An introduction to biostatistics: part 1

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Table of contents

Introduction to data analysis

Uncertainty

Probability
  Conditional probability
  Random variables
  Discrete random variables
  The law of large numbers
  The central limit theorem

Associations between variables

Relative risk

Odds ratio

Statistical inference
  Pearson’s chi-squared test
  Fisher’s exact test
  Inference for 2 groups
  Inference with more than 2 groups
Data Analysis

One should start by examining what sorts of variables one has.

We distinguish between categorical and continuous variables: only a few possible values for the first, and in principle, an infinite number of possible values for the second.

Univariate summaries: simple summaries of each variable.

Example: tables, median and quantiles.
Data Analysis

Let’s read in some data from a genetic association study:

```r
fms <- read.delim("http://www.biostat.umn.edu/~cavanr/FMS_data.txt")
```

```r
> ls()
[1] "fms"
```

```r
> dim(fms)
[1] 1397 347
```

Let’s take a look at the variable called Race

```r
> table(fms$Race)

African Am Am Indian  Asian Caucasian Hispanic Other
   44       1     97     791     52      49     49
```
Data Analysis

but we don’t have this for all subjects

```r
> table(is.na(fms$Race))

FALSE  TRUE
    1034    363
```

We also can see how 2 variables look together

```r
> table(fms$apoe_c472t,fms$Race)

          African Am Am Indian Asian Caucasian Hispanic Other
    CC        6   0   9   101      3       3     6
    CT        1   0   3    26      0       0    0
```
Uncertainty

If I collected a different data set I would produce different summaries.

To provide a way to think about this, we think of the data we see as a realization of a random process: the data set we have is only 1 of many we could have observed.

We model our observed data as values taken on by a random variable.
Probability

Probability is a mathematical framework that allows one to make statements about phenomena with uncertain outcomes.

For some experiment with an uncertain outcome (e.g. flipping a coin) the sample space is the collection of all possible outcomes (e.g. heads or tails).

The possible outcomes are called events and probability is a map from the collection of events to a number in the interval $[0, 1]$ in a way that larger values indicate the event is more likely.
We use notation like $A$ is an event and $P(A)$ is the probability of that event.

There are just a few rules that probabilities must satisfy

1. for all events, $A_i$, $0 \leq P(A_i) \leq 1$.
2. if $S$ is the collection of all possible events, $P(S) = 1$.
3. if $A_1$ and $A_2$ are mutually exclusive (i.e. they can’t both occur in one trial of the experiment) then $P(A_1 \text{ or } A_2) = P(A_1) + P(A_2)$.
So, for example, if an experiment only has 2 possible outcomes and they are equally likely then the probability of each is one half.

The *odds* on an event is the number of times you expect to see the event happen for every time it doesn’t happen.

So if the probability of an event is \( \frac{k}{N} \) the odds on that event are \( k \) to the \( N - k \).

\[
\begin{array}{c|c|c}
\text{CC} & \text{CT} \\
128 & 30 \\
\end{array}
\]

So the observed odds on CC is 128 to 30 and the observed probability of seeing CC is \( \frac{128}{128 + 30} \).
Conditional probability

Two events, \( A_1 \) and \( A_2 \) are \textit{independent} if
\[
P(A_1 \text{ and } A_2) = P(A_1)P(A_2).
\]
The conditional probability of event \( A_1 \) given that event \( A_2 \) has
occurred is given by \( P(A_1 \text{ and } A_2)/P(A_2) \) and is denoted
\( P(A_1|A_2) \).

So what is \( P(A_1|A_2) \) in terms of \( P(A_2|A_1) \)?

If 2 events are independent then \( P(A_1|A_2) = P(A_1) \): this is the
intuitive basis for understanding what independence means.
Random variables

A *random variable* is a quantity that takes on certain values with certain probabilities.

For example, if I toss a coin and assign $X$ the value of 0 if there is a head and 1 if there is a tail, then $X$ is a random variable.

The distribution of a random variable is the values that the random variable takes and the probability that it takes those values.

We model the data we observe as the result of a randomized experiment and consequently as random variables.
Simulating random variables

We can simulate random variables in R.

Simulating 100 fair coins in R

```r
> set.seed(234)
> rbinom(100, size=1, p=.5)
[1] 1 1 0 1 0 1 1 1 1 0 1 1 1 1 0 0 0 1 0 1 1 1 1 1 0 0 1 0 1 1 1 0 0 1 1 1 1
[38] 0 1 0 1 1 1 0 1 1 0 1 0 0 1 0 0 0 0 1 1 0 0 0 1 0 0 1 1 1 1 1 0 1 0
[75] 0 0 1 0 0 0 1 1 0 1 1 1 0 1 0 1 1 1 0 0 1 1 0 0 1 1
```

and 100 unfair coins,

```r
> set.seed(234)
> rbinom(100, size=1, p=.05)
[1] 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
[38] 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
[75] 0 0 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0 1 0 0 0 0
```
Properties of random variables

The *expectation* of a random variable depends on its distribution and can be calculated as follows:

if $X$ is the random variable then its expectation is given by

$$EX = \sum_k kP(X = k).$$

while the *variance* of a random variable is given by

$$\text{Var}X = \sum_k (k - EX)^2 P(X = k).$$

So if $X$ is 1 with probability $p$ and is otherwise 0 what is its expected value? Its variance?

