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The Effects of Smoking & The Analysis of Variance copy 2

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created: 2019-Oct-18

updated: 2019-Oct-18

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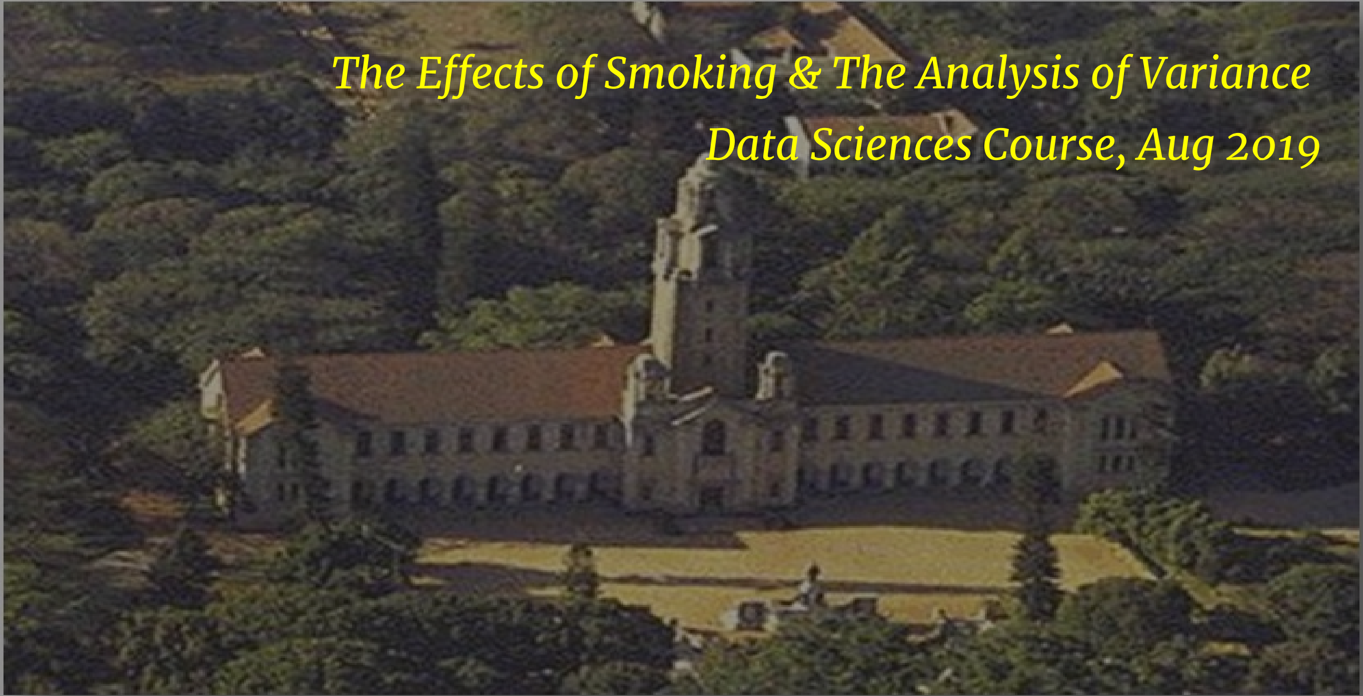
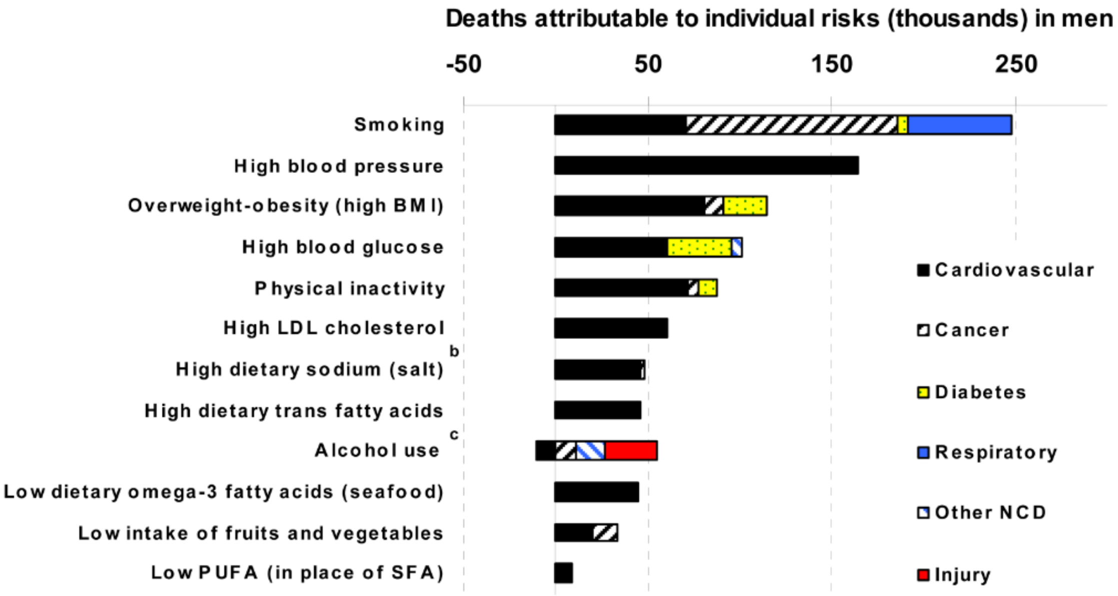


The Effects of Smoking & The Analysis of Variance

Data Sciences Course, Aug 2019

Ramesh Hariharan

Smoking leads Preventable Deaths in the US for Men



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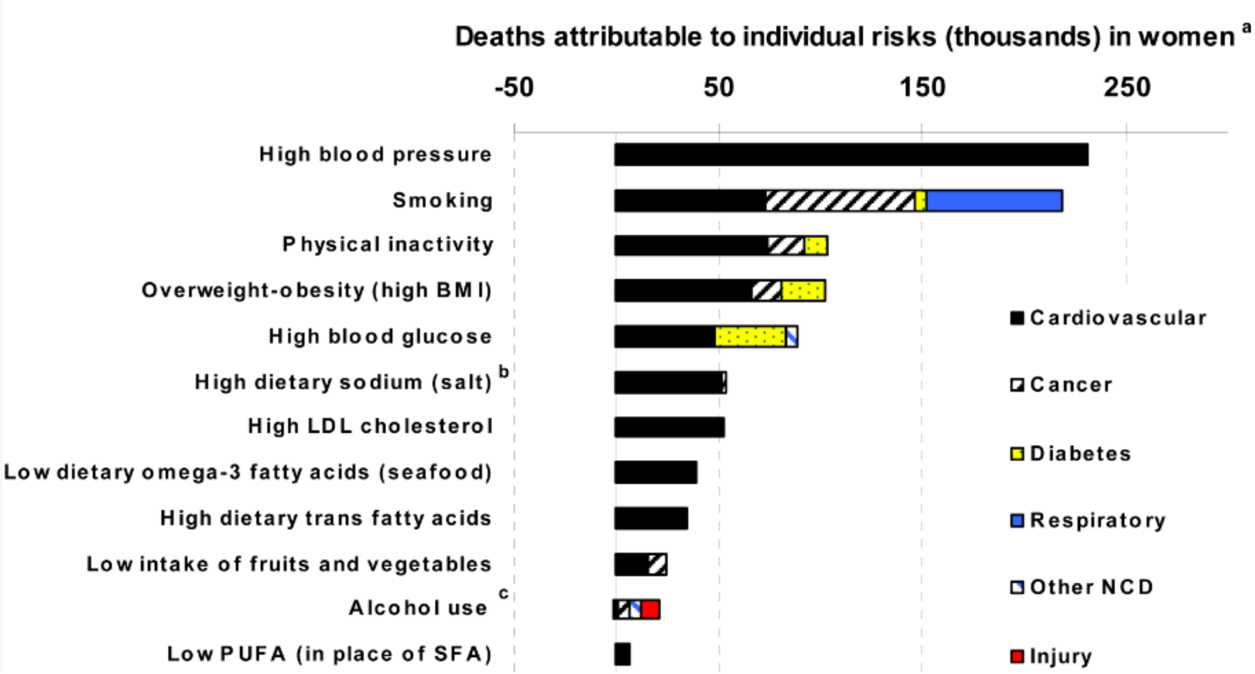
Smoking leads Preventable Deaths in the US for Women As Well



