Science, Statistics, and Getting it Right: Interactive Discussion of Common Problems

PISA 2017 Workshop

Sponsored by:
ASIP Committee for Career Development & Diversity
ASIP Education Committee

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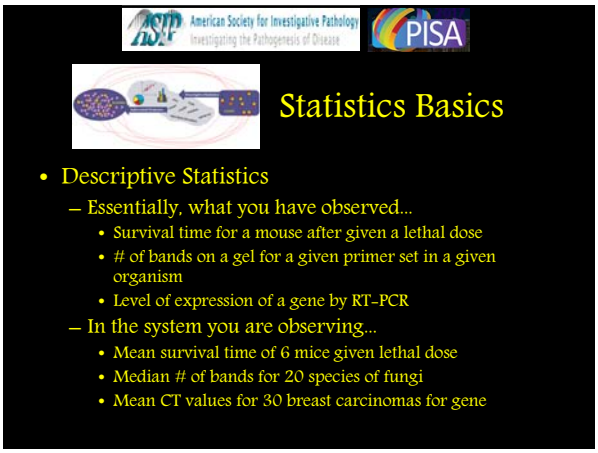
Special Thanks to Mark Sobel

Overview

- Introduction
- Large group discussion of cases
- Questions and parting thoughts

Why Statistics for Science?

- Science is careful observation of the natural world
  - Responses to perturbations/alterations
  - Changes in the presence of variable conditions
- First observation… "Is this true?"
- Second observation… "This may be true?"
- Nth observation… "This is most likely true!"
- Statistics allows us to determine if what we have observed given the number of observations we have made is "significant" (i.e., likely to represent the natural world)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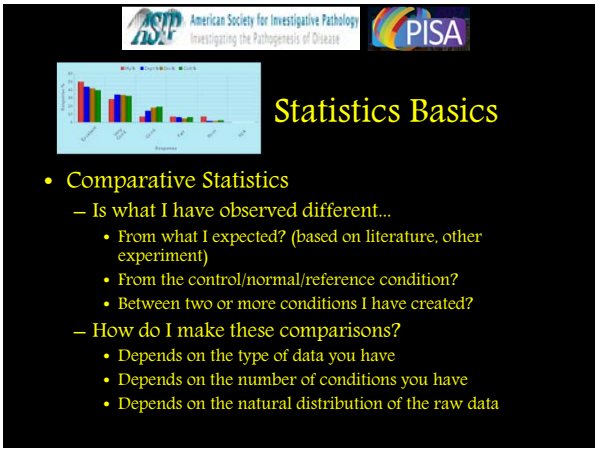
Statistics Basics
• Descriptive Statistics
– Essentially, what you have observed...
• Survival time for a mouse after given a lethal dose
• # of bands on a gel for a given primer set in a given organism
• Level of expression of a gene by RT-PCR
– In the system you are observing...
• Mean survival time of 6 mice given lethal dose
• Median # of bands for 20 species of fungi
• Mean CT values for 30 breast carcinomas for gene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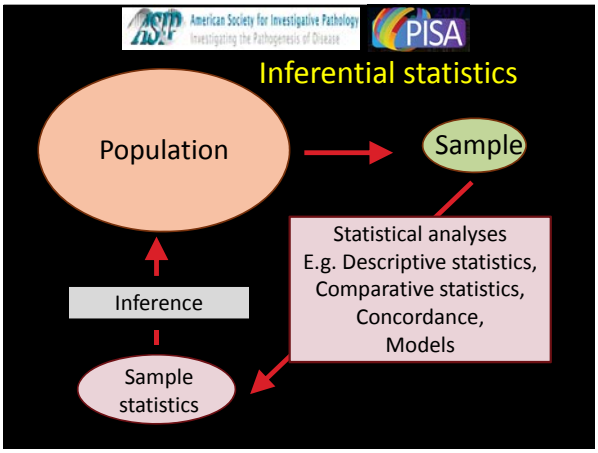
Statistics Basics
• Comparative Statistics
– Is what I have observed different...
• From what I expected? (based on literature, other experiment)
• From the control/normal/reference condition?
• Between two or more conditions I have created?
– How do I make these comparisons?
• Depends on the type of data you have
• Depends on the number of conditions you have
• Depends on the natural distribution of the raw data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Inferential statistics
Population
Sample
Statistical analyses
E.g. Descriptive statistics,
Comparative statistics,
Concordance,
Models
Inference
Sample
statistics

What test can we use?

What type of data do we have?

- Continuous dependent variable
  - Dependent variable is normally distributed → Parametric tests
  - Dependent variable is not normally distributed → Non-parametric tests
- Categorical dependent and independent variables
  - Unmatched/unpaired data for independent variables
  - Matched data for independent variables

Comparing 1 sample mean to a hypothesized mean	Comparing means in 2 independent samples	Comparing means in 2 paired observations	Comparing means in 3 or more independent samples
One sample t-test	Two independent samples t-test	Paired t-test	Analysis of variance (ANOVA)

What test should we use?

What type of data do we have?

