

# Introduction to Research Methods

Prepared for the Brilliant  
Mowhiba Students

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# Learning Outcomes

By the end of this workshop, participants will be able to:

- Evaluate the relevancy and importance of research in the development of man kind.
- Define key terms associated with scientific research

# Why Do Research?

- Answer questions
- Satisfy curiosity
- Correcting old/wrong ideas
- Further ideas
- Add to the body of knowledge

Why Do Clinical Research?

**HELPING MANKIND**

# Philosophy: The study of problems concerning anything

- Epistemology: The theory of knowledge
  - how did you come to know
  - What justifies knowledge?
  - How much truth, and how did you get to it to make it a belief (knowledge)?

- Methodology: The applied particular procedures and steps
  - Logic: Valid demonstration and inference.
  - Validity: best approximation to the truth of a given proposition, inference, and conclusion.

By the way, Do you know the difference  
between inference and conclusions?



Let's challenge you with a question



Go to [Mentee.com](https://www.mentee.com)



Which of the two Sentences is true? Or are they both true or both false?

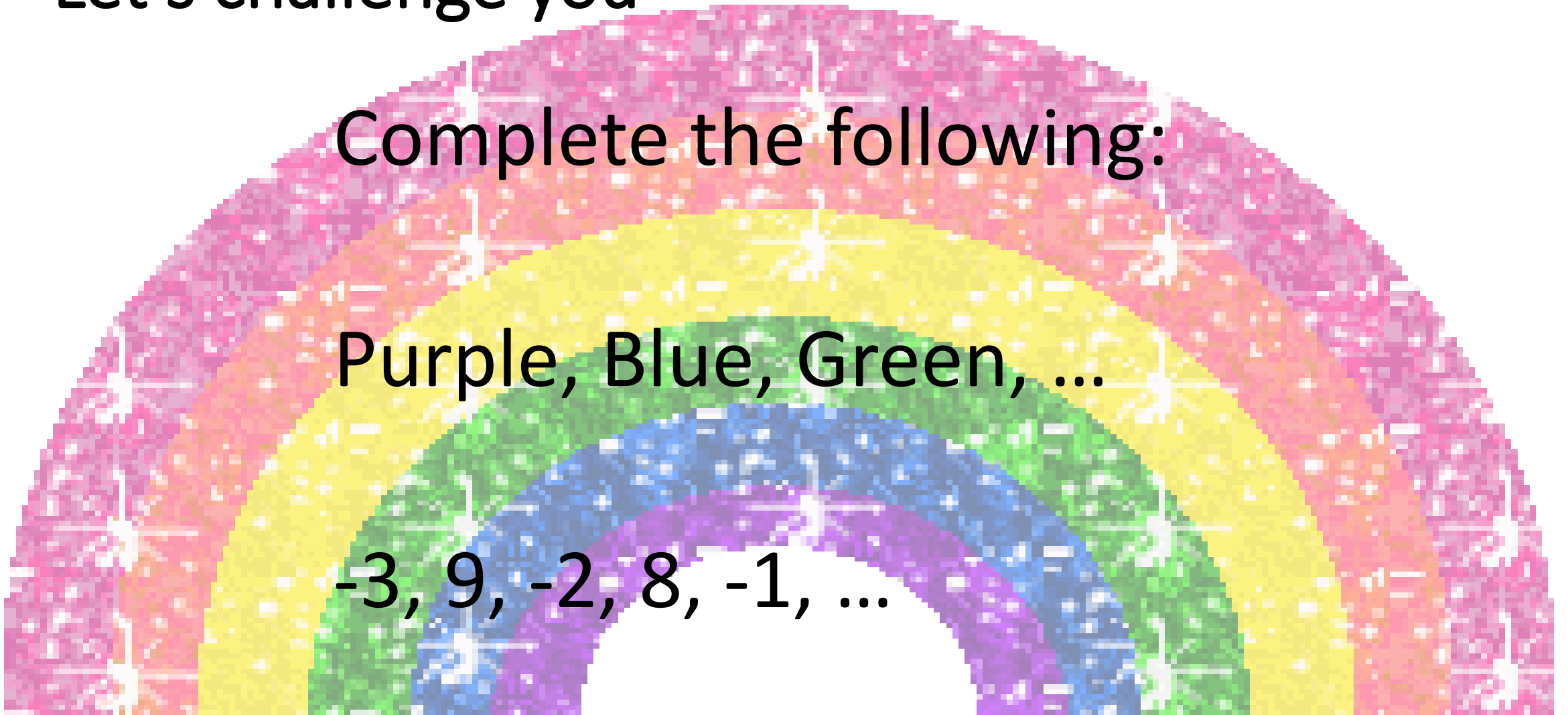
1. If all Mowhiba students have above-average IQ score and Mohammed has above average IQ score, then Mohammed is a Mowhiba student.
2. If all Mowhiba students have above-average IQ score and Mohammed is a Mowhiba student, then Mohammed has above average IQ score.

