

# Alzheimers Disease Brain Blood Samples

Janis Corona

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This analysis is done using the NCBI gene expression samples taken from a study that used the middle temporal gyrus side of the brain of frozen samples taken from Alzheimer's patients that totalled 78 samples of control and repeat microarray gene expression results. The file was able to be downloaded and unzipped complete with the platform gene symbol ID already attached. A separate file of the meta information that includes the age, gender, tissue type and disease as Alzheimer's Disease (AD) or control was made from the Series information for each sample. The age range for these samples of healthy and AD patients is from 70-95 years of age.

This study can be linked to with all the sample and meta information at:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109887> The link to the 41 mb text file of series of samples with ID that is too large for github, or my github account type is:

[ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE109nnn/GSE109887/matrix/GSE109887\\_series\\_matrix.txt.gz](ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE109nnn/GSE109887/matrix/GSE109887_series_matrix.txt.gz)

Open the original Sample ID values text file and write out as a csv file.

```
alz <- read.delim('GSE109887_series_matrix.txt', sep='\t', header=TRUE,
na.strings=c('', ' '), comment.char='!')
```

```
write.csv(alz, 'alzheimersamples.csv', row.names=FALSE)
```

```
head(alz)
```

```
## ID_REF GSM2973262 GSM2973263 GSM2973264 GSM2973265 GSM2973266 GSM2973267
## 1 7A5 6.436441 6.500922 6.513507 6.480839 6.478978 6.509372
## 2 A1BG 6.736136 6.649034 6.612224 6.559642 6.574546 6.471425
## 3 A1CF 6.545395 6.479783 6.588104 6.482503 6.466453 6.442931
## 4 A26C3 6.529725 6.504956 6.531813 6.549229 6.458589 6.602654
## 5 A2BP1 6.773307 7.675723 7.159857 7.334894 7.928057 7.427863
## 6 A2M 11.253437 10.758069 10.790378 11.280206 10.550165 11.015546
## GSM2973268 GSM2973269 GSM2973270 GSM2973271 GSM2973272 GSM2973273
GSM2973274
## 1 6.558079 6.539648 6.476178 6.384209 6.474428 6.506147
6.547314
## 2 6.601043 6.680376 6.619720 6.695035 6.636312 6.549436
6.631196
## 3 6.448482 6.511453 6.537003 6.485914 6.481272 6.470623
6.556559
## 4 6.551262 6.618060 6.473897 6.475091 6.494137 6.539573
```

6.504725						
## 5	7.753590	7.542770	6.962004	7.844319	7.045881	8.032980
7.021767						
## 6	10.374510	10.232084	11.219453	10.558733	11.928947	10.029828
11.261613						
##	GSM2973275	GSM2973276	GSM2973277	GSM2973278	GSM2973279	GSM2973280
GSM2973281						
## 1	6.482653	6.431043	6.467538	6.496945	6.455699	6.533167
6.502415						
## 2	6.637651	6.610124	6.629145	6.633296	6.574900	6.686115
6.594453						
## 3	6.500758	6.521914	6.561131	6.483240	6.495440	6.545356
6.540573						
## 4	6.574710	6.434496	6.499997	6.456096	6.506443	6.601116
6.455450						
## 5	7.237758	7.626402	7.702040	7.108037	8.086046	7.320380
7.262326						
## 6	10.869890	10.028131	10.710539	10.902636	10.507890	10.889177
10.479438						
##	GSM2973282	GSM2973283	GSM2973284	GSM2973285	GSM2973286	GSM2973287
GSM2973288						
## 1	6.444786	6.501353	6.477281	6.544612	6.400074	6.520184
6.539472						
## 2	6.571460	6.662062	6.610356	6.586472	6.590251	6.587090
6.597562						
## 3	6.462481	6.499808	6.487859	6.539648	6.521903	6.523406
6.469701						
## 4	6.449598	6.531614	6.493402	6.426615	6.542165	6.577142
6.421726						
## 5	7.229908	7.817468	7.986829	8.202127	7.727549	7.299809
8.396471						
## 6	10.736578	10.754942	9.644938	10.630864	9.750951	11.035407
10.141873						
##	GSM2973289	GSM2973290	GSM2973291	GSM2973292	GSM2973293	GSM2973294
GSM2973295						
## 1	6.424923	6.474643	6.589264	6.445287	6.529792	6.537969
6.459321						
## 2	6.680176	6.550771	6.679956	6.569058	6.675714	6.579930
6.559305						
## 3	6.530320	6.473031	6.517640	6.506745	6.516516	6.464695
6.556136						
## 4	6.533016	6.370309	6.488512	6.445287	6.455889	6.412834
6.477753						
## 5	8.017758	8.169229	8.477709	7.172333	7.303684	8.116424
8.008762						
## 6	10.213843	10.347774	10.304462	9.771581	11.610263	10.145408
9.672881						
##	GSM2973296	GSM2973297	GSM2973298	GSM2973299	GSM2973300	GSM2973301
GSM2973302						
## 1	6.418835	6.503889	6.485979	6.485081	6.530189	6.450836

