

**Science Skills Boot Camp**

**Workshop 2:  
Asking Scientific Questions**

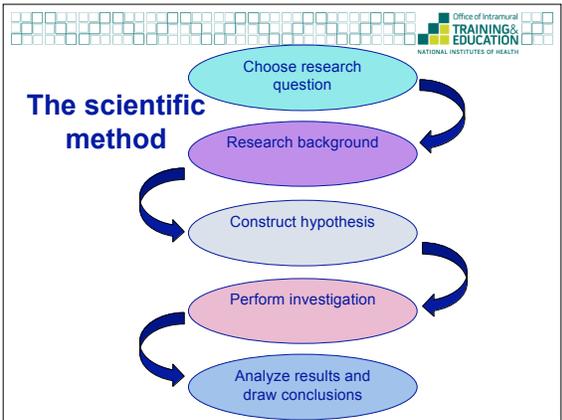
Keren Witkin, PhD

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“Science is a way of thinking  
much more than it is a body of  
knowledge.”

Carl Sagan

Where does this fit into the  
scientific method:

Prove that your  
hypothesis is true



Today's investigation:  
Phenylthiocarbamide (PTC)



The human gene TAS2R38 encodes  
taste receptor that detects PTC



<http://learn.genetics.utah.edu/content/begin/traits/ptc/>



Class data collection 1:

Taste the PTC paper and pick one  
description:

- ✧ This tastes like paper
- ✧ This tastes a little bitter
- ✧ This tastes horribly bitter

Please dispose of used PTC paper into the ziploc.



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Step 1:  
Choosing a  
research  
question

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## What makes a good research question?

- Testable
- Not too broad, but not too narrow
- Fits appropriately into the existing information
- Realistic in terms of resources
- Contributes to the field no matter what you find

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### Some possible questions:

- How does the sense of taste work?
- Do factors other than genetics affect the ability to taste PTC?
- How did PTC sensitivity evolve?
- Are supertasters cuter than non-tasters?
- Are there gender differences in PTC sensitivity?
- Do PTC tasters have a specific DNA polymorphism?

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Step 2:  
Researching  
background

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### Finding background information

- Primary literature
- Review articles
- Textbooks
- Your colleagues
- Internet resources

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### Review articles are a great place to start!

- Provide an overview of the field
- Often written by experts in the field
- Summarize many primary papers
- Often contain useful diagrams

**A** Open mitosis

The diagram illustrates the stages of open mitosis in a cell. It is divided into four main stages: Interphase, Metaphase, Anaphase, and Telophase. In Interphase, the cell contains a nucleus with a nucleolus and a centromere. In Metaphase, chromosomes align at the equatorial plate, and spindle fibers (microtubules) attach to the centromeres. In Anaphase, sister chromatids separate and move toward opposite poles. In Telophase, new nuclei form at each pole. The final stage is another Interphase, where the cell has two nuclei and is preparing for division. Labels include Centriole, Microtubule, Chromosome, and NE (Nucleolus).

Webster et al. Journal of Cell Science. 2009. 122: 1477-1486

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## Primary literature

- This is where the actual data is!
- Describes the experiments in detail
  - Introduction
  - Methods
  - Results
    - Figures
    - Tables
  - Discussion
  - References

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## All about PubMed

- Free database of biomedical and life sciences literature
- Maintained by the National Library of Medicine
- Contains over 20 million citations
- <http://www.ncbi.nlm.nih.gov/pubmed/>

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- Genetic variation in the hTAS2R38 taste receptor and brassica vegetable intake. Gorovic N, Alzai S, Tjermeland A, Overvad K, Vogel U, Abrechtson C, Poulsen HE. *Sensory J Clin Nutr*. 2011 Feb 21. [Epub ahead of print]. PMID: 21338274 [PubMed - as supplied by publisher]
- Bitter receptor gene (TAS2R38) P49A genotypes and their associations with aversion to vegetables and sweetest foods in Malaysian subjects. Ooi SX, Lee PL, Law HY, Say YH, Aza Paz J. *Clin Nutr*. 2010;19(4):491-8. PMID: 21147700 [PubMed - indexed for MEDLINE]
- Purification and characterization of phenoxidase from the hemocytes of *Eurygaster integriceps* (Hemiptera: Scutelleridae). Zobe A, Sandani AR, Malagoli D, Cenci Brodini Pasquale B. *Biochem Biophys Res Commun*. 2011 Jan;158(1):117-23. Epub 2010 Oct 20. PMID: 20970518 [PubMed - in process]
- Sensitivity to bitter and sweet taste perception in schoolchildren and their relation to dental caries. Furuguti TR, Pal-Federico RC, Maciel SM, Gomin-Junior A, Walter LR. *Oral Health Prev Dent*. 2010;8(3):253-9. PMID: 20818910 [PubMed - indexed for MEDLINE]