Let's challenge you

Complete the following:

Purple, Blue, Green, ...

-3, 9, -2, 8, -1, ...



# Source of Knowledge

- Deductive Reasoning
  - Making sense and logic
- Inductive Reasoning
  - Finding patterns



# Let's challenge yet another time

## Which question one is deductive and which is inductive?

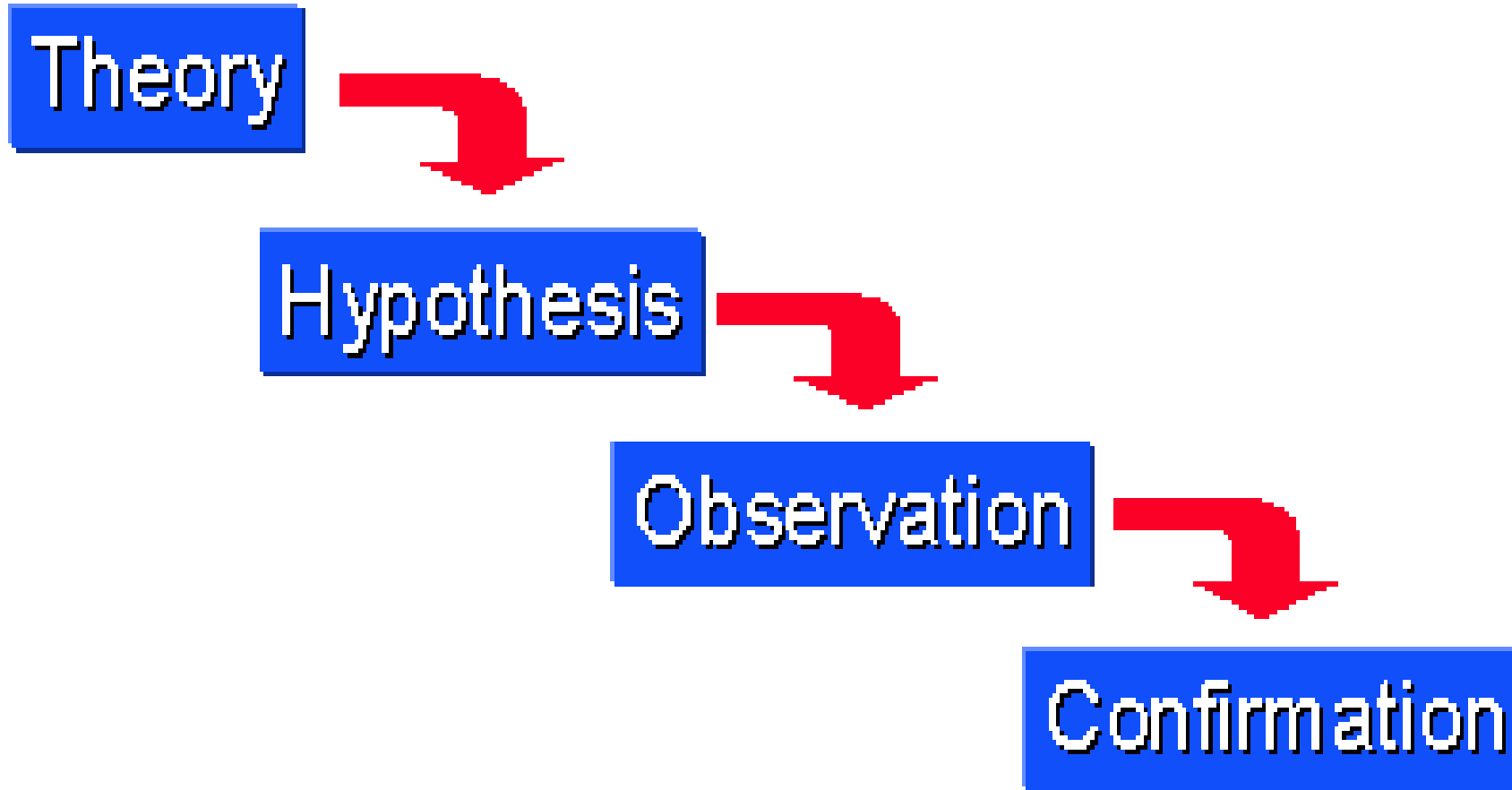
Complete the following:



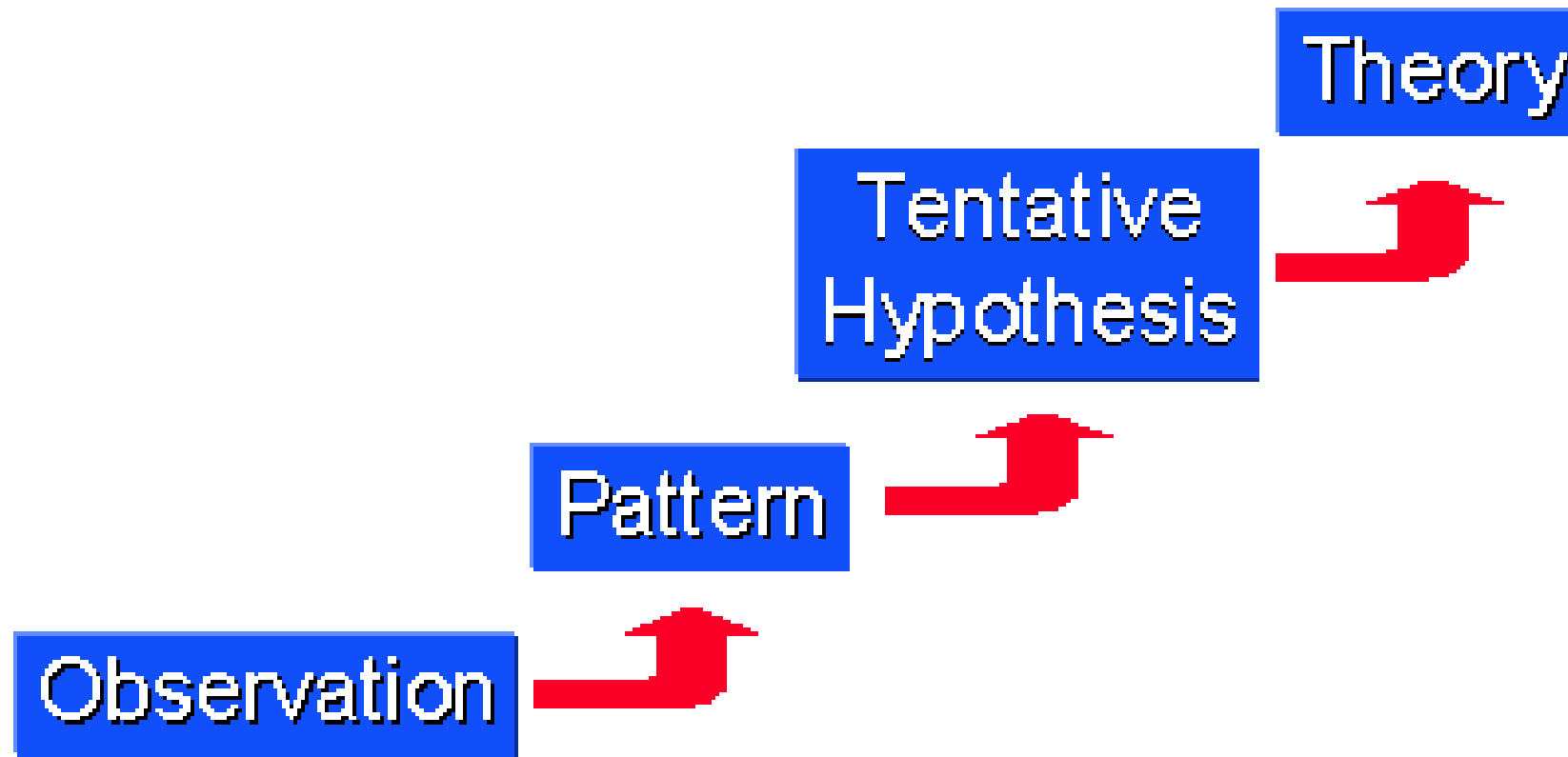
Which of the two Sentences  
true or both false?