6.489789						
## 2	6.616573	6.893967	6.556532	6.569898	6.896213	6.618346
6.637531						
## 3	6.481994	6.476141	6.525448	6.504118	6.471415	6.439531
6.522903						
## 4	6.443488	6.490153	6.502554	6.540274	6.610700	6.443019
6.483445						
## 5	7.650064	7.083986	7.212323	7.138866	6.818649	7.580220
6.876141						
## 6	11.445590	10.917707	10.639809	10.877692	11.223275	9.632867
10.446053						
##	GSM2973303	GSM2973304	GSM2973305	GSM2973306	GSM2973307	GSM2973308
GSM2973309						
## 1	6.450708	6.461540	6.470023	6.446682	6.580688	6.506489
6.335251						
## 2	6.593549	6.678117	6.591719	6.636234	6.648021	6.622491
6.577416						
## 3	6.503260	6.528314	6.487072	6.525948	6.540932	6.499622
6.479403						
## 4	6.592281	6.483814	6.582309	6.535979	6.521488	6.538749
6.492057						
## 5	7.380896	6.899655	8.136078	7.597110	7.000424	8.066728
6.978048						
## 6	11.354353	10.837673	9.561554	10.928431	10.706460	10.236279
10.857606						
##	GSM2973310	GSM2973311	GSM2973312	GSM2973313	GSM2973314	GSM2973315
GSM2973316						
## 1	6.435910	6.383139	6.504311	6.442846	6.495440	6.423849
6.439607						
## 2	6.715207	6.776508	6.606429	6.632080	6.614676	6.610304
6.641509						
## 3	6.589606	6.485514	6.522903	6.514882	6.493620	6.504014
6.494156						
## 4	6.473449	6.466644	6.498143	6.367646	6.512105	6.483955
6.578028						
## 5	6.770394	8.196010	8.175675	8.239208	7.745435	7.878781
8.135434						
## 6	11.231447	10.371160	9.715824	9.869439	10.131814	10.695212
10.372269						
##	GSM2973317	GSM2973318	GSM2973319	GSM2973320	GSM2973321	GSM2973322
GSM2973323						
## 1	6.476767	6.432943	6.567727	6.590029	6.518445	6.402109
6.462368						
## 2	6.647461	6.583375	6.641342	6.559408	6.603364	6.667564
6.643683						
## 3	6.522168	6.490471	6.612509	6.502178	6.495266	6.483240
6.539573						
## 4	6.577480	6.422356	6.485102	6.515997	6.489093	6.380886
6.519553						
## 5	7.967045	7.416130	7.679170	8.173208	7.996740	7.893309

```

8.566021
## 6 10.452866 10.354096 10.449631 9.839133 9.939212 10.397824
10.141047
## GSM2973324 GSM2973325 GSM2973326 GSM2973327 GSM2973328 GSM2973329
GSM2973330
## 1 6.429510 6.461409 6.404062 6.527984 6.519651 6.447827
6.462642
## 2 6.610161 6.570311 6.587222 6.672783 6.565616 6.645337
6.667337
## 3 6.473020 6.468053 6.550716 6.533538 6.514122 6.499146
6.540705
## 4 6.397061 6.451695 6.484181 6.441520 6.464654 6.473196
6.557970
## 5 8.215478 8.026517 7.850848 8.178894 7.749528 8.342166
8.141308
## 6 9.837524 10.109630 11.115016 9.570170 11.066659 9.929106
9.642897
## GSM2973331 GSM2973332 GSM2973333 GSM2973334 GSM2973335 GSM2973336
GSM2973337
## 1 6.536339 6.506450 6.429433 6.427672 6.488852 6.503583
6.508713
## 2 6.551605 6.557762 6.600702 6.648831 6.660864 6.617121
6.541810
## 3 6.491767 6.479403 6.535446 6.487697 6.515856 6.546361
6.457605
## 4 6.505190 6.585256 6.594342 6.523304 6.526753 6.492019
6.623703
## 5 8.334120 8.201316 7.891050 8.061283 7.794727 7.156839
6.903667
## 6 10.061636 11.116617 10.779104 10.415084 10.889901 11.026483
10.926880
## GSM2973338 GSM2973339
## 1 6.442999 6.465497
## 2 6.547078 6.611912
## 3 6.469584 6.521874
## 4 6.467831 6.453058
## 5 8.187274 7.777883
## 6 9.636975 10.613131

```

Lets read in the meta information for age, gender, and disease type as AD or control.

```

meta <- read.csv('AlzheimerAgeGenderTissueSamplesMeta.csv', sep=',',
                 header=TRUE, na.strings=c('', ' '))

```

```
head(meta)
```

```

## sampleID          GSM2973262
## 1 gender              M
## 2 age                91
## 3 disease            AD
## 4 tissue brain, middle temporal gyrus blood

```

##	GSM2973263	GSM2973264
## 1	M	F
## 2	87	82
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973265	GSM2973266
## 1	F	M
## 2	73	94
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973267	GSM2973268
## 1	M	F
## 2	72	90
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973269	GSM2973270
## 1	F	F
## 2	86	87
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973271	GSM2973272
## 1	M	M
## 2	92	81
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973273	GSM2973274
## 1	M	F
## 2	87	92
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973275	GSM2973276
## 1	F	M
## 2	95	75
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973277	GSM2973278
## 1	F	M
## 2	87	95
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973279	GSM2973280
## 1	M	F
## 2	90	77
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973281	GSM2973282
## 1	F	M
## 2	84	85
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood

##	GSM2973283	GSM2973284
## 1	M	F
## 2	89	89
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973285	GSM2973286
## 1	F	F
## 2	82	78
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973287	GSM2973288
## 1	M	F
## 2	70	86
## 3	AD	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973289	GSM2973290
## 1	F	F
## 2	75	94
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973291	GSM2973292
## 1	M	M
## 2	82	82
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973293	GSM2973294
## 1	M	F
## 2	73	77
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973295	GSM2973296
## 1	M	M
## 2	85	92
## 3	AD	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973297	GSM2973298
## 1	F	F
## 2	84	87
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973299	GSM2973300
## 1	M	F
## 2	86	92
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973301	GSM2973302
## 1	M	F
## 2	92	90
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood

##	GSM2973303	GSM2973304
## 1	F	F
## 2	82	82
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973305	GSM2973306
## 1	F	M
## 2	89	90
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973307	GSM2973308
## 1	M	M
## 2	87	78
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973309	GSM2973310
## 1	F	F
## 2	88	86
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973311	GSM2973312
## 1	F	M
## 2	88	86
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973313	GSM2973314
## 1	F	F
## 2	92	81
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973315	GSM2973316
## 1	M	F
## 2	82	92
## 3	AD	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973317	GSM2973318
## 1	F	M
## 2	81	89
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973319	GSM2973320
## 1	F	M
## 2	85	94
## 3	AD	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973321	GSM2973322
## 1	F	F
## 2	85	82
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood

	GSM2973323	GSM2973324
## 1	M	F
## 2	81	77
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973325	GSM2973326
## 1	F	M
## 2	81	79
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973327	GSM2973328
## 1	M	F
## 2	78	78
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973329	GSM2973330
## 1	M	M
## 2	79	86
## 3	control	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973331	GSM2973332
## 1	M	M
## 2	91	82
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973333	GSM2973334
## 1	M	F
## 2	84	91
## 3	AD	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973335	GSM2973336
## 1	F	M
## 2	87	86
## 3	control	AD
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973337	GSM2973338
## 1	M	M
## 2	88	81
## 3	AD	control
## 4	brain, middle temporal gyrus blood	brain, middle temporal gyrus blood
##	GSM2973339	
## 1	F	
## 2	85	
## 3	AD	
## 4	brain, middle temporal gyrus blood	

Transpose the meta to fields instead of rows

```
meta1 <- as.data.frame(t(meta))
names <- as.character(meta$sampleID)
```



```
colnames(meta1) <- names
meta2 <- meta1[,-1,]
head(meta2)
```

	gender	age	disease	tissue
## GSM2973262	M	91	AD brain, middle temporal gyrus	blood
## GSM2973263	M	87	AD brain, middle temporal gyrus	blood
## GSM2973264	F	82	AD brain, middle temporal gyrus	blood
## GSM2973265	F	73	AD brain, middle temporal gyrus	blood
## GSM2973266	M	94	AD brain, middle temporal gyrus	blood
## GSM2973267	M	72	AD brain, middle temporal gyrus	blood

Separate the data into gender and control or AD data sets respectively in each gender and for both genders.