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- Genetic variation in the hTAS2R38 taste receptor and brassica vegetable intake. Gorovic N, Alzai S, Tjermeland A, Overvad K, Vogel U, Abrechtson C, Poulsen HE. *Sensory J Clin Nutr*. 2011 Feb 21. [Epub ahead of print]. PMID: 21338274 [PubMed - as supplied by publisher]
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- Primary hemocyte culture of *Panaeus monodon* as an in vitro model for white spot syndrome virus titration, viral and immune related gene expression and cytotoxicity assays. Jose S, Mohandas A, Philip R, Binigi Singh B, J Invertebr Pathol. 2010 Nov;100(3):312-21. Epub 2010 Aug 31. PMID: 20807837 [PubMed - indexed for MEDLINE]
- Identification of non-taster Japanese macaques for a specific bitter taste. Suzuki N, Sugawara T, Matsui A, Go Y, Hira H, Imai H. *Primates*. 2010 Oct;51(4):285-9. Epub 2010 Jul 28.

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Results: 1 to 20 of 148

- Identification of non-taster Japanese macaques for a specific bitter taste. Suzuki N, Sugawara T, Matsui A, Go Y, Hira H, Imai H. *Primates*. 2010 Oct;51(4):285-9. Epub 2010 Jul 28. Department of Cellular and Molecular Biology, Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan.

Abstract: Bitter taste perception evolved as a key detection mechanism against the ingestion of bioactive substances, and is mediated by TAS2R gene family members in vertebrates. The most widely known and best studied bitter substance is phenylthiocarbamide (PTC), which is recognized by TAS2R38 and has a molecular structure similar to that of glucosinolates contained in Brassica plants. The "non-taster" phenotypic polymorphism (i.e., not sensitive to PTC-containing foods) has been identified in many primates, including humans. Here, we report genetic and behavioral evidence for the existence of "non-taster" Japanese macaques, which originated from a restricted region of Japan. Comparison of the sequences of the TAS2R38 gene of 333 Japanese and 55 macaque macaques suggested that the genotype appeared after the divergence of these two species, independently of the appearance of human and chimpanzee "non-tasters". This finding might give a clue for elucidating the ecological, evolutionary, and neurobiological aspects of bitter taste perception of primates, as related to the plants that they sometimes use as foods in their habitats.

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Journal Article: Behavioral genetics and taste: John D. Douglter

Journal Article: Age modifies the genotype-phenotype relationship for the bitter receptor TAS2R38

Book Chapter: Evolution of mammalian bitter taste

Journal Article: Hedonic Bitter Taste by Melanocyte-Independent Pathway

NEWS AND PERSPECTIVES

Identification of non-taster Japanese macaques for a specific bitter taste

Nami Suzuki, Takuo Sugawara, Atsushi Matsui, Yasuhito Go, Hirohisa Hirai and Hiroo Inai

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SUPPLEMENTALS (1) REFERENCES (1) EXPORT CITATION ABOUT

Abstract

Bitter taste perception evolved as a key detection mechanism against the ingestion of bioactive substances, and is mediated by TAS2R gene family members in vertebrates. The most widely known and best studied bitter substance is phenylthiocarbamide (PTC), which is recognized by TAS2R38 and has a molecular structure similar to that of glucosinolates contained in Brassica plants. The "non-taster" phenotypic polymorphism (i.e., not sensitive to PTC-containing foods) has been identified in many primates, including humans. Here, we report genetic and behavioral evidence for the existence of

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- Order through the NIH library
  - On NIH library Web site, click on "services" then "order a document"

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## Where else can you find scientific papers?

- EMBASE: Biomedical and pharmacological database
- SCOPUS: Broad coverage of scientific, technical, medical, and social science literature, including arts and humanities
- Web of Science: Coverage of ~12,000 journals and conference proceedings. Not limited to biomedical sciences.