- If all FAMS students have above average IQ, then Mohammed is a FAMS student, then Mohammed has above average IQ
- If all FAMS students have above average IQ, then Mohammed is a FAMS student

- Top-down
- More general to more specific
- Exploratory



- Bottom-up
- More specific to more general
- Confirmatory



# Who is controlling who???

- Studying the correlation between car accidents and texting  
Texting → car accidents
- The excellent grades of a student as a relationship with studying hours  
Studying → excellent grades
- The later side effects of steroid use with regards to heart muscle enlargement, heart failure and liver damage  
Steroids → heart and liver problems

# Some Definitions

## Variables

- **Independent** (Predictor): it is what you or nature is manipulating
- **Dependent** (Outcome): it is dependent on/effected by what you or nature manipulated
- Qualitative vs. Quantitative
- Subject (sample) vs. population



# Validity

- Internal Validity: Making the correct conclusion within the findings of your experiment

*Concluding the average IQ of Mowhiba Students by testing this group (you)*

# Validity

- External Validity: Making the correct generalization to subjects outside the study.

*IQ in KSA by testing this group*

# Validity

- External Validity: Making the correct generalization to subjects outside the study.

*IQ in Jeddah ~~by testing the classroom~~*

# Fishing for termites

- <https://www.youtube.com/watch?v=Sl9vgrc5yVo>

Once you found results, now what??

Share the knowledge  
(Passing on the ball)



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## MEKK2 regulates focal adhesion stability and motility in invasive breast cancer cells



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### ARTICLE INFO

#### Article history:

Received 30 July 2013

Received in revised form 14 January 2014

Accepted 24 January 2014

Available online 31 January 2014

#### Keywords:

MEKK2

Focal adhesion

Fibronectin

Kinase

### ABSTRACT

MEK Kinase 2 (MEKK2) is a serine/threonine kinase that functions as a MAPK kinase kinase (MAP3K) to regulate activation of Mitogen-activated Protein Kinases (MAPKs). We recently have demonstrated that ablation of MEKK2 expression in invasive breast tumor cells dramatically inhibits xenograft metastasis, but the mechanism by which MEKK2 influences metastasis-related tumor cell function is unknown. In this study, we investigate MEKK2 function and demonstrate that silencing MEKK2 expression in breast tumor cell significantly enhances cell spread area and focal adhesion stability while reducing cell migration. We show that cell attachment to the matrix proteins fibronectin or Matrigel induces MEKK2 activation and localization to focal adhesions. Further, we reveal that MEKK2 ablation enhances focal adhesion size and frequency, thereby linking MEKK2 function to focal adhesion stability. Finally, we show that MEKK2 knockdown inhibits fibronectin-induced Extracellular Signal-Regulated Kinase 5 (ERK5) signaling and Focal Adhesion Kinase (FAK) autophosphorylation. Taken together, our results strongly support a role for MEKK2 as a regulator of signaling that modulates breast tumor cell spread area and migration through control of focal adhesion stability.