Data tables of males and females for meta only:

```
row.names(alz) <- alz$ID_REF
alz1 <- alz[,-1]

row.names(meta) <- meta$sampleID
Meta <- meta[,-1]

names <- row.names(Meta)
Meta1 <- as.data.frame(t(Meta))
colnames(Meta1) <- names

fem <- grep('F', Meta1$gender)
mal <- grep('M', Meta1$gender)

Fem <- Meta1[fem,]
Mal <- Meta1[mal,]

Fem$sampleID <- as.factor(row.names(Fem))
Mal$sampleID <- as.factor(row.names(Mal))
```

Display the fields of these gender specific tables by meta information:

```
colnames(Fem)

## [1] "gender" "age" "disease" "tissue" "sampleID"

colnames(Mal)

## [1] "gender" "age" "disease" "tissue" "sampleID"
```

The same indices of each row name is the same as the alz1, alzheimer table colnames indices. So, use those same female and male indices values from the regex commands to separate the alzheimer table into male and female tables.

```
Females <- alz1[,fem]
Males <- alz1[,mal]
```

Data tables of control or AD for males and for females for meta only:

```
femControl <- grep('control', Fem$disease)
malControl <- grep('control', Mal$disease)

femAD <- grep('AD', Fem$disease)
malAD <- grep('AD', Mal$disease)

FemCtrl <- Fem[femControl,]
MalCtrl <- Mal[malControl,]

FemAD <- Fem[femAD,]
MalAD <- Mal[malAD,]
```

Display the AD or Control tables by gender for meta information:

```
colnames(FemCtrl)
## [1] "gender" "age" "disease" "tissue" "sampleID"

colnames(FemAD)
## [1] "gender" "age" "disease" "tissue" "sampleID"

colnames(MalCtrl)
## [1] "gender" "age" "disease" "tissue" "sampleID"

colnames(MalAD)
## [1] "gender" "age" "disease" "tissue" "sampleID"
```

Now use that same index information within each gender table derived from the alzheimer gene expression data:

```
Females_AD <- Fem[femAD,]
Females_control <- Fem[femControl,]

males_AD <- Mal[malAD,]
males_control <- Mal[malControl,]
```

Write these last tables to csv file:

```
write.csv(Females_AD, 'females_Alzheimers.csv', row.names=TRUE)
write.csv(Females_control, 'females_healthy_control.csv', row.names=TRUE)
write.csv(males_AD, 'males_Alzheimers.csv', row.names=TRUE)
write.csv(males_control, 'males_healthy_control.csv', row.names=TRUE)
```

What is the age range on these samples of healthy controls and AD patients?

```

age1 <- range(as.numeric(as.character(FemAD$age)))
age2 <- range(as.numeric(as.character(FemCtrl$age)))
age3 <- range(as.numeric(as.character(MalAD$age)))
age4 <- range(as.numeric(as.character(MalCtrl$age)))

char <- c('The minimum age: ', 'The maximum age: ')

AD_fem <- paste0(char,age1, sep='')
AD_mal <- paste0(char,age3, sep='')
ctrl_fem <- paste0(char,age2, sep='')
ctrl_mal <- paste0(char, age4, sep='')

adf <- c('Alzheimer females: ')
adm <- c('Alzheimer males: ')
cf <- c('healthy females: ')
cm <- c('healthy males: ')

ranges <- c(adf,AD_fem, adm, AD_mal, cf, ctrl_fem, cm, ctrl_mal)
ranges

## [1] "Alzheimer females: " "The minimum age: 73" "The maximum age: 95"
## [4] "Alzheimer males: " "The minimum age: 70" "The maximum age: 95"
## [7] "healthy females: " "The minimum age: 77" "The maximum age: 92"
## [10] "healthy males: " "The minimum age: 73" "The maximum age: 94"

```

The age range in all of this data for these healthy and AD patients is age 70 to age 95 years of age. Just useful to know when cross comparing blood microarray samples across the females from our overweight females using epigallocatechin (EGCG) in their 40s, the males from Russia who are also in their 40s with or without myocardial infarction (MI), the hemochromatosis (iron toxicity) males and females averaging their 40s in years of age with or without iron toxicity, and the flu vaccinated blood samples of males and females also in their 40s with or without antibiotic treatment combined with flu vaccination.

Lets do some data analysis of the means and fold change between the groups to create some additional statistical information on these Alzheimers blood samples and healthy controls of elderly patients. We will use dplyr for this and plot with ggplot2 later.

```
library(dplyr)
```

First lets look at the control and the AD tables within the gender specific tables: Females\_AD, Females\_control, males\_AD, males\_control. Get the means of each gene and compare into one table. This and these tables of data were already cleaned to only have one gene per row, due to not having to merge the platform fields to the series table and duplicate genes for missing data in the additional rows like previous studies mentioned earlier required.

Get the row means of each table and add to each table.

```

names <- row.names(Females_AD)
Females_AD1 <- rowMeans(Females_AD)

```

```

#colnames(Females_AD1) <- 'fem_AD_Mean'

Females_control1 <- rowMeans(Females_control)
#colnames(Females_control1) <- 'fem_ctrl_Mean'

males_AD1 <- rowMeans(males_AD)
#colnames(males_AD1) <- 'mal_AD_Mean'

males_control1 <- rowMeans(males_control)
#colnames(males_control1) <- 'mal_ctrl_Mean'

means <- cbind(Females_AD1, Females_control1, males_AD1, males_control1)
Means <- as.data.frame(means)
colnames(Means) <- paste(colnames(Means), '_Mean', sep='')
str(Means)

## 'data.frame':    31700 obs. of  4 variables:
## $ Females_AD1_Mean      : num  6.48 6.63 6.52 6.51 7.42 ...
## $ Females_control1_Mean: num  6.47 6.65 6.5 6.48 7.97 ...
## $ males_AD1_Mean        : num  6.48 6.6 6.5 6.51 7.51 ...
## $ males_control1_Mean   : num  6.48 6.62 6.51 6.5 8.01 ...

```

Now for the fold change values between the Means of the female control and AD patients, then for the male control and AD patients.

```

names <- row.names(Means)
FC_females <- Means %>% mutate(FC_fem_ctrl_AD =
                                Females_AD1_Mean/Females_control1_Mean)
str(FC_females)

## 'data.frame':    31700 obs. of  5 variables:
## $ Females_AD1_Mean      : num  6.48 6.63 6.52 6.51 7.42 ...
## $ Females_control1_Mean: num  6.47 6.65 6.5 6.48 7.97 ...
## $ males_AD1_Mean        : num  6.48 6.6 6.5 6.51 7.51 ...
## $ males_control1_Mean   : num  6.48 6.62 6.51 6.5 8.01 ...
## $ FC_fem_ctrl_AD        : num  1.001 0.998 1.004 1.005 0.931 ...

row.names(FC_females) <- names

names <- row.names(FC_females)
FC_both <- FC_females %>% mutate(FC_male_ctrl_AD =
                                males_AD1_Mean/males_control1_Mean)
row.names(FC_both) <- names
str(FC_both)

## 'data.frame':    31700 obs. of  6 variables:
## $ Females_AD1_Mean      : num  6.48 6.63 6.52 6.51 7.42 ...
## $ Females_control1_Mean: num  6.47 6.65 6.5 6.48 7.97 ...
## $ males_AD1_Mean        : num  6.48 6.6 6.5 6.51 7.51 ...
## $ males_control1_Mean   : num  6.48 6.62 6.51 6.5 8.01 ...