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## Also on the Internet: Bioinformatics tools

- GenBank
  - Free annotated collection of DNA sequences, run by NCBI (NIH)
  - <http://www.ncbi.nlm.nih.gov/genbank/>
  - Search by gene name to get DNA or protein sequence
  - "Blast" a DNA or protein sequence to find matches in the database

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NCBI Nucleotide

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View in the Sequence

Articles about the TAS2R38 gene

Bitter receptor gene (TAS2R38) P45A genotype and their associations with PTC taste (2010)

Taste genes associated with dentel cases. (2008 Feb 2010)

The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12 (2010)

Variation viewer

See a summary of TAS2R38 variants, including those of clinical significance.

Reference sequence information

RefSeq protein product

Homo sapiens taste receptor, type 2, member 38 (TAS2R38), mRNA

FASTA Statistics

LOCUS NM\_174817 1143 bp mRNA linear FR: 09-APR-2011

DEFINITION Homo sapiens taste receptor, type 2, member 38 (TAS2R38), mRNA.

ACCESSION NM\_174817

VERSION NM\_174817.4 GI:261489464

KEYWORDS

SOURCE Homo sapiens (human)

ORGANISM

Evansavia; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Euplorhini; Carnivora; Simiiformes; Hom.

REFERENCE

1 (bases 1 to 1143)

Seok,D.-H., Shu,C., Brezlin,P.A., Duke,F.T., Sanders,A.F., Campbell,M.J., Montgomery,G.W., Medland,S.E., Martin,N.G. and Kirich,A.L. The perception of quinine taste intensity is associated with common genetic variants in a bitter receptor cluster on chromosome 12. Hum. Mol. Genet. 19 (21), 4278-4285 (2010)

JOURNAL

2010712

PMID

20857512

ABSTRACT

GenetIP: Observational study and genome-wide association study of gene-disease association. (IPD) Navigator

REFERENCE

2 (bases 1 to 1143)

Medellin,F., Hanzlik,A., Brown,M., Cooper,M.E., DeGuzis,M.L., Heyan,P.J., Croux,P., McNeil,D.W. and Marziani,M.L. Taste genes associated with dental caries. J. Dent. Res. 89 (11), 1198-1202 (2010)

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### More bioinformatics tools

- Sequence alignment programs
- Structure prediction programs
- Gene expression and regulation databases
- Organism-specific databases
- Pathway analysis
- Promoter/SNP prediction

**And many others!**

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**Step 3:  
Constructing a hypothesis**

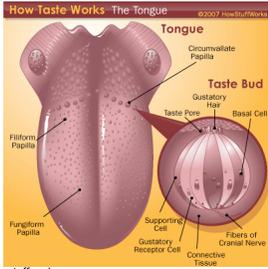
**Hypothesis:**  
Tentative statement predicting the outcome of an experiment

**If . . . , then . . .**

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### What we know about taste:

**Taste** is controlled by receptors in the tongue



*How Taste Works - The Tongue*  
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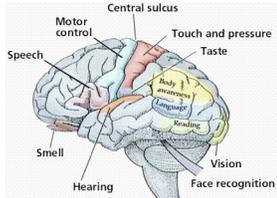
<http://health.howstuffworks.com>

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### What we know about taste:

**Taste perception** involves additional factors

- Smell
- Temperature
- Visual cues
- Texture



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### What we know about taste:

Studies show that smelling **mint** while eating makes it difficult to identify food by taste.



**Hypothesis:**  
**If** you smell mint while tasting PTC paper, **then** you will be less sensitive to the bitter taste.

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**Step 4:  
Perform investigation**

Planning

Doing

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## Planning experiments

**For each experiment:**

- Define your objective
- Plan your general strategy
- Decide on experimental details

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## Planning experiments

**For each experiment:**

- Define your objective
  - To determine whether strong smells decrease sensitivity to PTC paper
- Plan your general strategy
  - Participants will taste PTC paper and report on the taste. They will then smell mint while re-tasting PTC paper and report on the taste.
- Decide on experimental details
  - How many participants? What kind of mint? How will they rate PTC taste? What will be the controls?

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## What are some features of a good experiment?

- Has a clear purpose
- Answers one question definitively
- Has appropriate **controls**
- Has limited **variables**
- Has a large enough sample size
- Uses available reagents and equipment
- Can be repeated by you and others

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## Common controls

- **Positive controls**  
Show that everything is working well, and that your conditions are able to achieve a positive result, even if your samples turn out all negative
- **Negative controls**  
Show the base-line background in your experiment, using known samples that should produce a negative result

Sample

- + Sample

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## Our experiment today:

Each subject will taste PTC paper and report whether there is no taste, weak bitter taste, or strong bitter taste

**Experimental Group**

Half of the subjects will re-taste PTC paper while smelling mint and will report whether there is no taste, weak bitter taste or strong bitter taste

**Control Group**

Half of the subjects will re-taste PTC paper while smelling parsley and will report whether there is no taste, weak bitter taste or strong bitter taste

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- What are the controls in this experiment?
- What are the variables?
- Are there other controls that we're missing?