There are a number of commonly used probability models that are encountered, both discrete and continuous.
Discrete random variables

The *Bernoulli* distribution: a single success or failure random variable. Distribution only depends on the probability of a success.

The *binomial* distribution: the number of successes in $n$ independent trials where each trial is a success or failure (and all trials have the same success probability). Distribution depends on the success probability and the total number of trials.

The *multinomial* distribution is a generalization of the Binomial distribution to the case where each trial can have more than 2 outcomes. Distribution depends on success probabilities and number of trials.

The multinomial distribution is a natural model for nucleic acid sequences (since each position is one of 4 possible nucleotides).
The law of large numbers

Many possible models, none of which are particularly natural except perhaps the normal distribution (also called the Gaussian distribution).

Origin of the normal distribution: there are 2 critical results in probability theory.

The law of large numbers: if I have an infinitely long sequence of independent realizations of a random variable, then the sample mean will converge to the expectation of the random variable.

Hence if I compute the difference between the expectation and the sample mean it will go to zero.

> mean(rbinom(10,size=1,p=.6))
[1] 0.7
The central limit theorem

But, if I multiply this difference by the square root of the number of samples, the resulting product will behave like a random variable.

In fact one can determine the distribution of this random variable: this distribution is the normal distribution.

We often describe this in terms of the probability distribution of the sample mean, which is called its sampling distribution: \( \bar{x} \sim N(\mu, \sigma^2/n) \).

Here \( \mu \) is the expectation of the individual observations, \( \sigma^2 \) is their variance and \( n \) is the number of independent observations.

Can you use the expression for the approximate sampling distribution to verify the first claim on this slide?
Continuous random variables

For this reason, some argue, traits which are the outcome of many different factors, each with a small impact, are well modeled as normal random variables.

It frequently transpires that if the data are modeled as normally distributed, calculations that are otherwise impossible become “easy”.

Quantiles are typically used to summarize continuous variables.

> summary(fms$pre.BMI)

          Min. 1st Qu.  Median    Mean 3rd Qu.   Max.  NA's
Associations between variables

When you have 2 categorical variables, tables provide the most convenient way to summarize the association.

```r
> table(fms$resistin_c30t,fms$resistin_g540a)

       AA  GA  GG
     CC  75 281 356
     CT   0  11  10
```

When should one use row or column percents? Does it matter here?
Associations between variables

We frequently are interested in variables with 2 categories where one category indicates health status (e.g. have cancer).

Often we would like to know if some other dichotomous variable increases the likelihood that one has this health outcome (e.g. smoking).

The risk is the probability that one has a particular health outcome. If this risk is modified by another dichotomous variable, then we are often interested in how the risk varies with this other variable.
One can look at the difference in risk, 
\( P(\text{cancer}|\text{smoke}) - P(\text{cancer}|\text{don’t smoke}) \), but for rare health outcomes this will always be a small number.

More commonly we look at the ratio rather than the difference-this is called the *relative risk*.

To directly estimate \( P(\text{cancer}|\text{smoke}) \) we would need to set up a study where we enrolled many smokers and follow them in a *prospective* study.

This is expensive and time consuming, it is much easier to recruit subjects with cancer and ascertain if they smoke: a *retrospective* study.
Much like the relative risk is a ratio of probabilities, the odds ratio is a ratio of odds.

The big difference is that one can estimate the odds ratio using data from a retrospective study.

Moreover the odds ratio and relative risk are approximately equal when the event is rare.
Associations between variables

Let’s create a dichotomous variable that indicates if someone has a BMI greater than 30 and see if this is related to having metabolic syndrome.

```r
> table(fms$Met_syn,fms$pre.BMI>30)

   FALSE TRUE
0   688  53
1    37  39
```

How would you summarize this table?

Does a simple formula for the odds ratio exist?
Statistical inference

If I collected another data set in the same fashion as this one, do you think we would find a positive association?

To address this question, we frequently pose the question as follows: what is the probability that I would observe as big a difference as I have observed if there was not an association between these 2 variables?
About 9% of the subjects have metabolic syndrome and about 10% of subjects have a BMI greater than 30, so if these 2 variables are independent then the probability of both of these properties being true is about 1%.

However we have observed about 5% of our subjects having a BMI over 30 and having metabolic syndrome: is this simply too large to call chance variation?
Imagine simulating this table under the assumption of no association.