```

```
## $ FC_fem_ctrl_AD      : num  1.001 0.998 1.004 1.005 0.931 ...
## $ FC_male_ctrl_AD     : num  0.999 0.997 0.999 1.002 0.938 ...

write.csv(FC_both, 'FC_both.csv', row.names=TRUE)
```

Combine the data of the samples with IDs in the alz1 table with the stats.

```
both <- cbind(FC_both, alz1)

Both <- round(both, 3)

write.csv(Both, 'Both.csv', row.names=TRUE)
```

Top 100 genes Most expressed in fold change for females with AD:

```
top100_fem <- Both[order(Both$FC_fem_ctrl_AD, decreasing=TRUE)[0:100],]
a <- row.names(top100_fem)
a
```

## [1]	"SLC5A3"	"LOC339879"	"DDIT4L"	"SLC6A12"	"SLC7A2"
## [6]	"SFMBT2"	"LOC727908"	"CHST6"	"TEAD2"	"GPER"
## [11]	"FLJ38717"	"FCGBP"	"HEY2"	"ITPRIPL2"	"SIPA1"
## [16]	"DDX27"	"RAPGEF3"	"C15ORF52"	"CALD1"	"ITSN1"
## [21]	"AEBP1"	"PLXNB1"	"HS.37648"	"LOC100129828"	"NBPF8"
## [26]	"GSDMD"	"ITPKB"	"PTH1R"	"SYTL4"	"MYOT"
## [31]	"TMEM137"	"FAM65C"	"FZD9"	"VCAN"	
	"ZCCHC24"				
## [36]	"HS.505676"	"HS.534061"	"LOC100133019"	"LRRC32"	"AHNAK"
## [41]	"LYL1"	"NOTCH1"	"APLNR"	"HS.379253"	"PTRF"
## [46]	"RPPH1"	"C10RF110"	"INPPL1"	"LOC649841"	"DLC1"
## [51]	"ITGB5"	"RASL12"	"AKR1C3"	"FOXC1"	
	"LOC100132532"				
## [56]	"RAB13"	"WSCD1"	"LOC643287"	"ACACB"	"CUEDC1"
## [61]	"INPP5D"	"LOC100129681"	"LOC642031"	"TNFRSF1B"	"CFLAR"
## [66]	"FXYS5"	"LOC100131541"	"PDGFRB"	"RAXL1"	"ANGPT2"
## [71]	"LOC100131277"	"SLC15A3"	"FLNC"	"LOC648921"	"NACC2"
## [76]	"RUNDC2C"	"UBXN2A"	"VIL2"	"ZNF786"	
	"DCLRE1C"				
## [81]	"FLJ46906"	"HS.143018"	"LOC727948"	"HS.193767"	"LRCH4"
## [86]	"MT1H"	"SCRIB"	"KIF1C"	"LASS1"	
	"LOC100130598"				
## [91]	"NDE1"	"RN7SK"	"ZNF621"	"C9ORF130"	"CAPS"
## [96]	"EML3"	"HS.576072"	"KCNJ10"	"LOC100131096"	
	"LOC653158"				

Top 100 genes most expressed in fold change for males with AD:

```
top100_male <- Both[order(Both$FC_male_ctrl_AD, decreasing=TRUE)[0:100],]
b <- row.names(top100_male)
b
```

##	[1]	"RGS1"	"SLC5A3"	"LOC649362"	"TTR"	"CD44"
##	[6]	"FCGBP"	"SLC7A2"	"C100RF10"	"EMP1"	"ITPRIP"
##	[11]	"DDIT4"	"PLXNB1"	"ITPKB"	"S100A8"	
		"SERPINA3"				
##	[16]	"TEAD2"	"ADAMTS1"	"CCL2"	"ZFP36"	"HEY2"
##	[21]	"PTH1R"	"CHST6"	"GFAP"	"HLA-DRA"	
		"SLC6A12"				
##	[26]	"ACACB"	"GPR4"	"SRGN"	"CD14"	"CDKN1A"
##	[31]	"LRRC32"	"FOS"	"HLA-DMB"	"KIAA1881"	
		"TNFRSF1B"				
##	[36]	"HLA-DMA"	"MS4A6A"	"RAB13"	"CALD1"	"RASL12"
##	[41]	"MT2A"	"SYTL4"	"AHNAK"	"CEBPD"	"FAM65C"
##	[46]	"TGFB1"	"TMC6"	"CAPS"	"DDIT4L"	"ID3"
##	[51]	"ITGB2"	"LCP1"	"RNU1G2"	"S1PR3"	"SIPA1"
##	[56]	"GADD45G"	"GSDMD"	"NUPR1"	"ANLN"	"GEM"
##	[61]	"PDGFRB"	"VCAN"	"AEBP1"	"CD163"	"KIF1C"
##	[66]	"LOC387763"	"RUNDC2C"	"ZFP36L1"	"CFLAR"	"CTGF"
##	[71]	"IL8"	"PDK4"	"PLIN2"	"SLC16A9"	"CSF1R"
##	[76]	"IL17RB"	"LOC100133692"	"LOC643287"	"SCIN"	"TAGLN"
##	[81]	"TMEM106A"	"ADM"	"EDN1"	"FOXC1"	
		"GADD45B"				
##	[86]	"HSPA1A"	"MT1M"	"APLNR"	"APOLD1"	"C7"
##	[91]	"HS.37648"	"IGFBP5"	"MYOT"	"RN7SK"	
		"C15ORF52"				
##	[96]	"GJA4"	"GPER"	"ITGB5"	"LEP"	"PLXDC2"

Bottom 100 genes that are least expressed in fold change in females with AD:

```
bottom100_fem <- Both[order(Both$FC_fem_ctrl_AD, decreasing=FALSE)[0:100],]
c <- row.names(bottom100_fem)
c
```

##	[1]	"SYT1"	"CHGB"	"PCSK1"	"VGF"	"MAL2"
##	[6]	"RGS4"	"GABRA1"	"NPTX2"	"SST"	"STAT4"
##	[11]	"C10RF173"	"CBLN4"	"DYNC1I1"	"STXBP1"	"GNG2"
##	[16]	"RGS7"	"TSPAN13"	"EFCBP1"	"NELL2"	"HPRT1"
##	[21]	"TAGLN3"	"DCLK1"	"ST6GALNAC5"	"GLRB"	"BEX5"
##	[26]	"DIRAS2"	"SYP"	"KIAA1107"	"PAK1"	"PTPRN"
##	[31]	"SERPINI1"	"ZCCHC12"	"NELL1"	"DACH2"	"ELMOD1"
##	[36]	"NECAB1"	"NEFM"	"PARM1"	"RTN1"	"VAMP2"
##	[41]	"ENC1"	"TMEM16C"	"CREG2"	"STX1A"	"SVOP"
##	[46]	"CAP2"	"SCG2"	"UCHL1"	"CALY"	
		"KIAA0748"				
##	[51]	"NAP1L3"	"RIMBP2"	"YWHAG"	"GABRG2"	
		"LOC100128403"				
##	[56]	"NAP1L2"	"NAP1L5"	"STMN2"	"ADCYAP1"	"SCN3B"
##	[61]	"GAD1"	"HS.390250"	"INA"	"SCN2B"	
		"GPRASP2"				
##	[66]	"ITFG1"	"SNX10"	"SV2B"	"ATP6V1G2"	"GABBR2"
##	[71]	"GAD2"	"HPCA"	"NUDT11"	"LPPR4"	

```

"TMEM155"
## [76] "CAMK1G"      "EPHA4"      "GABRA5"      "MYT1L"
"HS.553187"
## [81] "CDH13"       "VSNL1"      "XK"          "CKMT1A"
"HS.31961"
## [86] "PCSK1N"      "PRKCG"      "SYT13"       "NPY"         "PRKCB"
## [91] "RASL11B"     "SYN2"       "CADPS"       "TSPYL1"
"C12ORF53"
## [96] "HSPB3"       "MKL2"       "PPP3R1"      "SNCA"
"C2ORF80"

