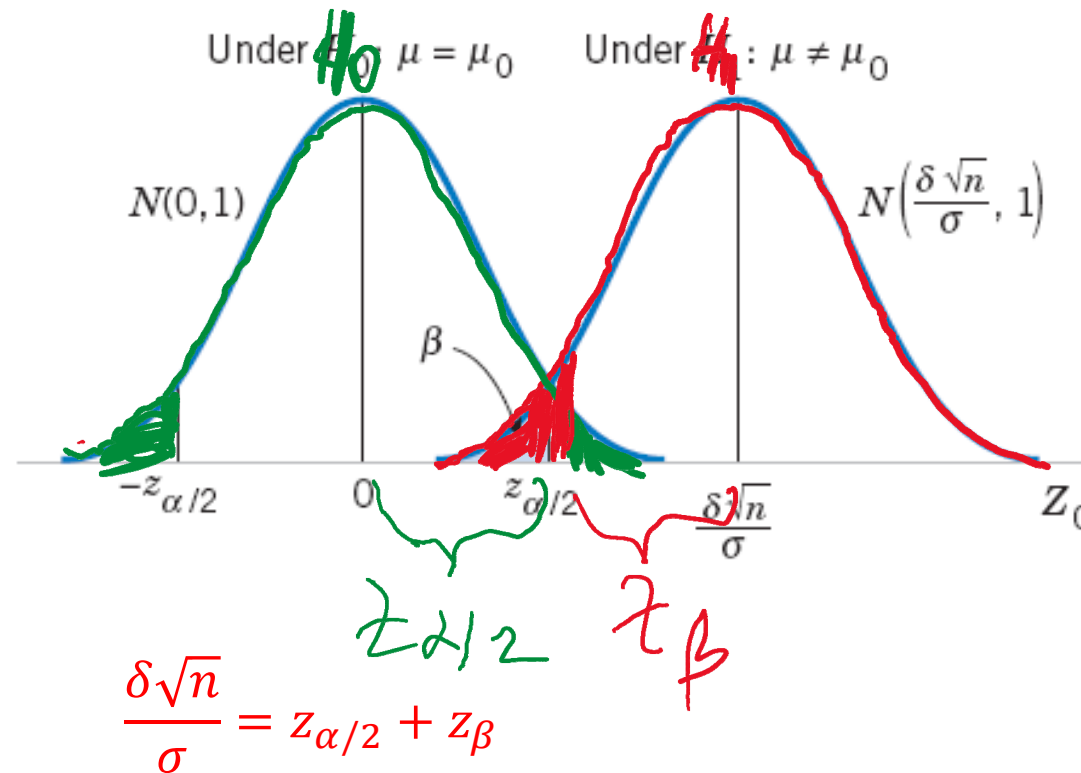
- For a given α use $\mu_0 \pm t_{\alpha/2, n-1} T$ to reject the null hypothesis

Type II Error and Choice of Sample Size

Assume you know the minimum $\delta = |\mu_1 - \mu_0|$ that you care about.

What is the minimal sample you should use to separate H_0 and H_1 hypotheses if your tolerance to type I and type II errors is α and β ?

Figure 9-9 The distribution of Z_0 under H_0 and H_1 .



$$n \cong \frac{(z_{\alpha/2} + z_{\beta})^2 \sigma^2}{\delta^2} \quad \text{where} \quad \delta = \mu - \mu_0 \quad (9-22)$$