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Differential Effects on Women

- Effects of smoking are more serious for women than for men
  - more vulnerable to cigarette smoke-induced respiratory diseases
  - adverse affects on fertility, early menopause, pregnancy complications
  - higher risk of type-2 diabetes
  - higher absolute risk for lung cancer
  - additional hazards such as breast cancer, ovarian cancer, and cancer of the cervix
- On the positive side, women have been found to have higher survival rates regardless of lung cancer type, stage and therapy

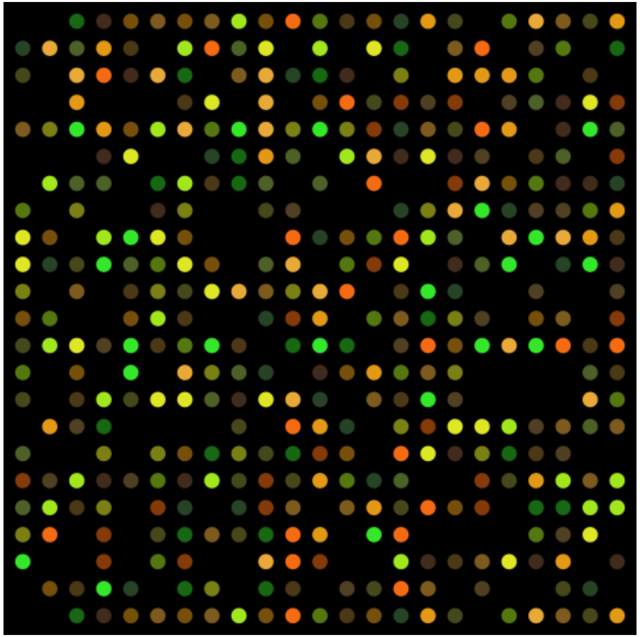
Genes & Gene Expression

- ~20,000 genes in each cell of the body, coded in (largely) read-only DNA
- Genes are transcribed to mRNA, then translated to protein
- Proteins react chemically to drive various biological functions
- The amount of gene-->mRNA transcription (gene expression) is dynamic, as a function of the cell type, the stimulus, age etc
- How does gene expression for the various genes respond to smoke?
- Which gene increase in expression, which decrease?
- Are these different between men and women?
- What biological functions do these differential genes influence?
- Does the disruption in these functions explain observed pathology due to smoking?

Measuring Gene Expression

- A gene is a double stranded sequence of A, C, G, Ts, very stable
- The two strands are complementary
- Could be thousands or tens of thousands of characters long
- mRNA is a single stranded sequence, but highly unstable, meant for temporary purposes
- mRNA can be extracted from a cell and converted to the complementary single stranded DNA (called cDNA)
- A probe is a shorter DNA sequence, ~25-100 characters long, complementary to this cDNA
- Many copies of a probe can be spotted on a glass surface, with different spots carrying probes for cDNA from different genes
- Typically use a few distinct probes per gene, so tens of thousands of spots
- mRNA converted to cDNA from a collection of cells is then poured on the glass slide
- cDNA from each gene gravitates towards its respective spot
- A cDNA molecule hybridized to its probe glows
- The glow at a spot is proportional to the amount of mRNA for that gene

A Microarray Picture

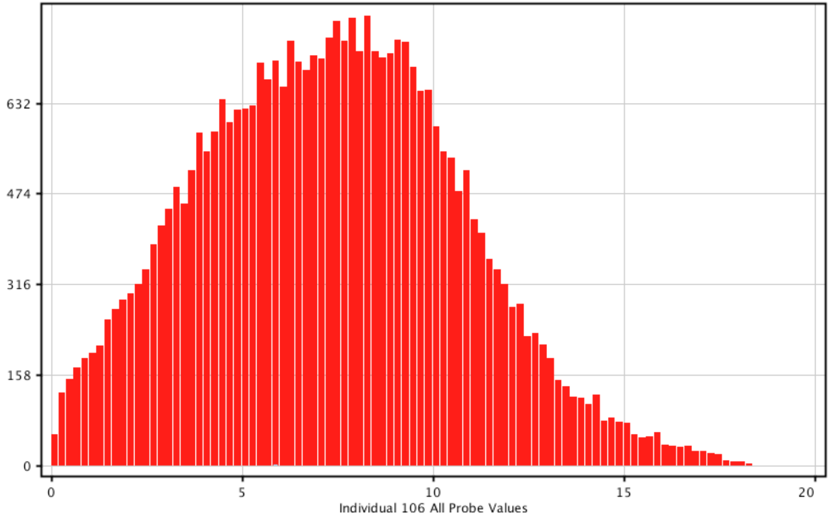


- Image analysis identifies each spot and measures its intensity
- For each probe, we now have the intensity
- And we also know the corresponding gene for each probe
- So, we now have one or more measurements of the expression level of each of the 20,000 genes
- And we can repeat this for multiple humans, some who are smokers, some non-smokers, some males, some females

The Data

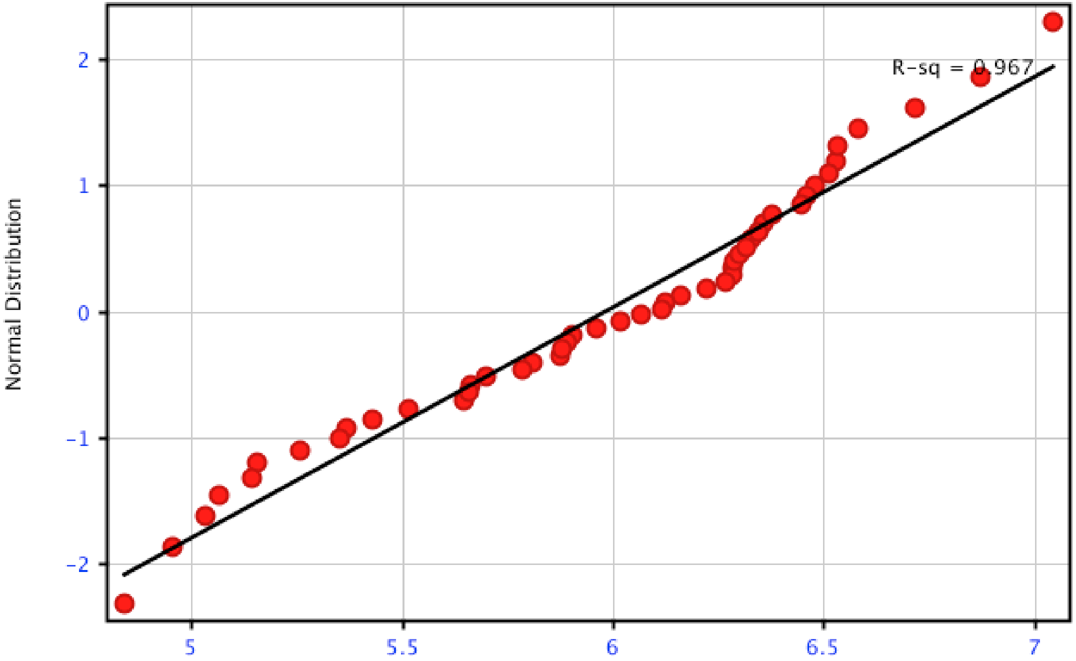
- Data is generated from white blood cells from 48 individuals reference
- A single file with 48 columns of data, plus some auxiliary columns, here
- Auxiliary columns: Probe name, Gene Symbol, Entrez Gene Id, ignore the rest
- A single gene (identified by a Gene Symbol or Entrez Gene Id) could have multiple probes
- Totally 41,094 probes
- Data Columns:
    - 12 Male Non-smokers (106-117)
    - 12 Male Smokers (118-129)
    - 12 Female Non-Smokers (130-141)
    - 12 Female Smokers (142-153)
- Values are logs to the base 2 of the original value
- There are some 0 values as well, due to thresholding low value before taking the log