```

Bottom 100 genes that are least expressed in fold change in males with AD:

```

bottom100_male <- Both[order(Both$FC_male_ctrl_AD, decreasing=FALSE)[0:100],]
d <- row.names(bottom100_male)
d

```

```

## [1] "VGF"      "PCSK1"    "RGS4"     "CHGB"     "STAT4"
## [6] "GABRA1"   "ST6GALNAC5" "ADCYAP1"  "MAL2"     "SYT1"
## [11] "CREG2"    "NPTX2"    "TMEM16C"  "CBLN4"    "SVOP"
## [16] "KIAA1107" "RGS7"     "BEX5"     "C10RF173" "NELL1"
## [21] "SST"      "PARM1"    "DYNC1I1"  "NAP1L5"   "NEFM"
## [26] "VAMP2"    "HS.390250" "PTPRN"    "HSPB3"
"LOC100128403"
## [31] "STXBP1"   "ENC1"     "GAD2"     "PAK1"     "ANO3"
## [36] "INA"      "LOC387856" "SLC30A3"  "STX1A"
"ZCCHC12"
## [41] "SCN2B"    "C2ORF80"  "CRYM"     "EPHA4"    "GLRB"
## [46] "HPRT1"    "KIAA0748" "STMN2"    "C11ORF87" "CAP2"
## [51] "GAD1"     "SNX10"    "EFCBP1"   "RPH3A"
"SERPINI1"
## [56] "DACH2"    "DCLK1"    "GPRASP2"  "SYP"
"TSpan13"
## [61] "VIP"      "C12ORF53" "CALY"     "OLFM3"    "SV2B"
## [66] "COPG2IT1" "DIRAS2"   "ELMOD1"   "NELL2"    "SCG2"
## [71] "SLC39A10" "GABRG2"   "SLITRK4"  "TAGLN3"
"TMEM155"
## [76] "CKMT1B"   "CYP26B1"  "GABBR2"   "CPNE4"
"HS.553187"
## [81] "MFF"      "PRKCG"    "TUBB2A"   "ELAVL4"   "LRFN5"
## [86] "NCALD"    "RIMBP2"   "RTN1"     "SLC6A17"  "SYT13"
## [91] "YWHAE"    "CCKBR"    "FGF12"    "G3BP2"    "HOPX"
## [96] "NAP1L3"   "NMNAT2"   "ZNF365"   "C20ORF103" "CADPS"

```

```

ab <- c(a,b)
topBoth <- unique(ab)

cd <- c(c,d)
bottomBoth <- unique(cd)

```

The unique top genes are:

topBoth

##	[1]	"SLC5A3"	"LOC339879"	"DDIT4L"	"SLC6A12"	"SLC7A2"
##	[6]	"SFMBT2"	"LOC727908"	"CHST6"	"TEAD2"	"GPER"
##	[11]	"FLJ38717"	"FCGBP"	"HEY2"	"ITPRIPL2"	"SIPA1"
##	[16]	"DDX27"	"RAPGEF3"	"C15ORF52"	"CALD1"	"ITSN1"
##	[21]	"AEBP1"	"PLXNB1"	"HS.37648"	"LOC100129828"	"NBPF8"
##	[26]	"GSDMD"	"ITPKB"	"PTH1R"	"SYTL4"	"MYOT"
##	[31]	"TMEM137"	"FAM65C"	"FZD9"	"VCAN"	
"ZCCHC24"						
##	[36]	"HS.505676"	"HS.534061"	"LOC100133019"	"LRRC32"	"AHNAK"
##	[41]	"LYL1"	"NOTCH1"	"APLNR"	"HS.379253"	"PTRF"
##	[46]	"RPPH1"	"C10RF110"	"INPL1"	"LOC649841"	"DLC1"
##	[51]	"ITGB5"	"RASL12"	"AKR1C3"	"FOXCI"	
"LOC100132532"						
##	[56]	"RAB13"	"WSCD1"	"LOC643287"	"ACACB"	"CUEDC1"
##	[61]	"INPP5D"	"LOC100129681"	"LOC642031"	"TNFRSF1B"	"CFLAR"
##	[66]	"FXVD5"	"LOC100131541"	"PDGFRB"	"RAXL1"	"ANGPT2"
##	[71]	"LOC100131277"	"SLC15A3"	"FLNC"	"LOC648921"	"NACC2"
##	[76]	"RUNDC2C"	"UBXN2A"	"VIL2"	"ZNF786"	
"DCLRE1C"						
##	[81]	"FLJ46906"	"HS.143018"	"LOC727948"	"HS.193767"	"LRCH4"
##	[86]	"MT1H"	"SCRIB"	"KIF1C"	"LASS1"	
"LOC100130598"						
##	[91]	"NDE1"	"RN7SK"	"ZNF621"	"C9ORF130"	"CAPS"
##	[96]	"EML3"	"HS.576072"	"KCNJ10"	"LOC100131096"	
"LOC653158"						
##	[101]	"RGS1"	"LOC649362"	"TTR"	"CD44"	
"C10ORF10"						
##	[106]	"EMP1"	"ITPRIP"	"DDIT4"	"S100A8"	
"SERPINA3"						
##	[111]	"ADAMTS1"	"CCL2"	"ZFP36"	"GFAP"	"HLA -
DRA"						
##	[116]	"GPR4"	"SRGN"	"CD14"	"CDKN1A"	"FOS"
##	[121]	"HLA-DMB"	"KIAA1881"	"HLA-DMA"	"MS4A6A"	"MT2A"
##	[126]	"CEBPD"	"TGFB1"	"TMC6"	"ID3"	"ITGB2"
##	[131]	"LCP1"	"RNU1G2"	"S1PR3"	"GADD45G"	"NUPR1"
##	[136]	"ANLN"	"GEM"	"CD163"	"LOC387763"	
"ZFP36L1"						
##	[141]	"CTGF"	"IL8"	"PDK4"	"PLIN2"	
"SLC16A9"						
##	[146]	"CSF1R"	"IL17RB"	"LOC100133692"	"SCIN"	"TAGLN"
##	[151]	"TMEM106A"	"ADM"	"EDN1"	"GADD45B"	"HSPA1A"
##	[156]	"MT1M"	"APOLD1"	"C7"	"IGFBP5"	"GJA4"
##	[161]	"LEP"	"PLXDC2"			