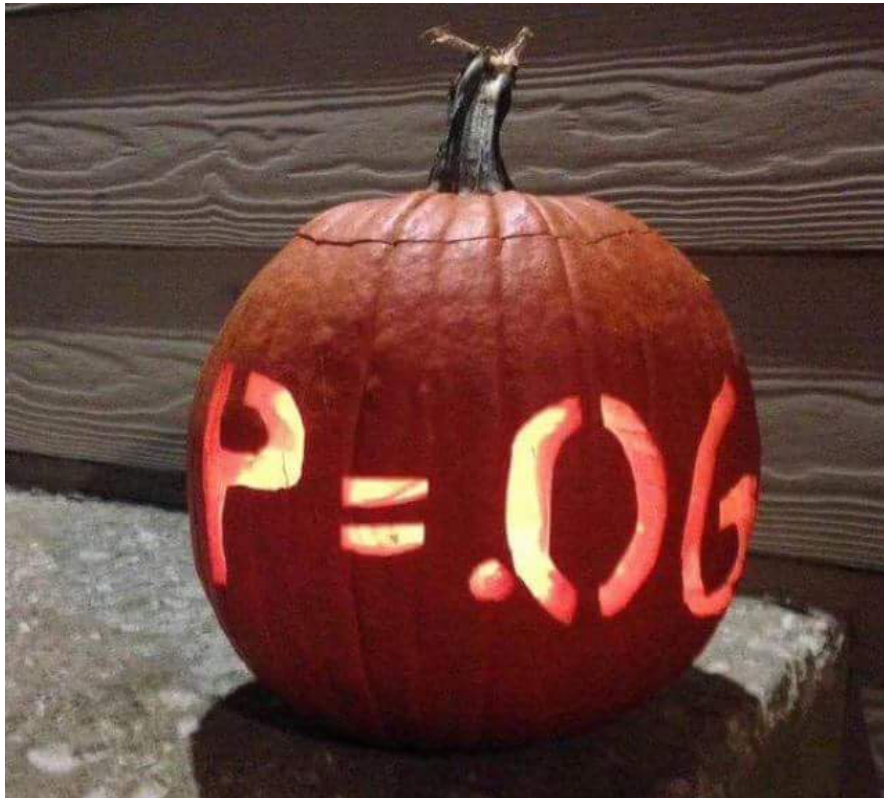
Standard notation to indicate P-value with

* ** ***
, ,

Table 11.1: A commonly adopted convention for reporting p values: in many places it is conventional to report one of four different things (e.g., $p < .05$) as shown below. I've included the "significance stars" notation (i.e., a * indicates $p < .05$) because you sometimes see this notation produced by statistical software. It's also worth noting that some people will write *n.s.* (not significant) rather than $p > .05$.

Usual notation	Signif. stars	English translation	The null is...
$p > .05$		The test wasn't significant	Retained
$p < .05$	*	The test was significant at $\alpha = .05$ but not at $\alpha = .01$ or $\alpha = .001$.	Rejected
$p < .01$	**	The test was significant at $\alpha = .05$ and $\alpha = .01$ but not at $\alpha = .001$.	Rejected
$p < .001$	***	The test was significant at all levels	Rejected

.....



Happy
Halloween!
(belated)

Credit: Trust me,
I'm a "Biologist"
Facebook community

<u>P-VALUE</u>	<u>INTERPRETATION</u>
0.001	]— HIGHLY SIGNIFICANT
0.01	
0.02	
0.03	
0.04	]— SIGNIFICANT
0.049	
0.050	]— OH CRAP. REDO CALCULATIONS.
0.051	
0.06	]— ON THE EDGE OF SIGNIFICANCE
0.07	
0.08	]— HIGHLY SUGGESTIVE, SIGNIFICANT AT THE $P < 0.10$ LEVEL
0.09	
0.099	]— HEY, LOOK AT THIS INTERESTING SUBGROUP ANALYSIS
≥ 0.1	