Distribution for a Single Individual



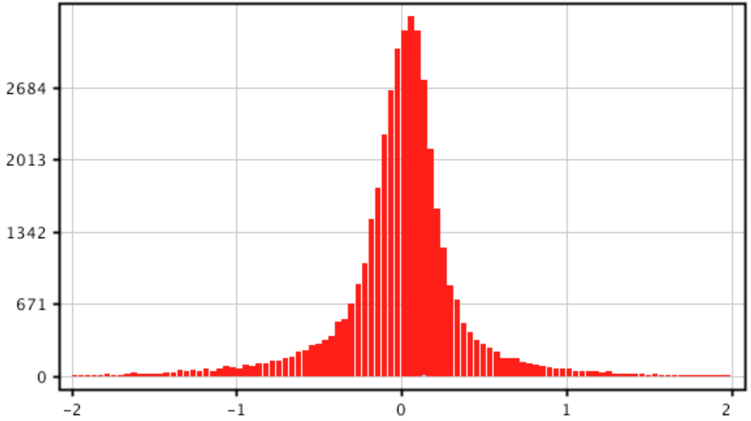
- Values go from roughly 0 to 20 on the log scale, so roughly 0-1,000,000 on the linear scale
- Median roughly 8.6, or 400 on the linear scale
- Distribution not quite Gaussian

Distribution for a Single Probe & Normality



- This Normal Probability Plot displays the 48 data points for probe A_24_P470079 against a corresponding number of (almost) equi-area-distant data points from a Gaussian $N(0,1)$ distribution ($\Phi^{-1}(\frac{i-0.5}{n})$)
- A straight line indicates the data is close to Gaussian
- This will be important later

Are Probe Variances the Same in the Various Groups?

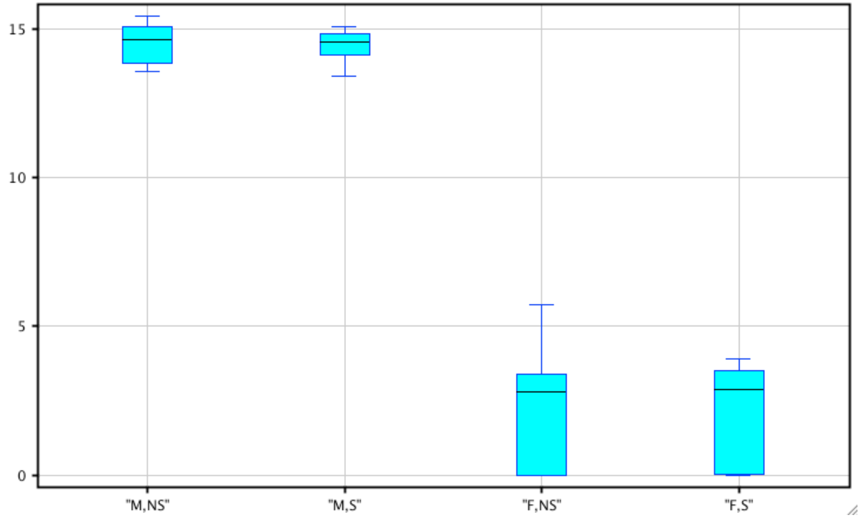


- Take the standard deviation across samples in a group for a single probe
- Take the difference of the above between two groups
- Plot the distribution of this quantity across all probes
- It turns out to be a distribution centered around 0, indicating standard deviations are similar in the various groups
- This will be important as well later

Differential Expression Analysis

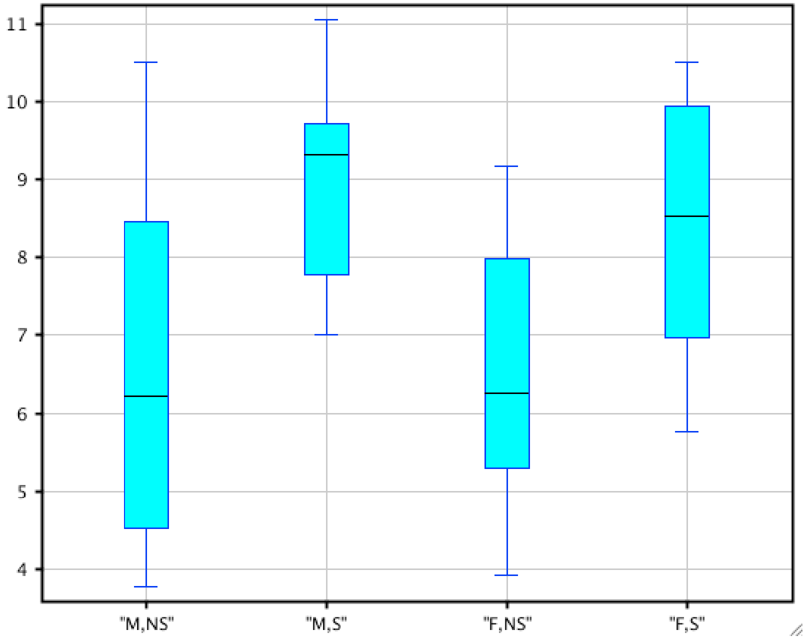
- Which of the ~20,000 genes behaves differently between
  - Males and Females, independent of Smoking Status
  - Smokers and Non-smokers, independent of Gender
  - Male Smokers and Non-smokers, vs Female Smokers and Non-smokers
- What do these genes tell us about our observations on females being more susceptible to smoking-related diseases but more robust to surviving smoking-related cancer?

Male vs Female Differential Response (*RPS4Y2*, A23_P324384)



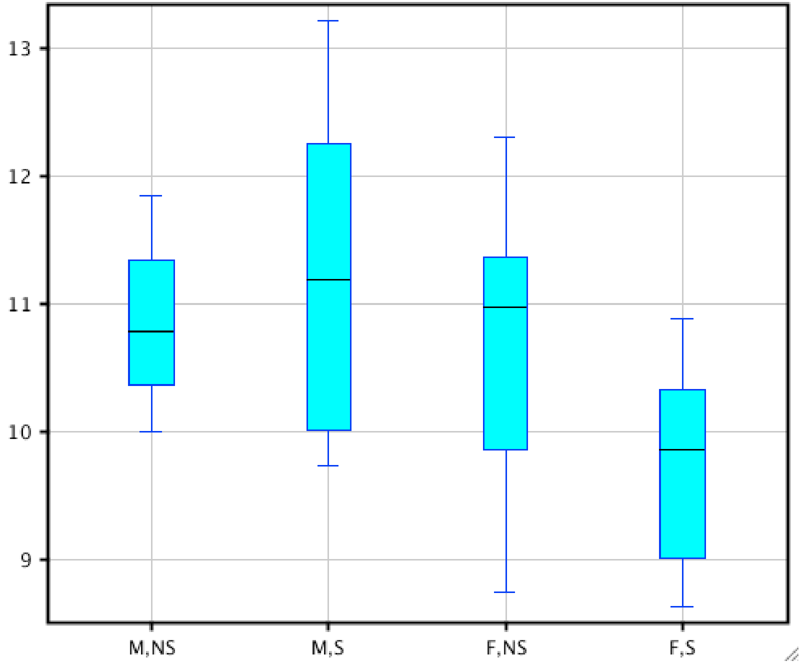
- The *RSPY2* gene is clearly different between males and females, regardless of smoking status
- The gene is located on the Y chromosome, which explains it
- There are 3 paralogs (similar copies) in the genome, one on the X chromosome and 2 on the Y chromosome
- Females express both copies on the X chromosome, while males express one on X and the two on Y, the latter to a lesser degree link