The unique bottom genes are:

bottomBoth



##	[1]	"SYT1"	"CHGB"	"PCSK1"	"VGF"	"MAL2"
##	[6]	"RGS4"	"GABRA1"	"NPTX2"	"SST"	"STAT4"
##	[11]	"C10RF173"	"CBLN4"	"DYNC1I1"	"STXBP1"	"GNG2"
##	[16]	"RGS7"	"TSPAN13"	"EFCBP1"	"NELL2"	"HPRT1"
##	[21]	"TAGLN3"	"DCLK1"	"ST6GALNAC5"	"GLRB"	"BEX5"
##	[26]	"DIRAS2"	"SYP"	"KIAA1107"	"PAK1"	"PTPRN"
##	[31]	"SERPINI1"	"ZCCHC12"	"NELL1"	"DACH2"	"ELMOD1"
##	[36]	"NECAB1"	"NEFM"	"PARM1"	"RTN1"	"VAMP2"
##	[41]	"ENC1"	"TMEM16C"	"CREG2"	"STX1A"	"SVOP"
##	[46]	"CAP2"	"SCG2"	"UCHL1"	"CALY"	
		"KIAA0748"				
##	[51]	"NAP1L3"	"RIMBP2"	"YWHAG"	"GABRG2"	
		"LOC100128403"				
##	[56]	"NAP1L2"	"NAP1L5"	"STMN2"	"ADCYAP1"	"SCN3B"
##	[61]	"GAD1"	"HS.390250"	"INA"	"SCN2B"	
		"GPRASP2"				
##	[66]	"ITFG1"	"SNX10"	"SV2B"	"ATP6V1G2"	"GABBR2"
##	[71]	"GAD2"	"HPCA"	"NUDT11"	"LPPR4"	
		"TMEM155"				
##	[76]	"CAMK1G"	"EPHA4"	"GABRA5"	"MYT1L"	
		"HS.553187"				
##	[81]	"CDH13"	"VSNL1"	"XK"	"CKMT1A"	
		"HS.31961"				
##	[86]	"PCSK1N"	"PRKCG"	"SYT13"	"NPY"	"PRKCB"
##	[91]	"RASL11B"	"SYN2"	"CADPS"	"TSPYL1"	
		"C12ORF53"				
##	[96]	"HSPB3"	"MKL2"	"PPP3R1"	"SNCA"	
		"C2ORF80"				
##	[101]	"ANO3"	"LOC387856"	"SLC30A3"	"CRYM"	
		"C11ORF87"				
##	[106]	"RPH3A"	"VIP"	"OLFM3"	"COPG2IT1"	
		"SLC39A10"				
##	[111]	"SLITRK4"	"CKMT1B"	"CYP26B1"	"CPNE4"	"MFF"
##	[116]	"TUBB2A"	"ELAVL4"	"LRFN5"	"NCALD"	
		"SLC6A17"				
##	[121]	"YWHAE"	"CCKBR"	"FGF12"	"G3BP2"	"HOPX"
##	[126]	"NMNAT2"	"ZNF365"	"C20ORF103"		

The top genes in common are those genes most expressed in fold change in females AND males who have Alzheimer:

```
A <- as.data.frame(a)
B <- as.data.frame(b)

topBoth <- merge(A,B,by.x='a', by.y='b')
topBoth$a
```

##	[1]	ACACB	AEBP1	AHNAK	APLNR	C15ORF52	CALD1	CAPS
##	[8]	CFLAR	CHST6	DDIT4L	FAM65C	FCGBP	FOXC1	GPB
##	[15]	GSDMD	HEY2	HS.37648	ITGB5	ITPKB	KIF1C	LOC643287

```
## [22] LRRC32      MYOT      PDGFRB    PLXNB1    PTH1R     RAB13     RASL12
## [29] RN7SK       RUNDC2C   SIPA1     SLC5A3    SLC6A12   SLC7A2    SYTL4
## [36] TEAD2       TNFRSF1B  VCAN
## 100 Levels: ACACB AEBP1 AHNAK AKR1C3 ANGPT2 APLNR C15ORF52 C10RF110 ...
ZNF786
```

The bottom genes in common are the least expressed genes in females AND males who have Alzheimer:

```
C <- as.data.frame(c)
D <- as.data.frame(d)

bottomBoth <- merge(C,D, by.x='c', by.y='d')
bottomBoth$c

## [1] ADCYAP1      BEX5          C12ORF53      C10RF173      C20RF80
## [6] CADPS        CALY          CAP2          CBLN4         CHGB
## [11] CREG2        DACH2        DCLK1         DIRAS2        DYNC1I1
## [16] EFCBP1       ELMOD1       ENC1          EPHA4         GABBR2
## [21] GABRA1       GABRG2       GAD1          GAD2          GLRB
## [26] GPRASP2      HPRT1        HS.390250     HS.553187     HSPB3
## [31] INA          KIAA0748     KIAA1107     LOC100128403  MAL2
## [36] NAP1L3       NAP1L5       NEFM          NELL1         NELL2
## [41] NPTX2        PAK1         PARM1         PCSK1         PRKCG
## [46] PTPRN        RGS4         RGS7          RIMBP2        RTN1
## [51] SCG2         SCN2B        SERPINI1      SNX10         SST
## [56] ST6GALNAC5   STAT4        STMN2         STX1A         STXBP1
## [61] SV2B         SVOP         SYP           SYT1          SYT13
## [66] TAGLN3       TMEM155      TMEM16C       TSPAN13       VAMP2
## [71] VGF          ZCCHC12
## 100 Levels: ADCYAP1 ATP6V1G2 BEX5 C12ORF53 C10RF173 C20RF80 CADPS ...
ZCCHC12
```