Credit: XKCD
comics

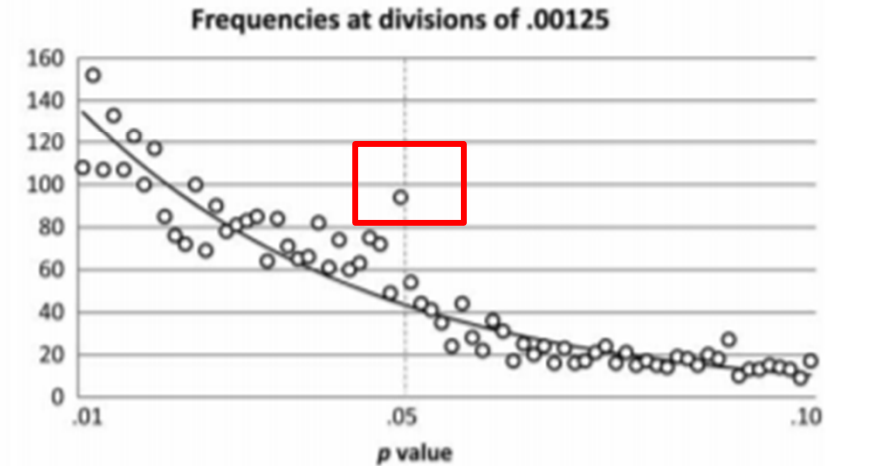
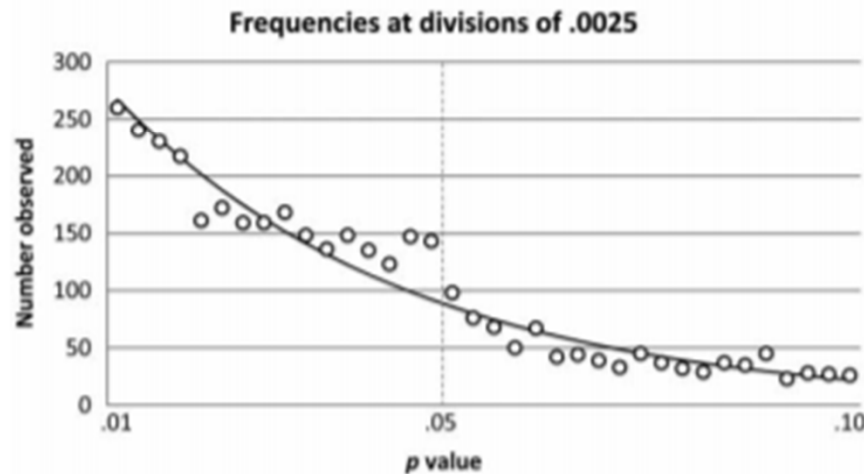
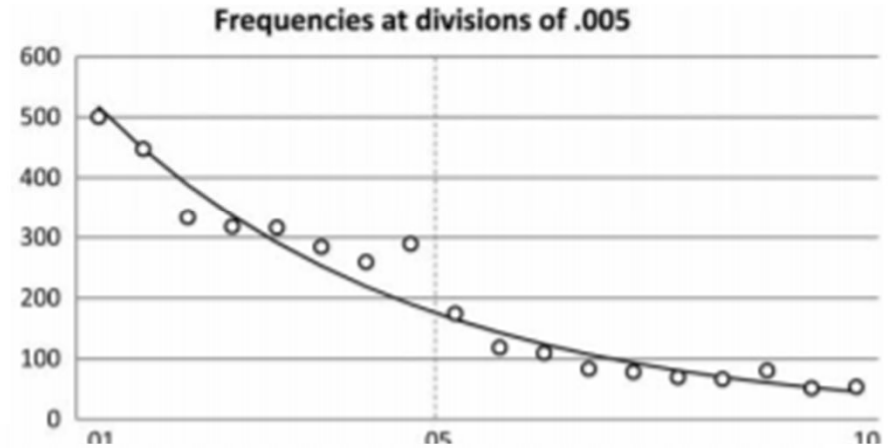
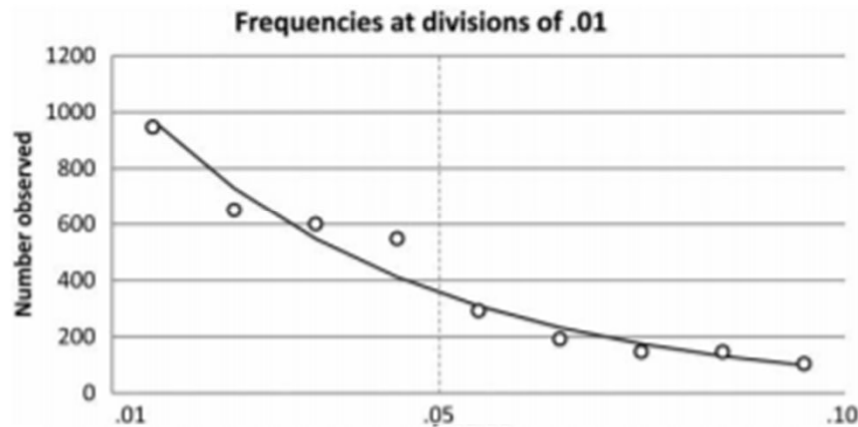
A peculiar prevalence of p values just below .05