Smoker vs Non-Smoker Diff. Response (*AHR*, A23_P215566)



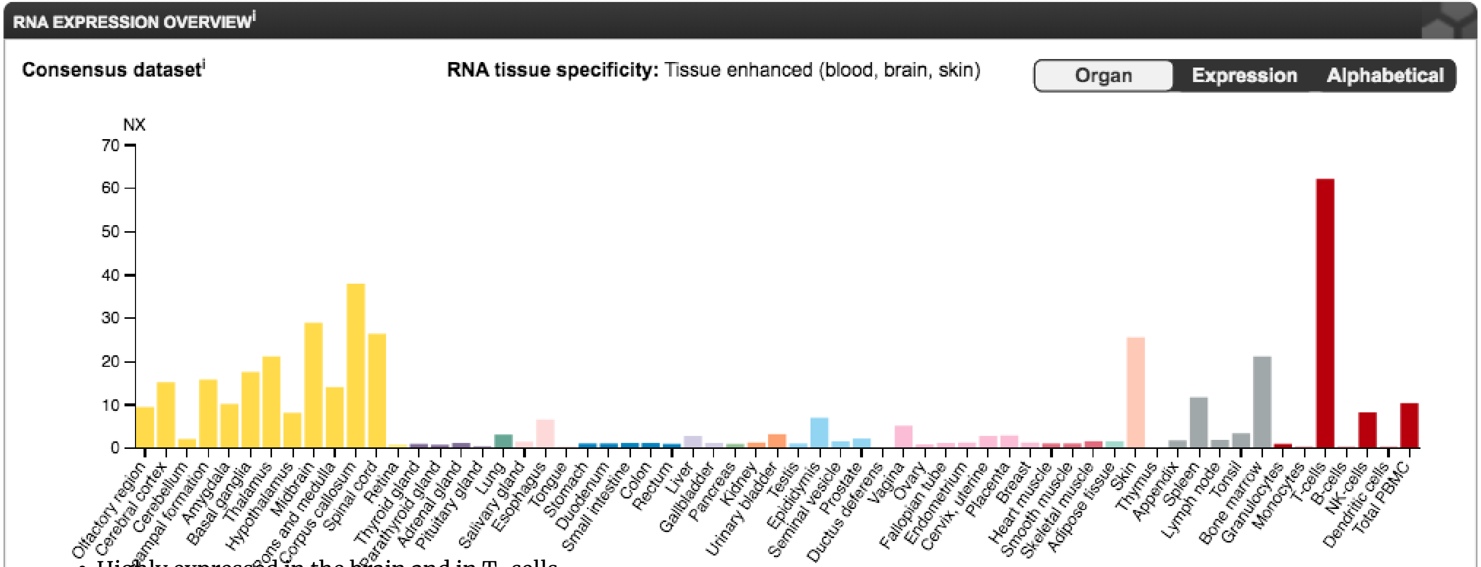
- The Aryl Hydrocarbon receptor (*AHR*) is a sensor of xenobiotic chemicals, such as those found in smoke
- It also causes the expression of other genes which metabolize (break down) these chemicals
- Side-effects of this breakdown include free radicals which cause DNA damage, which is widespread in smokers
- In addition, *AHR* expression is also found to be increased in many cancer cells, indicating a link to cancer

Gender x Smo. Status Diff. Response (*S1PR5*, A23_P107744)



- *S1PR5* is not so different between smokers and non-smokers in men
- Or between men and women non-smokers
- However, its expression appears quite reduced in female smokers relative to both female non-smokers and with males as a whole
- Little is known about the function of this gene

S1PR5, and Immune Response to Smoking in Women



RNA EXPRESSION OVERVIEW[i]

Consensus dataset[i]

RNA tissue specificity: Tissue enhanced (blood, brain, skin)

- Highly expressed in the brain and in T-cells
- Could play a role in immunity
- Indeed, it is known that changes in the immune system of women smokers are more pronounced than in men ref

Summary of Analysis

- Genes associated with cancer and the immune system are altered in both females and male smokers vs non-smokers
- Many more immune function genes were down-regulated in female smokers than in males; these differential changes in immune function in females could explain their greater susceptibility to several diseases
- Many genes associated with DNA repair, xenobiotic metabolism, free radical scavenging and natural killer cells cytotoxicity are down-regulated in female smokers relative to male smokers; these could explain the increased susceptibility of females to smoke-induced cancer
- There may also be some clues as to why females survive cancer better, though a conclusive answer is still not there (e.g., *CYP4F2*, *CYP4F12*)

Switching now to The Hypothesis Testing Problem

- Suppose you have an hypothesis, e.g.
    - Male and female heights have the same distribution, *versus*
    - Male and female heights do NOT have the same distribution
- How would you verify which is the case?
- You sample a few males and a few females *independently* at random, and measure their heights
- From this random sample, you could estimate the mean and variance of the underlying distribution(s) (*how*?)
- And then check if the means of the two distributions are the same or not (*how*?)

Sample Mean and Distribution Mean

- How does the sample mean relate to the mean of the underlying distribution?
- Let X be a random variable denoting the underlying distribution
- Let $E(X) = \mu, Var(X) = \sigma^2$
- Let $X_1, \ldots, X_n$ be the *independent* sample values (say heights of samples males)
- Then $E(X_i) = \mu, \forall i$
- $E(\Sigma X_i/n) = \Sigma_i E(X_i)/n = \Sigma_i \mu/n = \mu$ (note: Linearity of Expectation, regardless of independence)
- So the expected value of the sample mean is μ
- But how close does the sample mean get to μ?

Distribution of the Sample Mean

- The distribution of the sample mean (which is different from the underlying distribution) has mean μ
- What is the variance of this distribution?
- *This is where independence comes in*
- $Var(\Sigma X_i/n) = \Sigma_i Var(X_i)/n^2 = \sigma^2/n$ (note: Linearity of Variance, given independence)
- Variance $\propto \frac{1}{n}$, Std Dev $\propto \frac{1}{\sqrt{n}}$
- In fact, as n increases, the distribution of the sample mean gets closer to $N(\mu, \sigma/\sqrt{n})$ (the Normal distribution, regardless of the underlying distribution)

Estimating the Mean of the Underlying Distribution

- Suppose you want to estimate the underlying distribution mean so with greater than 95% probability you are within 10% of the actual number
  - Choose n so that $N(\mu, \sigma/\sqrt{n})$ has less than 5% outside $\mu(1 \pm 0.1)$
  - Then pick n samples independently at random
  - Take the sample mean as an estimate of the underlying distribution mean
- Catch: You need to know the variance σ^2!!
- How does one estimate the variance of the underlying distribution?