Lets look at three top expressed and three least expressed genes in common for females and males:

```
b3 <- as.character(bottomBoth$c[1:3])
t3 <- as.character(topBoth$a[1:3])

mix <- c(b3,t3)
mix

## [1] "ADCYAP1" "BEX5"      "C12ORF53" "ACACB"     "AEBP1"     "AHNAK"

Mix <- as.data.frame(mix)
mBoth <- Both
mBoth$gene <- row.names(mBoth)
MixBoth <- merge(Mix, mBoth, by.x='mix', by.y='gene')

toPlot <- MixBoth[,c(1,6,7)]
```

```

colnames(toPlot)[1] <- 'gene'
toPlot

##      gene FC_fem_ctrl_AD FC_male_ctrl_AD
## 1  ACACB      1.092      1.085
## 2 ADCYAP1      0.889      0.895
## 3  AEBP1      1.107      1.075
## 4  AHNAK      1.099      1.079
## 5   BEX5      0.877      0.907
## 6 C12ORF53     0.902      0.922

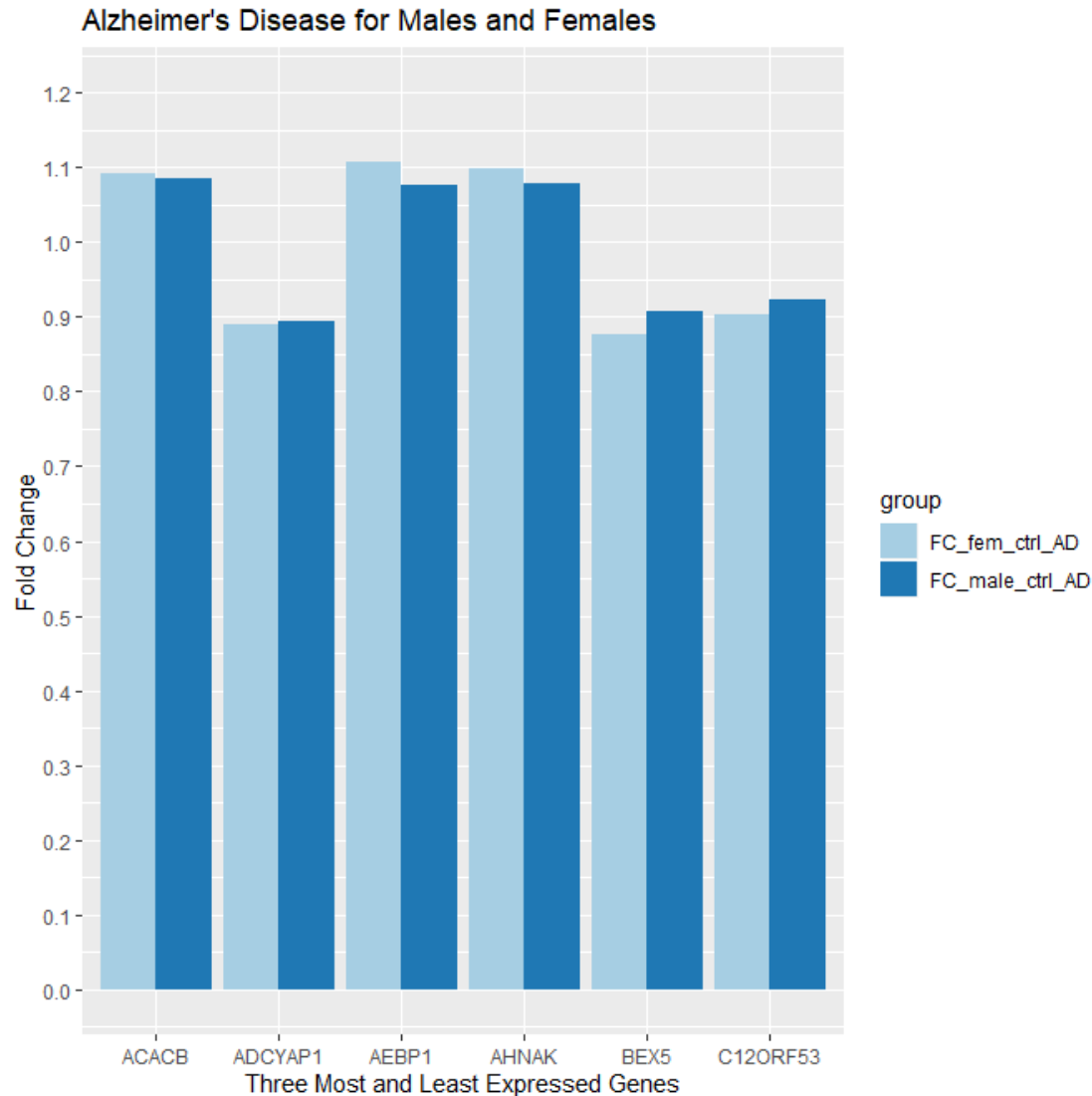
library(ggplot2)
library(tidyr)

to_plot <- gather(toPlot, 'group', 'foldChange', 2:3)
to_plot

##      gene      group foldChange
## 1  ACACB FC_fem_ctrl_AD      1.092
## 2 ADCYAP1 FC_fem_ctrl_AD      0.889
## 3  AEBP1 FC_fem_ctrl_AD      1.107
## 4  AHNAK FC_fem_ctrl_AD      1.099
## 5   BEX5 FC_fem_ctrl_AD      0.877
## 6 C12ORF53 FC_fem_ctrl_AD      0.902
## 7  ACACB FC_male_ctrl_AD      1.085
## 8 ADCYAP1 FC_male_ctrl_AD      0.895
## 9  AEBP1 FC_male_ctrl_AD      1.075
## 10 AHNAK FC_male_ctrl_AD      1.079
## 11  BEX5 FC_male_ctrl_AD      0.907
## 12 C12ORF53 FC_male_ctrl_AD      0.922

ggplot(data = to_plot, aes(x=gene, y=foldChange, fill=group)) +
  geom_bar(stat='identity', position=position_dodge()) +
  scale_y_continuous(breaks = seq(0, 1.2, by=.1), limits=c(0,1.2)) +
  scale_fill_brewer(palette='Paired') +
  ggtitle('Alzheimer\'s Disease for Males and Females') +
  ylab('Fold Change') +
  xlab('Three Most and Least Expressed Genes')

```



---

Some other data sets on diseases would be interesting to compare to this data on Alzheimer patients. The other data sets to compare this blood tissue type of microarray gene expression profiles are the: hemochromatosis (iron toxicity) from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121620>, epigallocatechin EGCG (green tea extract) use by overweight females in 40s found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74560>, myocardial infarction MI (heart disease) in Russian males in 40s found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE141512>, antibiotic treatment in flu vaccinated patients of males and females between 18-45 years of age found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120717> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120719>, and an immunization blood sample data set that only has healthy donors blood six days after receiving the

tetanus-diphtheria toxoids and acellular pertussis found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59697>.

Those data sets are needed for their stats with Fold Change values for each study respectively, except the tetanus shot because it is only healthy donor samples analyzing the tetanus-diphtheria toxoids as an antigen to test the B lymphocytes route in the plasma cells.

The females in the overweight study, the females in the flu study, and the females in the iron toxicity study could be cross compared to the females in this study on Alzheimer female patients. The males in the heart disease study, iron toxicity study, and flu study could be compared to the males in this Alzheimer study. And both genders from those studies could also be compared to both genders in this study.

The plan is to get the genes these studies all have in common and compare between the genders on fold change stats of those genes. That being iron toxicity fold change, and flu vaccinated fold change compared to added antibiotics (not compared to healthy non vaccinated patients) for comparison with Alzheimer patients. Overweight females using EGCG for weight loss is exclusive to the female comparisons, and the heart disease study was exclusive to male patients.

This study's data on fold change, means, and sample values is the **both.csv** file. The flu study had days 0,3,7, and 21 comparisons and both genders in the **FoldChange\_All.csv** file for that study. The iron toxicity fold change stats is in the **all-fc-grops.csv** file for the hemochromatosis study on both genders. The overweight females using EGCG to diet with the included fold change values for those using EGCG or EGCG+vitamin C+fish oil is in the **foldChange\_EGCG.csv** file. And the males who have heart disease of type MI is in the file **foldChange\_MI\_males.csv**. The tetanus immunization samples don't have the fold change because they are all healthy donors with no age data or gender data and taken 6 days after the tetanus shot, but it is useful to compare genes with healthy females and males in the healthy and control groups **tetanusImmunizationBlood.csv** is that file.

Lets read in those files with their respective and appropriately described names.

```
Alzheimer <- read.csv('both.csv', sep=',', header=T, na.strings=c('', ' '))
Heart_Disease_Males <- read.csv('foldChange_MI_males.csv', sep=',',
                                header=T, na.strings=c('', ' '))
TetanusImmunity <- read.csv('tetanusImmunizationBlood.csv', sep=',',
                             header=T, na.strings=c('', ' '))
Overweight_Females_EGCG <- read.csv('foldChange_EGCG.csv', sep=',',
                                     header=T, na.strings=c('', ' '))
flu_Vaccinated <- read.csv('FoldChange_All.csv', sep=',', header=T,
                           na.strings=c('', ' '))
iron_toxicity <- read.csv('all-fc-grops.csv', sep=',', header=T,
                          na.strings=c('', ' '))
```

Look at the column names and select the ones needed or that have fold change values to cross compare, or to compare means across samples as with the tetanus data set.