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MASICAMPO AND LALANDE



Credit: XKCD
comics

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WHY IS STEALING WRONG

WHY ARE DOGS AFRAID OF FIREWORKS
WHY IS THERE NO KING IN ENGLAND

WHY DO WHALES JUMP
WHY ARE WITCHES GREEN
WHY ARE THERE MIRRORS ABOVE BEDS

WHY DO I SAY UH

WHY IS SEA SALT BETTER

WHY ARE THERE TREES IN THE MIDDLE OF FIELDS

WHY IS THERE NOT A POKEMON MMO

WHY IS THERE LAUGHING IN TV SHOWS

WHY ARE THERE DOORS ON THE FREEWAY

WHY ARE THERE SO MANY SVCHOST.EXE RUNNING

WHY AREN'T THERE ANY COUNTRIES IN ANTARCTICA

WHY ARE THERE SCARY SOUNDS IN MINECRAFT

WHY IS THERE KICKING IN MY STOMACH

WHY ARE THERE TWO SLASHES AFTER HTTP

WHY ARE THERE CELEBRITIES

WHY DO SNAKES EXIST

WHY DO OYSTERS HAVE PEARLS

WHY ARE DUCKS CALLED DUCKS

WHY DO THEY CALL IT THE CLAP

WHY ARE KYLE AND CARTMAN FRIENDS

WHY IS THERE AN ARROW ON AANG'S HEAD

WHY ARE TEXT MESSAGES BLUE

WHY ARE THERE MUSTACHES ON CLOTHES

WHY ARE THERE MUSTACHES ON CARS

WHY ARE THERE MUSTACHES EVERYWHERE

WHY ARE THERE SO MANY BIRDS IN OHIO

WHY IS THERE SO MUCH RAIN IN OHIO

WHY IS OHIO WEATHER SO WEIRD

WHY ARE THERE BRIDESMAIDS

WHY DO DYING PEOPLE REACH UP

WHY AREN'T THERE VARIKOSE ARTERIES

WHY ARE OLD KINGDOMS DIFFERENT

WHY ARE THERE
SQUIRRELS



WHY IS PROGRAMMING SO HARD

WHY IS THERE A 0 OHM RESISTOR

WHY DO AMERICANS HATE SOCCER

WHY DO RHYMES SOUND GOOD

WHY DO TREES DIE

WHY IS THERE NO SOUND ON CNN

WHY AREN'T POKEMON REAL

WHY AREN'T BULLETS SHARP

WHY DO DREAMS SEEM SO REAL

Hypothesis testing: two samples

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

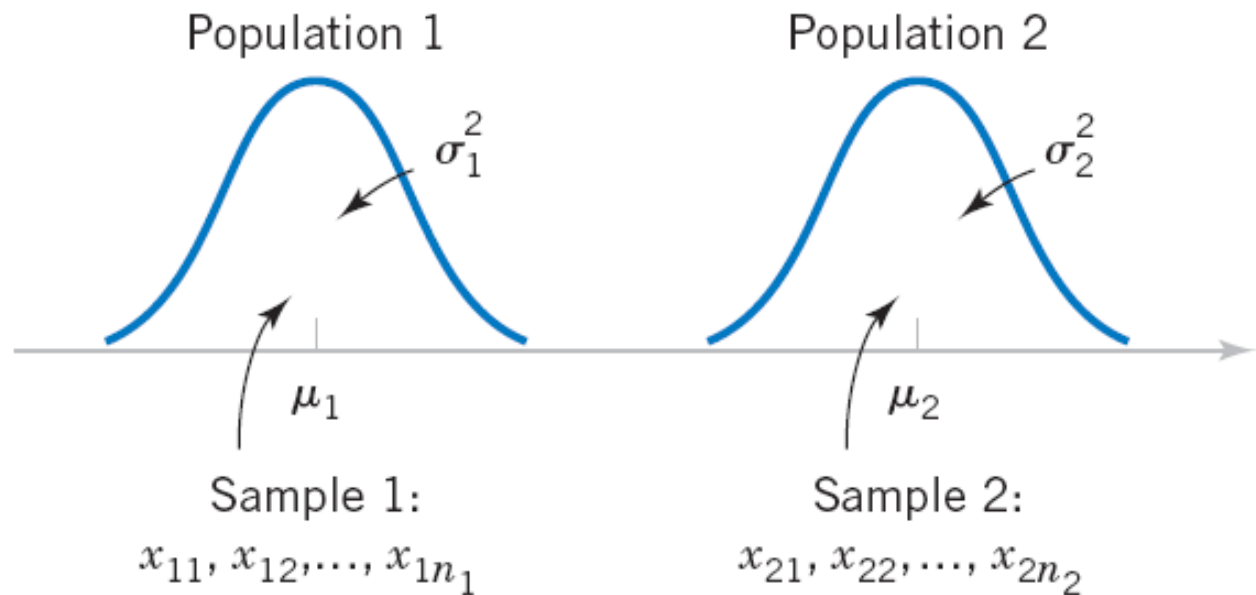


Figure 10-1 Two independent populations.

Figure 10-1 Two independent populations.

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

Assumptions

1. $X_{11}, X_{12}, \dots, X_{1n_1}$ is a random sample from population 1.