To do so we will assume that the margins of the table are just as we have observed them.

92 subjects have BMI greater than 30 and if there is no association between the 2 variables about 9% of these subjects will have metabolic syndrome, so conduct a few simulations

```r
> rbinom(1,92,p=.09)
[1] 10
> rbinom(1,92,p=.09)
[1] 6
> rbinom(1,92,p=.09)
[1] 8
```
These values are much smaller than our observed value of 39, in fact looking at a million of these variables we are never even close to the observed value of 39

```r
> summary(rbinom(1000000,92,p=.09))
   Min. 1st Qu.  Median    Mean 3rd Qu.   Max.  
    0.00    6.00    8.00    8.278   10.00    24.00
```

So what we are seeing doesn’t even occur 1 in a million times if these 2 variables are independent.
The process of using observed data to make statements about unobserved data (e.g. data we will observe in the future) is called statistical inference.

Frequently hypothesis tests are used to produce estimates of the probability of observing something more extreme than what we have observed.

Such probabilities are called *p-values* and when a *p*-value is less than 0.05 we say that there is a statistically significant association.

To test for an association between 2 dichotomous variables one uses a test procedure called Pearson’s $\chi^2$ test.
Pearson’s Chi-squared test

In R this is accomplished with the following code

```r
> chisq.test(table(fms$Met_syn,fms$pre.BMI>30))
```

Pearson’s Chi-squared test with Yates’ continuity correction
data:  table(fms$Met_syn, fms$pre.BMI > 30)
X-squared = 130.16, df = 1, p-value < 2.2e-16

Here the $p$-value is so small the software just reports that it is less than a very small number.
Pearson’s Chi-squared test

If there were fewer observations it would be more difficult to assess if there was an association:

```r
> table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30)

   FALSE TRUE
0     26    3
1      1    0
```

and if we conduct Pearson’s test we get a warning
Pearson’s Chi-squared test

```r
> chisq.test(table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30))
```

Pearson’s Chi-squared test with Yates’ continuity correction

data: table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30)
X-squared = 9.1766e-32, df = 1, p-value = 1

Warning message:
In chisq.test(table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30)): Chi-squared approximation may be incorrect
Fisher’s exact test

Pearson’s $\chi^2$ test, like many techniques in statistics, ultimately relies on the central limit theorem and so is only an approximation that is appropriate when the sample size is large.

Often times there are other techniques which are more appropriate when the sample size is small: here we can use a test called Fisher’s exact test.
Fisher’s exact test

That is also simple to implement in R

```r
> fisher.test(table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30))
```

Fisher’s Exact Test for Count Data

data:  table(fms$Met_syn[1:40], fms$pre.BMI[1:40] > 30)
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.0000  349.0909
sample estimates:
odds ratio
  0

Why not just always use this test?
Associations between variables: one continuous variable

While we can always dichotomize a continuous variable and conduct analyses as we have above, this is suboptimal as it disposes of information.

Boxplots are convenient visualization tools for comparing 2 or more continuous distributions.

```r
pdf("bxplt-BMI.pdf")
boxplot(fms$pre.BMI~fms$Met_syn)
dev.off()
```

Causation: look at the distribution of the response variable as it depends on the explanatory variable (this plot has this backwards).
Associations between variables: one continuous variable

We could test for a difference in the means across metabolic syndrome status

```r
> mean(fms$pre.BMI[fms$Met_syn==0 & !is.na(fms$Met_syn)],na.rm=T)
[1] 23.67998
> mean(fms$pre.BMI[fms$Met_syn==1 & !is.na(fms$Met_syn)],na.rm=T)
[1] 31.56039
```

A 2 sample *t*-test is an inferential procedure that tests for a difference in the means of 2 sets of measurements.
Associations between variables: one continuous variable

Here is the syntax for a 2 sample t-test.

> t.test(fms$pre.BMI[fms$Met_syn==0 & !is.na(fms$Met_syn)],
+ fms$pre.BMI[fms$Met_syn==1 & !is.na(fms$Met_syn)])

Welch Two Sample t-test

data: fms$pre.BMI[fms$Met_syn == 0 & !is.na(fms$Met_syn)] and
fms$pre.BMI[fms$Met_syn == 1 & !is.na(fms$Met_syn)]

t = -13.631, df = 85.794, p-value < 2.2e-16
Associations between variables: one continuous variable

When the categorical variable has multiple levels and we are interested in the hypothesis of no difference among the groups we can use a technique called analysis of variance (ANOVA).

```r
> a1=aov(fms$pre.BMI~fms$Race)
> summary(a1)

            Df Sum Sq Mean Sq F value Pr(>F)
fms$Race     5 142.00  28.40  1.3640 0.2361
Residuals 1025 21388.00   20.87

The basic idea is to compare how much variability there is among the means across the levels of the categorical variable to how much variability one sees within the levels of the categorical variable.