Estimating the Variance of the Underlying Distribution

- Would sample variance estimate the variance of the underlying distribution?
- Sample variance = $\frac{1}{n}\Sigma_i(X_i - \Sigma_j X_j/n)^2$
- $E[\frac{1}{n}\Sigma_i(X_i - \Sigma_j X_j/n)^2]$
- $= \frac{1}{n}E[\Sigma_i X_i^2 + \Sigma_i(\Sigma_j X_j/n)^2 - 2\Sigma_i X_i(\Sigma_j X_j/n)]$
- $= \frac{1}{n}E[\Sigma_i X_i^2 - (\Sigma_j X_j)^2/n] = \frac{1}{n}E[\Sigma_i \frac{n-1}{n} X_i^2 - \frac{2}{n}\Sigma_{i\neq j} X_i X_j]$
- $= \frac{1}{n}[\frac{n-1}{n}\Sigma_i E(X_i^2) - \frac{2}{n}\Sigma_{i\neq j} E(X_i X_j)]$
- $= \frac{1}{n}[(n-1)E(X_1^2) - (n-1)\mu^2]$ (note $E(X_i X_j) = E(X_i)E(X_j)$ by independence)
- $= \frac{n-1}{n}[E(X_1^2) - \mu^2] = \frac{n-1}{n}\sigma^2$ (note: not quite the distribution variance)

An Unbiased Estimator for the Distribution Variance

- The sample variance underestimates the variance of the underlying distribution!
- $E[\frac{1}{n}\Sigma_i(X_i - \Sigma_j X_j/n)^2] = \frac{n-1}{n}\sigma^2$
- Use $\frac{1}{n-1}\Sigma_i(X_i - \Sigma_j X_j/n)^2$ instead!
- In summary
    - the sample mean is an unbiased estimator for the distribution mean
    - $\frac{n-1}{n}$ times the sample variance is an unbiased estimator for the distribution variance
    - pick n so the distribution of the sample mean is tight around the distribution mean (based on the estimated distribution variance), or based on *what you can afford*

Are the Male and Female Distributions *Significantly* Different?

- You take an independent random sample of males, and a separate sample of females; measure heights in each sample and find the sample means $\hat{\mu}_M$ and $\hat{\mu}_F$ (*the hat typically indicates sample as opposed to distribution*)
- Are the two distributions different, i.e., are the sample means significantly different? (Assumption: variances are the same)
- What does signficant mean?
- $|\hat{\mu}_M - \hat{\mu}_F| > \Delta$ for some suitable Δ?
- If σ is large, then even if the two distributions were the same, $\hat{\mu}_M$ and $\hat{\mu}_F$ could be quite different (recall sample mean $\sim N(\mu, \sigma/\sqrt{n})$)
- So perhaps $\frac{|\hat{\mu}_M - \hat{\mu}_F|}{\sqrt{\hat{\sigma}_M^2 + \hat{\sigma}_F^2}} > \Delta$?

Test Statistics and P-Values

- $\hat{t} = \frac{|\hat{\mu}_M - \hat{\mu}_F|}{\sqrt{\hat{\sigma}_M^2 + \hat{\sigma}_F^2}}$ is called a *test statistic*
- This statistic has a certain distribution T (imagine sampling many many times and seeing what values of the statistic you get)
- T depends on the two underlying distribution(s) and the sample size
- The probability that $T \geq \hat{t}$ assuming that the two underlying distributions are the same (i.e., same means) is called the *p-value*
- A small p-value indicates that the two underlying distributions are likely to be different
- The conventional cut-off is 0.05
- To derive a p-value, one must first define a statistic (as a function of the sample values) and then obtain the distribution of this statistic assuming identical underlying distributions

The ANOVA Approach for Heights

- Two models:
    - The gender-specific model: Males $h = \mu_M + \epsilon$, females $h = \mu_F + \epsilon$, where ϵ is random variable with some distribution (to be determined) with mean 0
    - The universal model: $h = \mu + \epsilon$ (we will call this the *null model*)
- Now we sample females with height $\hat{h}_1, \ldots, \hat{h}_n$ and males $\hat{h}_{n+1}, \ldots, \hat{h}_m$
- From this sample, we determine the values of μ_M, μ_F that minimize $\alpha^2 = \mathbf{min}_{\mu_F, \mu_M} [\sum_{i=1}^{n} (\hat{h}_i - \mu_F)^2 + \sum_{i=n+1}^{m} (\hat{h}_i - \mu_M)^2] = \sum_{i=1}^{m} \hat{\epsilon}_i^2$ using standard linear regression
- Next we do the same for the null model: find μ that minimizes $\alpha'^2 = \mathbf{min}_{\mu} [\sum_{i=1}^{m} (\hat{h}_i - \mu)^2] = \sum \hat{\epsilon}_i^2$
- The statistic we use is $F = \frac{\hat{\alpha'^2}}{\hat{\alpha^2}} - 1 = \frac{\hat{\alpha'^2} - \hat{\alpha^2}}{\hat{\alpha^2}}$ is called the F-statistic (with a small modification later)
    - If it is large, then the null model is a less likely candidate from which the data at hand could have been drawn
    - What is the distribution of this statistic under the null model? How do we get a p-value?

The Gender-Specific Model in Matrix Form

- $$\begin{pmatrix} \hat{h}_1 \\ \hat{h}_2 \\ \vdots \\ \hat{h}_n \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 1 & 0 \\ \vdots & \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} \mu_F \\ \mu_M \end{pmatrix} + \begin{pmatrix} \hat{\epsilon}_1 \\ \hat{\epsilon}_2 \\ \vdots \\ \hat{\epsilon}_n \end{pmatrix} \tag{1}$$

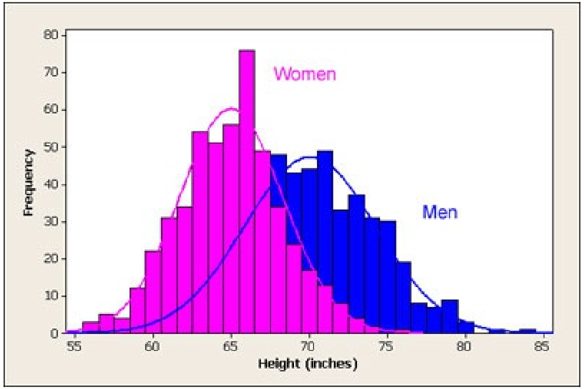
*There are m 1's and $n-m$ 0's in the first column

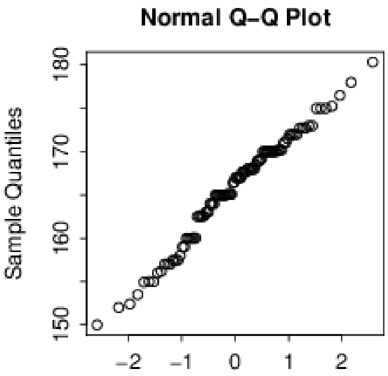
The Universal (Null) Model in Matrix Form

- $$\begin{pmatrix} \hat{h}_1 \\ \hat{h}_2 \\ \vdots \\ \hat{h}_n \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \\ 1 \\ \vdots \\ 1 \\ 1 \\ 1 \end{pmatrix} \left(\mu \right) + \begin{pmatrix} \hat{\epsilon}_1 \\ \hat{\epsilon}_2 \\ \vdots \\ \hat{\epsilon}_n \end{pmatrix} \tag{2}$$

- Further, we will need some assumption of the distribution from which $\hat{\epsilon}_i$'s are drawn; typical assumption is $N(0, \sigma^2)$, but needs to be justified

The $N(0, \sigma^2)$ Assumption





- Adult male heights mean 70in, s.d 4in link
- Adult female heights mean 65in, s.d 3.5in link
- Both appear Gaussian as shown by the Q-Q Plot link

Linear Regression to Minimize Sum of Squared Errors

- For each model, A and A', in turn (shown below for A only)
    - $\vec{\hat{h}} = A\vec{x} + \vec{\hat{\epsilon}}$
    - $(\vec{\hat{h}} - A\vec{x})^T(\vec{\hat{h}} - A\vec{x}) = \vec{\hat{\epsilon}}^T\vec{\hat{\epsilon}} = \Sigma_i \hat{\epsilon}_i^2$
    - To minimize the RHS over $\vec{x}$, we need to solve $A^T\vec{\hat{h}} = A^TA\vec{x}$
    - The best $\vec{x} = (A^TA)^\dagger A^T\vec{\hat{h}}$, where † is the pseudoinverse
    - The minimum value of $\Sigma_i \hat{\epsilon}_i^2$ then is $(\vec{\hat{h}} - A(A^TA)^\dagger A^T\vec{\hat{h}})^T(\vec{\hat{h}} - A(A^TA)^\dagger A^T\vec{\hat{h}}) = \vec{\hat{h}}^T(I - A(A^TA)^\dagger A^T)\vec{\hat{h}}$ $\ldots\mathbf{1}$
    - The above follows because $(I - A(A^TA)^\dagger A^T)$ is idempotent (X is idempotent if $XX = X$).