- Interval or continuous dependent variable
  - Dependent variable is normally distributed → Parametric tests
  - Dependent variable is not normally distributed → Non-parametric tests
- Categorical dependent and independent variables
  - Unmatched/unpaired data for independent variables
  - Matched data for independent variables

Comparing 1 sample median to a hypothesized median	2 independent samples	Paired or correlated observations	3 or more independent samples
One sample median test	Wilcoxon-Mann Whitney test	Wilcoxon signed rank test	Kruskal Wallis test

What test should we use?

What type of data do we have?

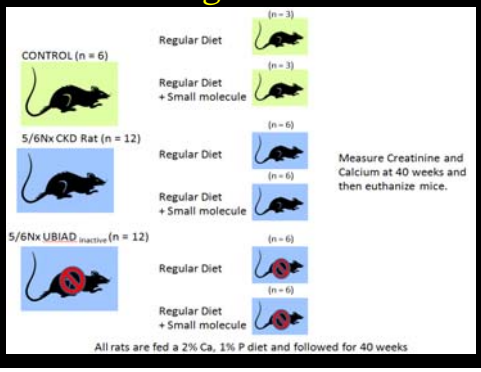
- Interval or continuous dependent variable
  - Dependent variable is normally distributed
  - Dependent variable is not normally distributed
- Categorical dependent and independent variables
  - Unmatched/unpaired data for independent variables
  - Matched data for independent variables

Comparing 1 sample proportion to a hypothesized proportion	Independent samples	Paired or correlated observations
Binomial test	Chi-square test, Fisher's exact test	McNemar's test

What Statistics Are (and are not)

- Description of the dataset we have and its parameters √
- Mathematical assessment of the significance of an observed result √
- Relevance of our data and findings to other reports in the literature √
- Tool for showing the importance of our work

Vignette 1



Vignette 1

- Common experimental model with:
  - Control: natural history of mouse with the wild-type genetic background
  - Experimental Model: Disease-specific mouse
  - Knockout: Loss of one specific gene (non-lethal)
  - Conditions: Regular diet or + small molecule
- Common challenge:
  - Small number of each group
  - Statistics of small numbers

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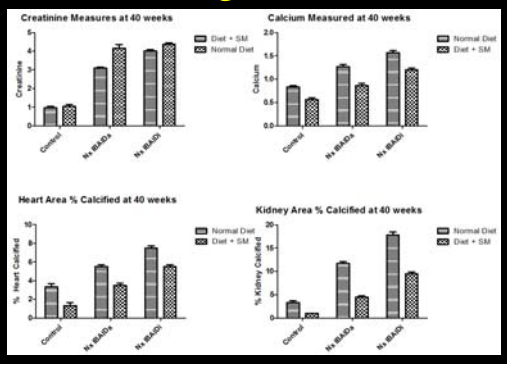
Vignette 1



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Vignette 1

- What kind of variables do you have?
- What kind of test can you perform?
- What kind of result are you looking for?
- How do you perform the test?
- What is the result of a two way ANOVA?

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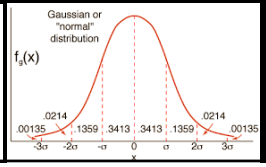
Vignette 1

- What kind of variables do you have?

Mouse (categorical) : controls, NX UBAID+, NX UBAID-
Diet (categorical): normal, normal + small molecule
Creatinine (continuous), Calcium (continuous),
heart % area calcified (continuous), kidney % area calcified (continuous)

Categorical vs. Continuous variables



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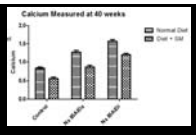
Vignette 1

- What kind of test can you perform?

Differences in UBIAD mouse calcium levels (excluding control):
Continuous variable, two groups = T-test (GD) or Rank Sum (NND)
Difference in mouse % heart calcified (all mice):
Continuous variable , three groups = ANOVA (GD) or Kruskal-Wallis (NND)
Difference in diet creatinine levels (excluding knock out):
Continuous variable, two groups = T-test (GD) or Rank Sum (NND)
Difference by mouse and diet in calcium level (all mice):
Continuous variable, three by two groups: two way ANOVA (GD) or others*



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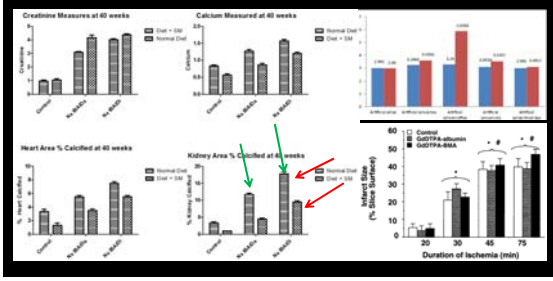
Vignette 1

- What kind of result are you looking for?



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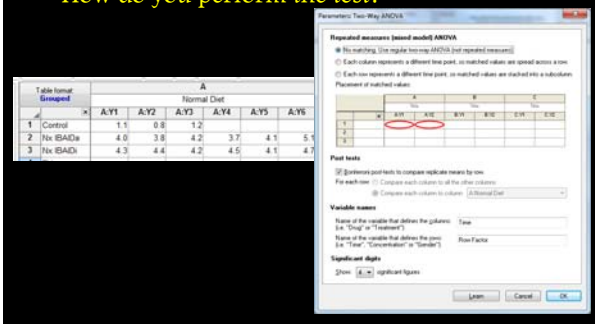
Vignette 1

- How do you perform the test?