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## Funding

This work was supported by the National Institutes of Health [Grant CA120881 (to B.C.)] and the American Cancer Society, Illinois Div. [Grant 160485 (to B.C.)].

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# Conceiving the Research Question



# Why Do Researchers Ask?

- Resolve a problem
  - Clear Uncertainty
  - Make money
  - Curiosity
  - In health, help mankind
- 
- *To answer an uncertainty about something in the population that you (the researcher) want to resolve by measuring/experimenting on subjects that represent the population.*

# Where Do Questions Come From?

- Literature
  - Journals
  - Old Data
  - Media
- Looking for New ideas or techniques
  - Skepticism
  - Don't be shy

- Imagination
  - Keeping an open mind
  - Creativity
  - SiFi movies and TV
- Advisors
  - *(I believe this is the simplest most important)*

# Characteristics of Good Research

**FINER**

- **F**easible: can it be done
  - Subjects
  - Techs
  - Cost
  - Time
  - Scope (*early recognition and backing off*)

- **I**nteresting

- Must pass the “*So what?*” Test

- **N**ovel

- Don't reinvent the wheel unless in a new way

- **E**thical

- **R**elevant

- Must pass the “*Why should my grandmother care?*” Test

# When Thinking about a question...

- Background and Significance
- Design
- Study Subjects: excluded or included according to a criteria.
- Variables
  - Independent Variables (Predictor): What is being manipulated
  - Dependent Variables (Outcome): What is measured
- Statistical Tools

## First Exercise

Write your research question  
and why it is a FINER question

# Literature Review



# Why Review the Literature?

## 1) What's been done so far

- Should provide you with the following:
  - The latest trends in your topic
  - Available databases that you can think about using
  - Any gaps that you may answer
  - A concept for how you will answer your question

## 2) Level of Existing Knowledge and theory

- Determine the level of knowledge
  - Descriptive
  - Explanatory
  - Predictive
- How knowledge is generated
- The methodology applied to gather data
- Boundaries of the study being reviewed
- How relevant to your topic

### 3) Relevance and rational to your investigation

- A well written lit review should
- Very good story, that...
- Flows directly into your research plan, and...
- Supports your choice of topic due to its relevance and choice of study design due to its innovation

# How to Conduct a Lit Review

- When?
  - Start early to get an idea or to fine tune one that you already have.
  - Keep searching while conducting research
  - In case using databases and previous results it can be part of your results
  - And always remember, three months in the lab will save you one hour in the library.

# What?

- Databases: Good to direct your thought, find gaps and can be used as part of the results
- Journals: Latest work and techniques
- Books: Basic fundamental concepts

# How?

- Set Parameters
  - Your topic
  - Related topics
  - Related methods
  - Start from a central point and branch off
  - Use good keywords

# Organization makes a world of difference

- You are not going to read everything at first
- Abstract:
  - directly related: good for latest findings
  - Indirectly related: good for techniques, approach, sitting
- Take short notes to remind yourself what's useful in that paper
- Highlight key points
- Periodically skim over the papers you thought useful.
- Learn the names of the authors

## Evaluate the paper critically

- Be as harsh as you want
- Ask your advisor if you are correct in your evaluation
- If possible contact the author



- Write up
  - Start with an outline
  - Specific points
  - Smooth flow
  - Fill in the large details then smaller and smaller..

# The Hypothesis

# What is a Hypothesis

- A statement
- Claiming a tentative fact
- But still needs to be tested
- A provisional idea whose merit requires evaluation (wiki)
- Specific version of the research question that summarizes the main elements of the study.

# Types of hypotheses

- **Null Hypothesis:** There is no association between the variables.
- **Alternative Hypothesis:** There is an association
- **One sided (one tailed):** The association is directional
- **Two sided (two-tailed):** only states that there is an association

# When are they used?

- One-Sided

- When the results are meaningful in one direction only
- When other studies indicated similar results
- Effect of smoking on lungs

## Two-Sided

When both sides could be interesting and can be published regardless to the outcome as meaningful results.