The F-Statistic & P-value

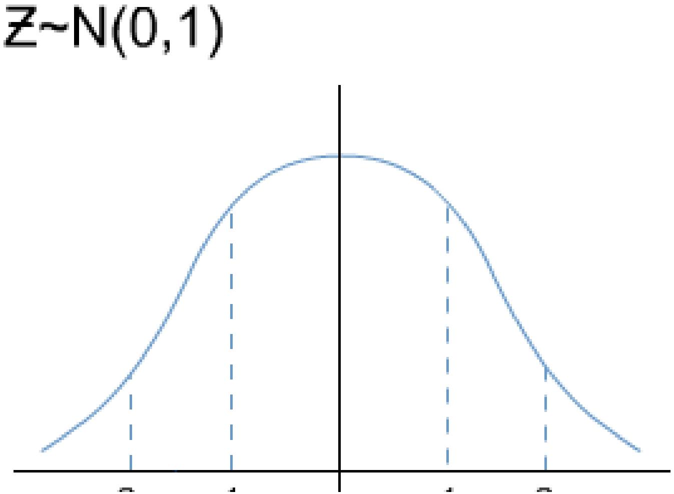
- The two A's of interest are:
- F-Statistic$= \frac{\vec{\hat{h}}^T (I - A'(A'^T A')^\dagger A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I - A(A^T A)^\dagger A^T)\vec{\hat{h}}} - 1 = \frac{\vec{\hat{h}}^T (A(A^T A)^\dagger A^T - A(A^T A)^\dagger A^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I - A(A^T A)^\dagger A^T)\vec{\hat{h}}}$
- To compute the p-value, we need the distribution of this statistic under the assumption that the universal (null) model holds, i.e., each $\hat{\epsilon}_i$ is drawn independently from $N(0, \sigma^2)$, i.e., $\hat{h}_i$ is drawn from $N(\mu, \sigma^2)$.
- A small p-value means the universal (null) model is unlikely to support the F-statistic derived from the data at hand, so the universal (null) model can be rejected
- Additionally, since we have evidence that heights are distributed as Gaussian and with the same/similar variance for males and females, then a low p-value shows that the gender-specific model is likely

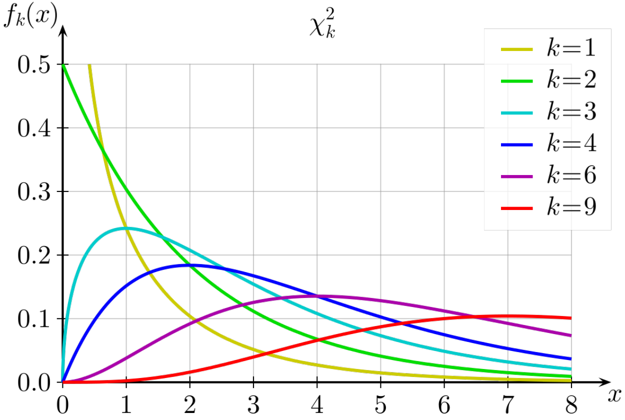
$$A = \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 1 & 0 \\ \vdots & \\ 0 & 1 \\ 0 & 1 \\ 0 & 1 \end{pmatrix}, A' = \begin{pmatrix} 1 \\ 1 \\ 1 \\ \vdots \\ 1 \\ 1 \\ 1 \end{pmatrix} \quad (3)$$

Distribution of the Num/Denom under the Null Model

- What is the distribution of $\vec{\hat{h}}^T f(X,Y)\vec{\hat{h}}$, where $f(X,Y) = X(X^TX)^\dagger X^T - Y(Y^TY)^\dagger Y^T$?
- $\vec{\hat{h}}^T f(X,Y)A^T\vec{\hat{h}} = \sigma^2 \times \frac{\vec{\hat{h}}-A'\mu}{\sigma}^T f(X,Y)\frac{\vec{\hat{h}}-A'\mu}{\sigma}$ (because $f(X,Y)A'\mu = \vec{0}$) $\ldots\mathbf{2}$
- $\vec{\hat{y}} = \frac{\vec{\hat{h}}-A'\mu}{\sigma}$ has entries that are independently sampled from a standard normal $N(0,1)$
- Claim: If the column space of Y is a subspace of the column space of X, and ALL eigenvalues of $X(X^TX)^\dagger X^T, Y(Y^TY)^\dagger Y^T$ are either 0 or 1 then $\ldots\ldots\mathbf{3}$
  - Distribution of $\vec{\hat{y}}^T f(X,Y)\vec{\hat{y}}$ is that of $\vec{\hat{y}}^T \Sigma\vec{\hat{y}}$ where Σ is the diagonal eigenvalue matrix of $f(X,Y)$ with $rank(X) - rank(Y)$ 1 eignvalues
  - Which is the sum of squares of $rank(X) - rank(Y)$ independent $N(0,1)$ random variables, or a Chi Square distribution with $rank(X) - rank(Y)$ degrees of freedom
  - $E[\vec{\hat{y}}^T f(X,Y)\vec{\hat{y}}] = rank(X) - rank(Y)$

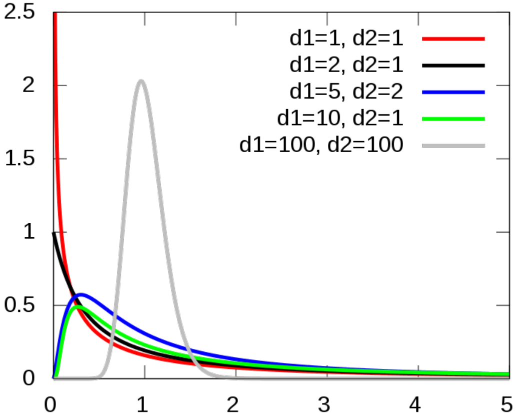
Chi Square Distributions for various Degrees of Freedom





Distribution of the F-Statistic under the Null Model

- Modified F-statistic (note the second term with degrees of freedom) $= \frac{\vec{\hat{h}}^T (A(A^TA)^{\dagger}A^T - A'(A'^TA')^{\dagger}A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I-(A(A^TA)^{\dagger}A^T)\vec{\hat{h}}} * \frac{n-rank(A)}{rank(A')-rank(A)}$
- the numerator is Chi Square distributed with $rank(A) - rank(A')$ degrees of freedom, the denominator is Chi Square distributed with $n - rank(A)$ degrees of freedom, and the two are independent
- the statistic is distributed as the F-distribution, with parameters $rank(A') - rank(A) = 1$ and $n - rank(A) = n - 2$
- Closed-form and calculators are commonly available for the F-distribution. The p-value is the total area to the right of the value calculated from the given data.