Vignette 1

- What is the result of a two way ANOVA?

Table Analyzed	Calcium			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	0.57	0.4388		
Column Factor	25.28	< 0.0001		
Row Factor	60.75	< 0.0001		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Column Factor	***	Yes		
Row Factor	***	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	0.01800	0.009000	0.8526
Column Factor	1	0.8008	0.8008	75.87
Row Factor	2	1.925	0.9623	91.17
Residual	24	0.2533	0.01056	
Number of missing values	6			
Bonferroni posttests				
Normal Diet vs Diet + SM				
Row Factor	Normal Diet	Diet + SM	Difference	95% CI of diff.
Control	0.8333	0.5667	-0.2667	-0.4826 to -0.05077
Nx IBAIDa	1.267	0.8667	-0.4000	-0.5527 to -0.2473
Nx IBAIDi	1.567	1.200	-0.3667	-0.5193 to -0.2140
Row Factor	Difference	t	P value	Summary
Control	-0.2667	3.179	P < 0.05	*
Nx IBAIDa	-0.4000	6.743	P<0.001	***
Nx IBAIDi	-0.3667	6.181	P<0.001	***

Vignette 1: Interim Summary

- When designing an experiment, determine what the data will look like and what statistical tests will be used.
- When possible, calculate a sample size based on 80% power and alpha of 0.05
  - Normally distributed data
  - If non-normal, can approximate with normal
- For any given question, there is usually only one "correct" statistical test

Vignette 2

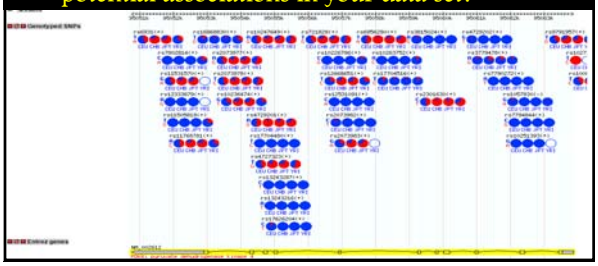
- 1175 healthy subjects (43% Caucasian, 33% African or African American, 24% Hispanic/Latino)
- College students provided DNA sequencing and detailed questionnaire
- Follow up survey every 5 years for the next 25 years
- Sequence each patient to 40X coverage, comparative genomic hybridization
  - Mutations included insertions/deletions, single nucleotide polymorphisms, and gene duplication.
- Questionnaire: diabetes, hypertension, malignancy, infections, diet, and exercise habits.
- Free pedometer connected to the internet
- Pyruvate dehydrogenase kinase 4 (PDK4) mutation in mice shown to decrease activity and lead to obesity

Vignette 2

- How would you go about investigating any potential associations in your data set?
- What considerations (statistical) are important in thinking about this question?
- How should the pedometer data be parsed at the individual variable level for analysis?

Vignette 2

- How would you go about investigating any potential associations in your data set?



Vignette 2

- What considerations (statistical) are important in thinking about this question?

Number of Samples: 1175 samples (n) but 100K+ variables! - > Hypothesis generation…

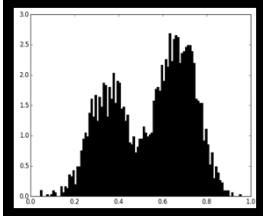
Distribution of SNPs: HapMap vs. our data set… New SNPs?

Other variables: Levels of exercise during college, lifestyle, etc.

Censored Data: Highly active individuals die of unrelated causes

Vignette 2

- How should the pedometer data be parsed at the individual variable level for analysis?

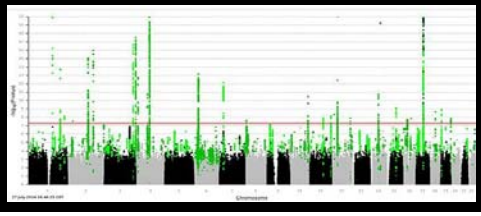


	Major Allele	Minor Allele
<50%	A	B
>50%	C	D

Vignette 2

	Major Allele	Minor Allele
<50%	A	B
>50%	C	D

- Analysis:
  - Fishers Exact test for EACH SNP (100K+)
  - Alternative: T-test for each SNP (pedometer)



Vignette 2: Interim Summary

- Genetic studies have a common problem:
  - n (number of patients) << k (number of variables)
- Same or similar statistical tests with special modifications (corrections)
- Should be considered "hypothesis generating" exercises
  - Always need experimental or epidemiological confirmation and, in best cases, biological evidence
- Gene expression data have similar challenges with equivalent solutions

Summary and Conclusions

- Your observations (science) are always important...
  - Positive OR Negative results
- If your experiments were designed correctly...
  - Including statistics BEFORE you begin
- And you complete them in a reproducible manner...
  - Repetition by others is required to get to the Nth observation!

Questions?