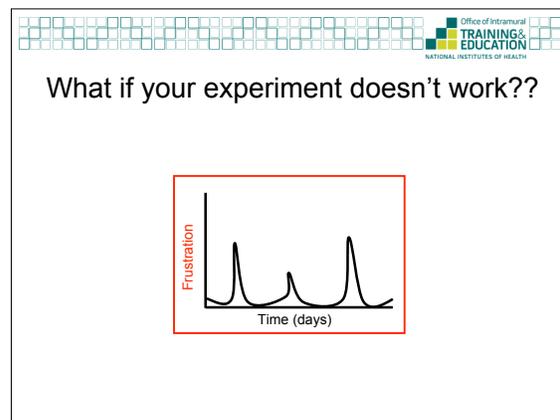


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- ### Tips for good experiments
- Be prepared
  - Be organized
  - Be meticulous
    - Work deliberately and carefully
    - Follow protocol closely
    - Note any deviations from protocol
  - Minimize bias
  - Document everything

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- ### Learning a new technique
- Find a protocol
  - Read it carefully
  - Consult with your mentor or other experts
  - Make or acquire reagents ahead of time
  - Learn how to use required equipment
  - Do a "dry run"
  - Allow plenty of time for the first run

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- ### Making or acquiring reagents
- If ordering reagents, do it as early as possible
  - Research how each reagent should be used and stored
  - If making up solutions
    - Make sure you know what solvent to use
    - Brush up on molarity and serial dilutions, if necessary
    - Check and re-check all calculations

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- ### Common mistakes
- Doing huge experiments with too many samples
  - Not thinking carefully about your controls before you start
  - Waiting until the last minute before tracking down reagents
  - Forgetting to grow up the cells you need ahead of time



**When your experiment doesn't work:  
Troubleshooting**

- Identify possible sources of error
  - Reread protocol
  - Check calculations
  - Consider whether reagents or equipment might be suspect
  - Think about repeating experiment as is
- Consult with mentor
- Consider whether your hypothesis might be flawed
- Don't get frustrated!

**Class data collection- Part 2**

1. Taste the PTC paper while smelling the mint or parsley and pick one description:
  - ◇ This tastes like paper
  - ◇ This tastes a little bitter
  - ◇ This tastes horribly bitter
2. Dispose of all waste into the ziploc
3. Class data will be collected and recorded on the board

**Step 5:  
Analyzing Results and  
Drawing Conclusions**

- How many people changed their responses after smelling the mint?
- How many people changed their responses in the control group?
- Can we draw any conclusions?
- Does our data suggest future experiments?
- How do we want to present this data?

**Raw data: Recorded in your notebook**

Subject	Mint or parsley	Before	After
1	M	No	No
2	M	Strong	Strong
3	M	Strong	Weak
4	M	Weak	Weak
5	P	No	No
6	P	Weak	Strong
7	P	Weak	Weak

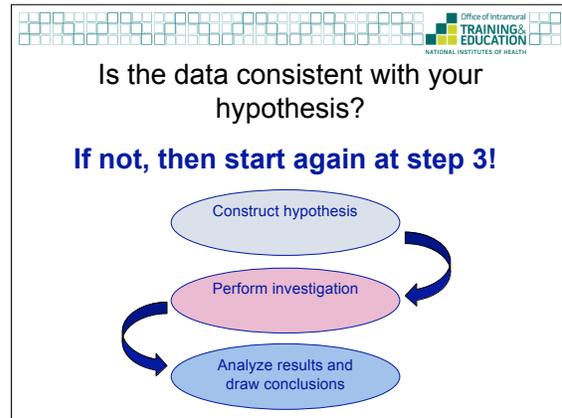
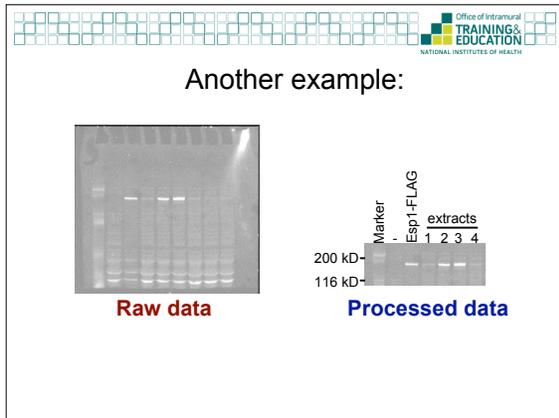
**Other types of raw data:**  
Gels  
Blots  
Photographs  
Observations