Proof of Claims toward the Distribution of the F-Statistic

- $A^T A\vec{x} = \vec{y}$ has a solution for any vector $\vec{y}$ in the column space of A^T, i.e., $\vec{x} = (A^T A)^\dagger \vec{y}$
    - $A^T A\vec{x}$ is clearly in the column space of A^T
    - Therefore, the column space of $A^T A$ is a subspace of the column space of A^T
    - It suffices to show that the column spaces of $A^T A$ and A^T have the same dimension; that would imply equality of the two spaces
    - Since nullspace of $A^T A$ and A are the same, the column spaces of $A^T A$ and A have the same dimension
    - The column space of A has the same dimension as the row space of A
    - It follows that the column space of $A^T A$ has the same dimension as the column space of A^T

Proof of Claims toward the Distribution of the F-Statistic

- $A^T A\vec{x} = \vec{y}$ has a solution for any vector $\vec{y}$ in the column space of A^T, i.e., $\vec{x} = (A^T A)^\dagger \vec{y}$
- $\implies$ $A(A^T A)^\dagger A^T \vec{y} = \vec{y}$ for any vector $\vec{y}$ in the column space of A (i.e., $\vec{y} = A\vec{z}$), and 0 for all vectors $\vec{y}$ orthogonal to this column space. Ditto for A'
- $\implies$ Eigenvalues of $A(A^T A)^\dagger A^T$ are 0,1 with exactly $rank(A)$ 1's. Ditto for A'
- $\implies$ All vectors in the column space of A are eigenvectors of $A(A^T A)^\dagger A^T$. Ditto for A'
- $\implies$ $(I - A(A^T A)^\dagger A^T)(I - A(A^T A)^\dagger A^T) = (I - A(A^T A)^\dagger A^T)$ because $A(A^T A)^\dagger A^T A = A$. Ditto for A'
- $\implies$ $A(A^T A)^\dagger A^T A'\mu = A'(A'^T A')^\dagger A'^T A'\mu$, where $A'\mu$ is the non- gender specific model (because $A'\mu$ is in the column space of A', which in turn is in the column space of A). Ditto for A'

Proof of Claims toward the Distribution of the F-Statistic

- $\implies A(A^TA)^\dagger A^T, A'(A'^TA')^\dagger A'^T$
  - have $rank(A')$ orthonormal eigenvectors in common with eigenvalue 1 (because the column space of A' is a contained in that of A)
  - have $rank(A) - rank(A')$ orthonormal eigenvectors in common, but with eigenvalue 1 for $A(A^TA)^\dagger A^T$ and 0 for $A'(A'^TA')^\dagger A'^T$ (these are in the column space of A but orthogonal to that of A')
  - have $n - rank(A)$ orthonormal eigenvectors in common with eigenvalue 0 (these are orthogonal to the column spaces of both A and A')
- $\implies A(A^TA)^\dagger A^T = V\Sigma V^T, A'(A'^TA')^\dagger A'^T = V\Sigma' V^T$, where Σ, Σ' are the corresponding diagonal eigenvalue matrices and the columns of V are the orthonormal eigenvectors
- $\implies A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T = V(\Sigma - \Sigma')V^T$, where $\Sigma - \Sigma'$ has only $rank(A) - rank(A')$ 1's

Proof of Claims toward the Distribution of the F-Statistic

- $\implies I - A(A^TA)^\dagger A^T = V(I-\Sigma)V^T$, where $I-\Sigma$ has only $n - rank(A)$ 1's
- $\implies \vec{\hat{y}}^T(A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T)\vec{\hat{y}} = \vec{\hat{y}}^T V(\Sigma - \Sigma')V^T\vec{\hat{y}}$, where $\vec{\hat{y}}$ is a vector whose entries are chosen independently from $N(0,1)$
- $\implies \vec{\hat{y}}^T(I - A(A^TA)^\dagger A^T)\vec{\hat{y}} = \vec{\hat{y}}^T V(I-\Sigma)V^T\vec{\hat{y}}$, where $\vec{\hat{y}}$ is a vector whose entries are chosen independently from $N(0,1)$
- Since the columns of V are orthonormal, we could rotate the coordinate axes so V becomes I. What happens to $\vec{\hat{y}}$ in the process?
- Because of spherical symmetry, $\vec{\hat{y}}$ remains a vector whose entries are chosen independently from $N(0,1)$!
- $\implies \vec{\hat{y}}^T(A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T)\vec{\hat{y}}$ has the same distribution as $\vec{\hat{y}}^T(\Sigma - \Sigma')\vec{\hat{y}}$
- $\implies \vec{\hat{y}}^T(I - A(A^TA)^\dagger A^T)\vec{\hat{y}}$ has the same distribution as $\vec{\hat{y}}^T(I-\Sigma)\vec{\hat{y}}$

Proof of Claims toward the Distribution of the F-Statistic

- $\vec{\hat{y}}^T(\Sigma - \Sigma')\vec{\hat{y}}$ is the sum of squares of a subset of the entries of $\vec{\hat{y}}$
- Likewise for $\vec{\hat{y}}^T(I - \Sigma)\vec{\hat{y}}$
- The two subsets are disjoint because the 1s in Σ' are a subset of the 1s in Σ
- $\implies$
  - $\vec{\hat{y}}^T(A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T)\vec{\hat{y}}$ is distributed as the sum of squares of $rank(A) - rank(A')$ independent $N(0,1)$ random variables
  - $\vec{\hat{y}}^T((I - A(A^TA)^\dagger A^T)\vec{\hat{y}}$ is distributed as the sum of squares of $n - rank(A)$ independent $N(0,1)$ random variables
  - The two distributions are independent

Summarizing ANOVA for Heights

- Sample females with height $\hat{h}_1, \ldots, \hat{h}_n$ and males $\hat{h}_{n+1}, \ldots, \hat{h}_m$
- Calculate F-statistic $\hat{f} = \frac{\vec{\hat{h}}^T (A(A^T A)^\dagger A^T - A'(A'^T A')^\dagger A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I - (A(A^T A)^\dagger A^T)\vec{\hat{h}}} * \frac{n - rank(A)}{rank(A) - rank(A')}$
- Calculate the area to the right of $\hat{f}$ in the density plot of the F-distribution with $rank(A) - rank(A')$ and $n - rank(A)$ degrees of freedom; this is the p-value
- Reject the universal (null) model if p-value is small
- Note, the proofs above hinge on the following two facts
    - We have two models A, A'
    - The column space of the null model A' is contained in that for A
    - The $\hat{\epsilon}$'s in the null model are all sampled from $N(0, \sigma^2)$ independently
- Further given there is reason to believe that heights are Gaussian and with equal/similar variance across genders, unlikeliness of the universal model translates to likeliness of the gender-specific model, with distinct means for males and females

Many Groups

- The two A's of interest are:

$$A = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{pmatrix}, A' = \begin{pmatrix} 1 \\ 1 \\ 1 \\ \vdots \\ 1 \\ 1 \\ 1 \end{pmatrix} \tag{4}$$

- F-Statistic$= \frac{\vec{\hat{h}}^T (I - A'(A'^T A')^\dagger A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I - A(A^T A)^\dagger A^T)\vec{\hat{h}}} - 1 = \frac{\vec{\hat{h}}^T (A(A^T A)^\dagger A^T - A(A^T A)^\dagger A^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I - A(A^T A)^\dagger A^T)\vec{\hat{h}}}$
- The rest of the process is identical to the two-groups case above
- Rejection of the null model suggests that the underlying group means are not all the same

Two-Way ANOVA

- Two dimensions of groups (or *factors*)
    - Status: Smokers vs Non-Smokers
    - Gender: Males vs Females
- There are three questions now
    - Are the means the same for Males and Females?
        - Derive f_{Gender} as above.
    - Are the means the same for Smokers vs Non-Smokers?
        - Derive f_{Status} as above.
    - Are the individual means for each of the 4 Status x Gender groups just a simple additive combination of the Status and the Gender means?
        - What about this?