Exercise for children with DMD

# Characters of Good Hypotheses

- Specific and focused: state the test, subject and outcome. Go to the point
- Short: do not tell a story of how and why
- Simple: One predictor and one outcome
- Written in advance: helps keeping the research concise and focused

Some Bad examples...and a couple of good ones... find them 😊

Innate immune receptors participating in bacterial recognition pathways, such as Toll-like receptors (TLRs) on the cell surface and NOD-like receptors (NLRs) in the cytosol, sense the types of bacteria present in Bacterial Vaginosis, and play a role in pathogenesis of this common condition.

Decreased expression of CD59 allows the binding of C5b-7 and of C9 leading to destruction of red and white blood cells in hyperhemolysis syndrome of acutely and chronically diseased patients.

Since SBP1 was evidenced to be an autoantigen, the presence of this autoantigen could mean possible risk for cancer; hence, the detection of this antibody against SBP could also suggest the risk for ovarian cancer.

The length of the telomere in HIV infected subjects is comparable to uninfected elderly individuals.

The diagnosis of ovarian cancer involves the examination of the cell nucleus morphology. The nucleus undergoes morphological changes as it differentiates into a cancerous cell. These tumor-related transformations are recognized as an early event in malignancy and result in modification of nuclear matrix proteins at the molecular level. An autoimmune response is triggered when the nuclear matrix proteins are released into circulation.

- individual's with sarcopenia will have more insulin resistance than individuals without sarcopenia.
- Influenza infection during pregnancy does not increase the risk of developing schizophrenia.



- Studies have reported anti-ovarian autoantibodies in sera of humans and hens with ovarian cancer. Researchers have also observed nuclear morphological changes associated with early stage ovarian cancer in hens. Thus it is hypothesized that anti-nuclear matrix protein antibodies will predict nuclear morphological changes in cells associated with early ovarian cancer in the spontaneous laying hen and will be correlated with ovarian tumor angiogenesis.

- There is an association between the use of statins and the prevention of secondary myocardial infarctions.
- caffeine consumption can increase one's risk of certain types of cancer, specifically colon cancer.
- Prolonged use of oral contraceptives such as those containing DRSP or LNG significantly increases the incidence of deep vein thrombosis.

## Exercise 2

Turn your research question into a valid alternative hypothesis and state the null

# Statistical Error

## Type I

- False Positive
- Rejection of the null
- Erroneous acceptance of the alternative hypothesis

## Type II

False Negative

Failure to reject the null hypothesis

Erroneous rejection of the alternative hypothesis

# Statistical Significance

- The probability of committing a type I error (false positive) is known as the alpha ( $\alpha$ ) value
- Small value that gives an upper limit of the chance of incorrectly rejecting the null hypothesis
- ~0.01- 0.05

The probability of committing a type II error (false negative) is known as the beta ( $\beta$ ) value

Failure to reject the null when it is actually incorrect

~0.05 -0.20

# Depends on the situation

- Studying the efficacy of biopsies to diagnose OVCA

- $\alpha = 0.20$

- 20 out of every 100 women will be subjected to a dangerous and invasive technique for nothing

Using CA125 as a biomarker to detect early stage OVCA

$\beta = 0.30$

30 out of every 100 women with OVCA will be misdiagnosed/under diagnosed

# Jury Decision

- |   |                                    |
|---|------------------------------------|
| • Innocence: -----  | Null                               |
| • Guilt: -----  | Alternative                        |
| • Beyond reasonable doubt: ---                              | Significance ( $\alpha$ )          |
| • Acquit an innocent person or<br>convect a criminal: ----- | Correct inference                  |
| • Convect an innocent person:                               | Type I error ( <i>false pos</i> )  |
| • Acquit a criminal: -----                                  | Type II error ( <i>false neg</i> ) |

# POWER

- The quantity of  $1-\beta$  is power
- The probability of correctly rejecting the null hypothesis and accepting the alternative
- A power of 0.90 means that the samples will fall under the predicted outcome by the alternative hypothesis 90% of the time.