**Processed data:**

	Parsley		Mint	
	Before	After	Before	After
No taste	12	12	13	13
Weakly bitter	27	25	23	24
Strongly bitter	11	13	14	13

**PTC tasting results**

Category	Before	After
no taste (control)	12	12
no taste (mint)	13	13
weakly bitter (control)	27	25
weakly bitter (mint)	23	24
strongly bitter (control)	11	13
strongly bitter (mint)	14	13



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Data vs interpretation

**Data:** 48/50 study participants reported no change in PTC taste sensitivity after smelling mint, compared to 49/50 in the control group.

**Interpretations:**

- Smell does not affect taste
- Smelling mint does not affect taste
- The smell of mint does not affect PTC taste sensitivity

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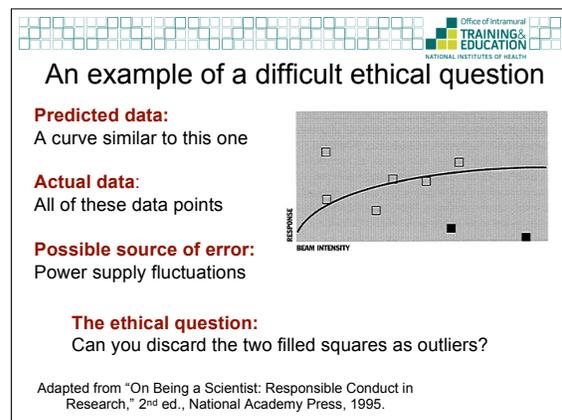
**Question:**

How could we design an experiment to better test the broader conclusion that **smell does not affect taste?**

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Does your data mean what you think it means?

- Is it statistically significant?
- Are you doing the right statistical analysis?
- Do you have a large enough sample size or enough repetitions?
- Are there alternative explanations?
- Are there confounding factors?



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### Pitfalls in data analysis

- Only considering specific data points
- Over-interpretation of data
- Ignoring confounding factors
- Using too small of a sample size

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### Scientific misconduct

- **Falsifying data**  
Can happen accidentally when you “process” data
- **Fabricating data**  
**This is always wrong!**
- **Plagiarism**  
Includes using other’s IDEAS as well as WORDS

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### Same basic scientific method no matter what kind of research you do

- Basic research
- Clinical research
- Translational research
- Social and behavioral research
- Epidemiology
- Computational research
- Mathematical modeling

```

    graph TD
      A(Choose question) --> B(Research background)
      B --> C(Construct hypothesis)
      C --> D(Perform investigation)
      D --> E(Analyze results and draw conclusions)
      E --> A
  
```

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**Does the data support my hypothesis??**

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### Special considerations for epidemiologists

- Are you surveying an appropriate population?
- Do you have enough study participants?
- Are you using the appropriate analytical tools?
- Have you considered potential alternative explanations?
  - Confounding factors
  - Bias
  - Chance
  - Reverse causality

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### Special considerations for clinical research

- Bioethics
- Professionalism
- Confidentiality
- Possibility of health risks for investigator
- Critical to have appropriate study design and meticulous technique
  - Institutional Review Board (IRB)
  - Data Safety Monitoring Board
  - Double-blind studies

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More on **bioethics**: The 7 major ethical principles that guide clinical research

**Each study must have:**

- Social and clinical value
- Scientific validity
- Fair subject selection
- Favorable risk-benefit ratio
- Independent review
- Informed consent
- Respect for potential and enrolled subjects

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Special considerations when using animals in research

- Oversight by the NIH Office of Laboratory Animal Welfare
- Must have an Animal Care and Use Protocol
- Ensuring humane and responsible use
  - Carefully designed experiments
  - Minimizing the number of animals used
  - Avoiding/minimizing pain and stress
  - Appropriate housing conditions
  - Appropriate sedation or anesthesia
  - Veterinary care, when necessary

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Ethical considerations for all kinds of scientists

- Honesty
- Objectivity
- Integrity
- Carefulness
- Respect for intellectual property
- Responsible publication
- Respect for colleagues

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Last but not least . . .

Communicate your results