2. $X_{21}, X_{22}, \dots, X_{2n_2}$ is a random sample from population 2.
3. The two populations represented by X_1 and X_2 are independent.
4. Both populations are normal.

$$E(\bar{X}_1 - \bar{X}_2) = E(\bar{X}_1) - E(\bar{X}_2) = \mu_1 - \mu_2$$

$$V(\bar{X}_1 - \bar{X}_2) = V(\bar{X}_1) + V(\bar{X}_2) = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}$$

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

The quantity

$$Z = \frac{\bar{X}_1 - \bar{X}_2 - (\mu_1 - \mu_2)}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \quad (10-1)$$

has a $N(0, 1)$ distribution.

10-2: Inference for a Difference in Means of Two Normal Distributions, Variances Known

10-2.1 Hypothesis Tests for a Difference in Means, Variances Known

usually $\Delta_0 = 0$

Null hypothesis: $H_0: \mu_1 - \mu_2 = \Delta_0$

Test statistic:
$$Z_0 = \frac{\bar{X}_1 - \bar{X}_2 - \Delta_0}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \quad (10-2)$$

Alternative Hypotheses	P-Value	Rejection Criterion For for Fixed-Level Tests
$H_1: \mu_1 - \mu_2 \neq \Delta_0$	Probability above $ z_0 $ and probability below $- z_0 $, $P = 2[1 - \Phi(z_0)]$	$z_0 > z_{\alpha/2}$ or $z_0 < -z_{\alpha/2}$
$H_1: \mu_1 - \mu_2 > \Delta_0$	Probability above z_0 , $P = 1 - \Phi(z_0)$	$z_0 > z_\alpha$
$H_1: \mu_1 - \mu_2 < \Delta_0$	Probability below z_0 , $P = \Phi(z_0)$	$z_0 < -z_\alpha$

10-2.1 Hypotheses Tests on the Difference in Means, Variances Unknown

Case 2: $\sigma_1^2 \neq \sigma_2^2$

If $H_0: \mu_1 - \mu_2 = \Delta_0$ is true, the statistic

$$T_0^* = \frac{\bar{X}_1 - \bar{X}_2 - \Delta_0}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \quad (10-15)$$

is distributed as **t-distribution** with degrees of freedom given by

$$v = n_1 + n_2 - 2,$$

or more generally

Manhattan plot for Genome-Wide Association Study (GWAS)