# P-Value

- The chance (%age) of null is true
- The null hypothesis is rejected in favor of its alternative **only** if the  $P$  value is smaller than  $\alpha$  (significance)
- In other words, the results cannot be explained by the alternative hypothesis alone, but it could NOT be explained by chance as well.
- The smaller the  $P$ -value the better.

# Sampling

- Cannot test the entire population of a disease or condition
- Must rely on testing a sample that best represents the population
- Generalize the conclusion back (truth in the sample) to the population (truth in the universe)

Hypothesis: Becker Muscular Dystrophy (BMD) severity is  
Dependent on the location of the mutation within the  
dystrophin rod gene

Targeted Population

BMD patients exhibiting a range of symptoms severity

Accessible Population

Patients with (BMD) being treated at the KAUH

Intended Sample

The number of patients asked to participate in the study

Actual Sample (subjects)

The number of patients who participate in the study

# Selection Criteria

Inclusion Criteria: List of the main characteristics of the targeted population

- Demographic: Becker muscular dystrophy 15-55
- Clinical: Mild to sever symptoms
- Geographic: Jeddah - KAUH
- Temporal: Over a defined 6 months period
- *problem: what is the definition of mild or sever?*

## Exclusion Criteria

- Specific list applied to eligible sample (meeting inclusion criteria) but also meeting few more criteria to “*not to*” include them in the study.
- The more exclusion criteria, the more the restrictions, the smaller the sample.
  - Pain hypersensitivity
  - Overly medicated on pain reducers
  - Using new experimental steroid therapy

## Exercise 3

Select your inclusion and exclusion  
criteria

# DESIGNING THE STUDY

- Cohort: A group of soldiers marching together
- A group of subjects tested together
  - Prospective cohort
  - Retrospective cohort

# Prospective

- Assemble the cohort
- *selected babies with low birth weight*
- Measure predictor variables and potential confounders
- *measured their weight (baseline), find all possible confounders*
- Follow-up the cohort and measure outcomes
- *measured their weight and IQ as they develop*



# Retrospective

- ID cohort
- *Aortic aneurysm*
- *Collect predictor*
- *Age, sex, size of aneurysm, other diseases*
- *Measure the outcome*
- *When did it rupture?*

# INCIDENCE

- Cohort Studies are best for reporting incidence (risk)

The number of People who gets a disease

Number of people at risk X time at risk

*The number of people who develop skin cancer*

*# of people working w/ radiation X time of exposure*

*= The risk of development skin cancer due to radiation exposure*

# CROSS-SECTIONAL

- All the measurements in one occasion or within a short period of time.
- Best for describing variables and their distribution patterns.
- *The number of CMV+ donors in Chicago*
- Can be used for examining associations
- *an association between childhood obesity and TV-watching.*

# PREVALENCE

- A proportion or percentage of individuals who have the conditions (being tested) at one given point of time.

The number of people who have a condition

People at risk

*West Nile Virus cases in Chicago*

*Chicago population*

*= prevalence of WNV in Chicago*

# Case-Control Studies

- Cannot yield to incidence or prevalence but provides association between the predictor and outcome.
- Best for assessing risk factors
- The risk of HIV or Hepatitis from injection drug use
- CAUSALITY

# How...

- In general...
- Must assemble a test group
- Case = subjects with the problem assessed
- Must assemble a reference group
- Control = subjects showing no problem
- Compare the prevalence amongst the two groups

# Prospective Case-Control

1) ID subjects (case and control)

*Second-hand smoking and non-smoking mothers*

2) Measure/administer the predictor

*# cigarettes exposed/day*

3) Measure/assess the outcome

*Birth Weight and IQ*

# Retrospective Case-Control

1) ID subjects (case and control)

*subjects with good vs. poor post transfusion complications due to blood age*

3) You have the outcome

2) Look backward to find differences in predictors that caused the two populations

*Analyze stored data on age of blood, HgbA1C, ...*



## Exercise 4

Select your study design

ANY QUESTIONS?