Status x Gender Model

- $$\begin{pmatrix} \hat{h}_1 \\ \hat{h}_2 \\ \vdots \\ \hat{h}_n \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} g_{S,M} \\ g_{S,F} \\ g_{NS,M} \\ g_{NS,F} \end{pmatrix} + \begin{pmatrix} \hat{\epsilon}_1 \\ \hat{\epsilon}_2 \\ \vdots \\ \hat{\epsilon}_n \end{pmatrix} \qquad (5)$$

Status + Gender (Null) Model

- $$\begin{pmatrix} \hat{h}_1 \\ \hat{h}_2 \\ \vdots \\ \hat{h}_n \end{pmatrix} = \begin{pmatrix} 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 1 \end{pmatrix} \begin{pmatrix} g_S \\ g_{NS} \\ g_M \\ g_F \end{pmatrix} + \begin{pmatrix} \hat{\epsilon}_1 \\ \hat{\epsilon}_2 \\ \vdots \\ \hat{\epsilon}_n \end{pmatrix} \tag{6}$$

F-Statistic for Status x Genter Interaction

- $$A = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \end{pmatrix}, A' = \begin{pmatrix} 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 1 \end{pmatrix} \tag{7}$$

- $\boldsymbol{rank(A) = 4, rank(A') = 3}$, the column space of $\boldsymbol{A}$ contains the column space of $\boldsymbol{A'}$

- F-statistic for Status x Gender $f_{Status\hat{},Gender} = \frac{\vec{\hat{h}}^T(A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T(I-(A(A^TA)^\dagger A^T)\vec{\hat{h}}} * \frac{n-rank(A)}{rank(A)-rank(A')}$

The Two-Way ANOVA Process

- Compute F-statistic for Status x Gender & obtain the p-value
- If too low, then reject the additive (null) distribution, i.e., it is unlikely that the data can be supported by the means for the individual groups being a sum of the Status-wise and Gender-wise means
- Otherwise
    - Do the one way ANOVA process to see if the universal (null) model that assumes the same means for all Genders can be rejected
    - Separately, ditto for Status

Onw-Way Repeated Measures

- A single group of individuals measured repeatedly over time
- Does time make a systematic difference in measurement across multiple individuals?
    - E.g., measurements $0, 10, 20$ at timepoint 1 and $2, 12, 22$ at timepoint 2
    - The variation among individuals within each time point is large and the difference between the two timepoints pales in comparison to this variation; so conventional one-way ANOVA will not reject the universal (null) model
    - However, there is indeed a systematic effect of time: measurements increase by 2 for each individual
- Use Individual and Time as two factors
    - Use a Universal (on Time) model as the null (individuals have different underlying means but these do not change with time)
    - Compared to an additive model of Individual and Time effects

F-Statistic for One Way Repeated Measures

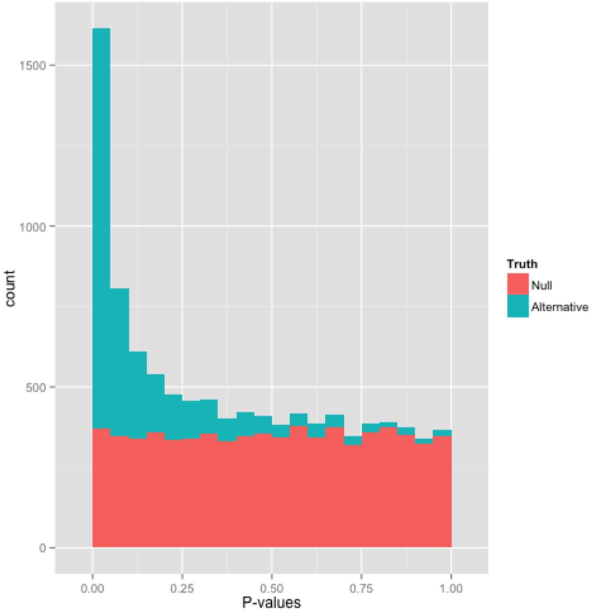
- $$A = \begin{pmatrix} 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 1 & 0 & 1 \end{pmatrix}, A' = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ 0 & 0 & 1 \end{pmatrix} \tag{8}$$
- The first 3 columns of A are individual effects, the last two are time
- A' has only individual effect columns
- $rank(A) = 4, rank(A') = \mathbf{3}$, the column space of A contains the column space of A'
- F-statistic $f_{\widehat{Status,Gender}} = \frac{\vec{\hat{h}}^T (A(A^TA)^\dagger A^T - A'(A'^TA')^\dagger A'^T)\vec{\hat{h}}}{\vec{\hat{h}}^T (I-(A(A^TA)^\dagger A^T)\vec{\hat{h}}} * \frac{n-rank(A)}{rank(A)-rank(A')}$

Correction for Multiple Testing

- Recall, for each gene
  - we independently derive a p-value under the null model
  - this p-value is the probability that the drawing samples for this gene from the null model yields as high an F-statistic as is obtained from the data at hand, thus c
  - note: this p-value has a uniform distribution between 0 and 1
- But, we have $n = 20,000$ genes
- Assume n_0 of these satisfy the null model (n_0 is the majority in practice)
- For these n_0 genes, assume p-values are drawn independently from a uniform distribuion (?)
- So the smallest of these will have an expected value of $\frac{1}{n_0}$, which could be as low as 0.00005!
- Which means that many of these n_0 genes will have the null model rejected, falsely; expected number $n_0 q$ if q is the cut-off.
- The probability that even one of these n_0 genes passes the cut-off is $1 - (1 - q)^{n_0} \;\; n_0 q$. Using q/n_0 instead of q as the cut-off ensures that this probability is less than q

Estimating n_0

- Note, we don't know n_0
- But p-values for these genes can be assumed to be independent and uniformly distributed in 0..1
- The other genes will have p-values biased towards 0
- Draw the histogram of all the p-values (use a suitable bin size, say 0.1)
- If you see bias closer to 0 (greater density near 0 than near 1), use the density closer to 1 to estimate n_0
- Otherwise, use n as a conservative estimate of n_0



link

False Discovery Rate (FDR)

- Sort all the p-values
- Suppose he ith smallest p-value is p_i
- The expected number of false positives from the n_0 genes with p-values smaller than x is $n_0 p_i$
- The fraction of false positives, roughly speaking, is expected to be $\frac{n_0 p_i}{i}$.
- Control this fraction at say cut-off q
- So pick the largest i such that $\frac{n_0 p_i}{i} \leq q$
- Or, in other words, pick the largest i such that $p_i < \frac{qi}{n_0}$
- Estimate n_0 as above
- Among all genes which pass this test, you can show that the expected false positive fraction is at most q

The Asignment

- The data file is here
- Your goal is to identify genes which respond differently to smoke in men vs women (Smoking Status x Gender model vs the Smoking Status + Gender null)
    - Use the above 2-way ANOVA framework to generate p-values for each row
    - Draw the histogram of p-values
    - See if a better (than n) estimate for n_0 is derivable from this histogram; justify your estimate
    - Use an FDR cut-off of 0.05 to shortlist rows
    - Create a shortlist of gene symbols from these rows
    - Intersect with the following gene lists: Xenobiotic metabolism, Free Radical Response, DNA Repair, Natural Killer Cell Cytotoxicity
    - Report intersection counts for each list, split into four groups; going down in women smokers vs non-smokers/going up in women smokers vs non-smokers x ditto for men