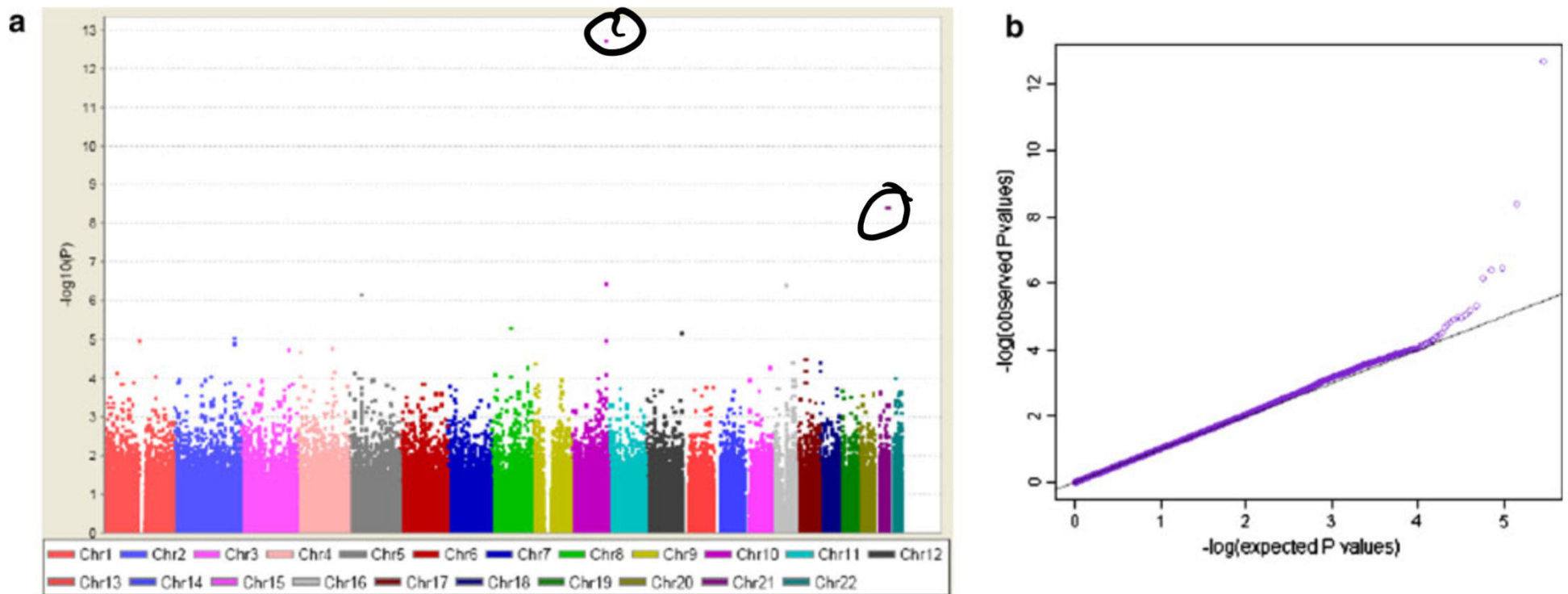


Fig. 1 Genome-wide association results comparing 2,702 cases and 5,726 controls: **a** Manhattan and **b** quantile–quantile plots of $-\log_{10}$ transformed P values of 285,984 SNPS genotyped

Li J., et al. A combined analysis of genome-wide association studies in breast cancer. *Breast Cancer Res Treat.* 2011;126: 717–727

Multiple null hypotheses: Bonferroni correction

- What if you have **m independent null hypotheses**?
Say you have **m=25,000 genes** in a genome?
- What is the probability that **at least one** of the **null-hypotheses** will be shown to be **false** at significance threshold α_1 ?
- Answer:
Family-Wise Error Rate
or **$\text{FWER} = 1 - (1 - \alpha_1)^m \approx m\alpha_1$**
- If $m=20$ and $\alpha_1=0.05$,
 $\text{FWER} = 0.6415$
- If you want to get **$\text{FWER} < \alpha$** , use
 $\alpha_1 = \alpha/m$

Carlo Emilio Bonferroni
(1892 –1960)
Italian mathematician
who worked on
probability theory.



Example 10-7

Chocolate and Cardiovascular Health

An article in *Nature* (2003, Vol. 424, p. 1013) described an

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Is there ev
plasma an

Plasma antioxidants from chocolate

Dark chocolate may offer its consumers health benefits the milk variety cannot match.

There is some speculation that dietary flavonoids from chocolate, in particular (–)epicatechin, may promote cardiovascular health as a result of direct antioxidant effects or through antithrombotic mechanisms^{1–3}. Here we show that consumption of plain, dark chocolate (Fig. 1) results in an increase in both the total antioxidant capacity and the (–)epicatechin content of blood plasma, but that **these effects are markedly reduced when the chocolate is consumed with milk or if milk is incorporated as milk chocolate**. Our findings indicate that milk may interfere with the absorption of antioxidants from chocolate *in vivo* and may therefore negate the potential health benefits that can be derived from eating moderate amounts of dark chocolate.

To determine the antioxidant content of different chocolate varieties, we took dark chocolate and milk chocolate prepared from the same batch of cocoa beans and defatted them twice with *n*-hexane before extracting them with a mixture of water, acetone and acetic acid (70.0:29.8:0.2 by volume). We measured their *in vitro* total antioxidant capacities using the ferric-reducing antioxidant potential (FRAP) assay⁴; FRAP

reduced iron per 100 g for dark and milk chocolate, respectively. Volunteers must therefore consume twice as much milk chocolate as dark chocolate to receive a similar intake of antioxidants.

We recruited 12 healthy volunteers (7 women and 5 men with an average age of 32.2 ± 1.0 years (range, 25–35 years). Subjects were non-smokers, had normal blood lipid levels, were taking no drugs or vitamin supplements, and had an average weight of 65.8 ± 3.1 kg (range, 46.0–86.0 kg) and body-mass index of 21.9 ± 0.4 kg m^{–2} (range, 18.6–23.6 kg m^{–2}). On different days, following a crossover experimental design, subjects consumed **100 g dark chocolate, 100 g dark chocolate with 200 ml full-fat milk, or 200 g milk chocolate** (containing the equivalent of up to 40 ml milk).

One hour after subjects had ingested the chocolate, or chocolate and milk, we measured the total antioxidant capacity of their plasma by FRAP assay. Plasma antioxidant levels increased significantly after consumption of dark chocolate alone, from $100 \pm 3.5\%$ to $118.4 \pm 3.5\%$ (*t*-test, $P < 0.001$), **returning to baseline values ($95.4 \pm 3.6\%$) after 4 h** (Fig. 2a). There was



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†Plant Products and Human Nutrition Group, Graham Kerr Building, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Figure 1 Stack of benefits? Unlike its milky counterpart, dark chocolate may provide more than just a treat for the tastebuds.

could be due to the formation of secondary bonds between chocolate flavonoids and milk proteins^{6,7}, which would reduce the biological accessibility of the flavonoids and therefore the chocolate's potential antioxidant properties *in vivo*.

Our findings highlight the possibility

Vol. 424
↓

Sweet matlab exercise #1

- Download **dark_vs_milk_chocolate_analysis_template.m** at the course website. **Correct all ??** In the file
- `dark=[118.8 122.6 115.6 113.6 119.5 115.9 115.8 115.1 116.9 115.4 115.6 107.9];`
- `milk=[102.1 105.8 99.6 102.7 98.8 100.9 102.8 98.7 94.7 97.8 99.7 98.6]`
- Use Z-statistics to calculate **P-value** of the null hypothesis H_0 that **milk = dark** against H_1 that **dark > milk**. **$P_value_z=2*[1-normcdf(|Z|)]$**
- Repeat using T-statistics. # of degrees of freedom is **$dof=2*(n-1)$**
 $P_value_t=2*tcdf(|T|, dof)$

Sweet matlab exercise #1

- `dark=[118.8 122.6 115.6 113.6 119.5 115.9 115.8 115.1 116.9 115.4 115.6 107.9];`
- `milk=[102.1 105.8 99.6 102.7 98.8 100.9 102.8 98.7 94.7 97.8 99.7 98.6]`
- `x_dark=mean(dark) % sample mean dark chocolate`
- `x_milk=mean(milk) % sample mean milk chocolate`
- `s_dark=std(dark) % sample std dark chocolate`
- `s_milk=std(milk) % sample std milk chocolate`
- `n=12 % sample size of both dark and milk`
- `std_xdiff=sqrt(s_dark.^2./2+s_milk.^2./n) % std diff x`
- `z_stat=(x_dark-x_milk)./std_xdiff % z-statistic`
- `P_value_z=erfc(z_stat./sqrt(2))./2 % P-value of null true`
- `% P_value_z=9.9629e-34`
- `dof=(n-1)+(n-1) % # of degrees of freedom`
- `P_value_t=tcdf(z_stat,dof,'upper') % P-value of null true`
- `%P_value_t= 1.8417e-11`

Credit: XKCD
comics

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WHY DON'T BOYS LIKE ME

WHY IS THERE ALWAYS A JAVA UPDATE

WHY ARE THERE RED DOTS ON MY THIGHS

WHY IS LYING GOOD

WHY ARE THERE
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WHY IS THERE AN OWL IN MY BACKYARD

WHY IS THERE AN OWL OUTSIDE MY WINDOW

WHY IS THERE AN OWL ON THE DOLLAR BILL

WHY DO OWLS ATTACK PEOPLE

WHY ARE AK 47s SO EXPENSIVE

WHY ARE THERE HELICOPTERS CIRCLING MY HOUSE

WHY ARE THERE GODS

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WHY IS STEALING WRONG

WHY DO WHALES JUMP
WHY ARE WITCHES GREEN
WHY ARE THERE MIRRORS ABOVE BEDS
WHY DO I SAY UH
WHY IS SEA SALT BETTER

WHY ARE THERE TREES IN THE MIDDLE OF FIELDS
WHY IS THERE NOT A POKEMON MMO
WHY IS THERE LAUGHING IN TV SHOWS
WHY ARE THERE DOORS ON THE FREEWAY
WHY ARE THERE SO MANY SVCHOST.EXE RUNNING
WHY AREN'T THERE ANY COUNTRIES IN ANTARCTICA
WHY ARE THERE SCARY SOUNDS IN MINECRAFT
WHY IS THERE KICKING IN MY STOMACH
WHY ARE THERE TWO SLASHES AFTER HTTP

WHY ARE THERE CELEBRITIES
WHY DO SNAKES EXIST
WHY DO OYSTERS HAVE PEARLS
WHY ARE DUCKS CALLED DUCKS
WHY DO THEY CALL IT THE CLAP
WHY ARE KYLE AND CARTMAN FRIENDS
WHY IS THERE AN ARROW ON AANG'S HEAD
WHY ARE TEXT MESSAGES BLUE
WHY ARE THERE MUSTACHES ON CLOTHES
WHY ARE THERE MUSTACHES ON CARS
WHY ARE THERE MUSTACHES EVERYWHERE
WHY ARE THERE SO MANY BIRDS IN OHIO
WHY IS THERE SO MUCH RAIN IN OHIO
WHY IS OHIO WEATHER SO WEIRD

WHY ARE THERE BRIDESMAIDS
WHY DO DYING PEOPLE REACH UP
WHY AREN'T THERE VARICOSE ARTERIES
WHY ARE OLD KINGDOMS DIFFERENT

WHY ARE THERE
SQUIRRELS



WHY IS PROGRAMMING SO HARD
WHY IS THERE A 0 OHM RESISTOR
WHY DO AMERICANS HATE SOCCER
WHY DO RHYMES SOUND GOOD
WHY DO TREES DIE
WHY IS THERE NO SOUND ON CNN
WHY AREN'T POKEMON REAL
WHY AREN'T BULLETS SHARP
WHY DO DREAMS SEEM SO REAL

WHY IS SEX
SO IMPORTANT



WHY ARE THERE SO MANY CROWS IN ROCHESTER, MN
WHY IS PSYCHIC WEAK TO BUG
WHY DO CHILDREN GET CANCER
WHY IS POSEIDON ANGRY WITH ODYSSEUS
WHY IS THERE ICE IN SPACE
WHY ARE DOGS AFRAID OF FIREWORKS
WHY IS THERE NO KING IN ENGLAND