The Alzheimer column names are:

```
colnames(Alzheimer)
```

```
## [1] "X" "Females_AD1_Mean"
"FC_fem_ctrl_AD"
## [4] "males_AD1_Mean" "males_control1_Mean"
## [7] "FC_male_ctrl_AD" "GSM2973262" "GSM2973263"
## [10] "GSM2973264" "GSM2973265" "GSM2973266"
## [13] "GSM2973267" "GSM2973268" "GSM2973269"
## [16] "GSM2973270" "GSM2973271" "GSM2973272"
## [19] "GSM2973273" "GSM2973274" "GSM2973275"
## [22] "GSM2973276" "GSM2973277" "GSM2973278"
## [25] "GSM2973279" "GSM2973280" "GSM2973281"
## [28] "GSM2973282" "GSM2973283" "GSM2973284"
## [31] "GSM2973285" "GSM2973286" "GSM2973287"
## [34] "GSM2973288" "GSM2973289" "GSM2973290"
## [37] "GSM2973291" "GSM2973292" "GSM2973293"
## [40] "GSM2973294" "GSM2973295" "GSM2973296"
## [43] "GSM2973297" "GSM2973298" "GSM2973299"
## [46] "GSM2973300" "GSM2973301" "GSM2973302"
## [49] "GSM2973303" "GSM2973304" "GSM2973305"
## [52] "GSM2973306" "GSM2973307" "GSM2973308"
## [55] "GSM2973309" "GSM2973310" "GSM2973311"
## [58] "GSM2973312" "GSM2973313" "GSM2973314"
## [61] "GSM2973315" "GSM2973316" "GSM2973317"
## [64] "GSM2973318" "GSM2973319" "GSM2973320"
## [67] "GSM2973321" "GSM2973322" "GSM2973323"
## [70] "GSM2973324" "GSM2973325" "GSM2973326"
## [73] "GSM2973327" "GSM2973328" "GSM2973329"
## [76] "GSM2973330" "GSM2973331" "GSM2973332"
## [79] "GSM2973333" "GSM2973334" "GSM2973335"
## [82] "GSM2973336" "GSM2973337" "GSM2973338"
## [85] "GSM2973339"
```

The heart disease column names are:

```
colnames(Heart_Disease_Males)
```

```
## [1] "X" "Symbol"
## [3] "FC_MI_males" "HealthyMale_Means"
## [5] "MI_Male_Means" "healthy_Males_GSM4205364"
## [7] "healthy_Males_GSM4205363" "healthy_Males_GSM4205362"
## [9] "healthy_Males_GSM4205361" "healthy_Males_GSM4205360"
## [11] "healthy_Males_GSM4205359" "MI_Males_GSM4205358"
## [13] "MI_Males_GSM4205357" "MI_Males_GSM4205356"
## [15] "MI_Males_GSM4205355" "MI_Males_GSM4205354"
## [17] "MI_Males_GSM4205353"
```

The tetanus immunization after six days taken from their blood has the following column names:

```
colnames(TetanusImmunity)
```

```
## [1] "X" "GENE_SYMBOL" "GSM1443061" "GSM1443062"
## [5] "GSM1443063" "GSM1443064" "GSM1443065" "GSM1443066"
## [9] "Tetanis_Means"

min(TetanisImmunity[,3:9])

## [1] 0.02

max(TetanisImmunity[,3:9])

## [1] 8508456098
```

Take the log2 scale of the tetanis data set to make the values the same scale as the flu vaccinated and other samples that used log2 scale.

```
TetanisImmunity1 <- log2(TetanisImmunity[,3:9]+1)#avoid negative values
TetanisImmunity1$GENE_SYMBOL <- TetanisImmunity$GENE_SYMBOL
min(TetanisImmunity1[,1:7])

## [1] 0.02856915

max(TetanisImmunity1[,1:7])

## [1] 32.98625
```

The EGCG overweight females column names are:

```
colnames(Overweight_Females_EGCG)

## [1] "X" "FC_egcg_quer"
## [3] "FC_egcg" "DE_EGCG"
## [5] "DE_Quercentin" "Pre_Means"
## [7] "Post_EGCG_Means" "Post_EGCG_Quercentin_Means"
## [9] "pre_GSM1923000" "pre_GSM1923004"
## [11] "pre_GSM1923010" "pre_GSM1923012"
## [13] "pre_GSM1923007" "pre_GSM1923020"
## [15] "pre_GSM192998" "pre_GSM1922995"
## [17] "pre_GSM1923002" "pre_GSM1923008"
## [19] "pre_GSM1923015" "pre_GSM1923018"
## [21] "pre_GSM1923022" "pre_GSM1923017"
## [23] "post_EG_GSM1923001" "post_EG_GSM1923005"
## [25] "post_EG_GSM1923011" "post_EG_GSM1923013"
## [27] "post_EG_GSM1923021" "post_EG_GSM1923006"
## [29] "post_EG_GSM1923014" "post_EG_GSM192996"
## [31] "post_EQ_GSM1923003" "post_EQ_GSM1923009"
## [33] "post_EQ_GSM1923016" "post_EQ_GSM1923019"
## [35] "post_EQ_GSM1923023" "post_EQ_GSM192997"
```

The flu vaccinated (treated with antibiotics treatment or not as 't' or 'nt' respectively) for each of the initial day 0, day 3, day 7, and day 21 profiles from three treated and three not treated samples have the following column names:

```
colnames(flu_Vaccinated)
```

```
## [1] "Gene" "FC_t1"
## [3] "FC_t3" "FC_t7"
## [5] "FC_t21" "FC_nt1"
## [7] "FC_nt3" "FC_nt7"
## [9] "FC_nt21" "FCB_1"
## [11] "FCB_3" "FCB_7"
## [13] "FCB_21" "T0_Mean"
## [15] "T1_Mean" "T3_Mean"
## [17] "T7_Mean" "T21_Mean"
## [19] "NT0_Mean" "NT1_Mean"
## [21] "NT3_Mean" "NT7_Mean"
## [23] "NT21_Mean" "GSM3409106_29_day_0"
## [25] "GSM3409107_29_day_1" "GSM3409108_29_day_3"
## [27] "GSM3409004_29_day_7" "GSM3409105_29_day_21_screening"
## [29] "GSM3409006_30_day_0" "GSM3409007_30_day_1"
## [31] "GSM3409008_30_day_3" "GSM3409009_30_day_7"
## [33] "GSM3409005_30_day_21_screening" "GSM3409013_05_day_0"
## [35] "GSM3409014_05_day_1" "GSM3409015_05_day_3"
## [37] "GSM3409016_05_day_7" "GSM3409012_05_day_21_screening"
## [39] "GSM3409161_33_day_0_no" "GSM3409162_33_day_1_no"
## [41] "GSM3409163_33_day_3_no" "GSM3409111_33_day_7_no"
## [43] "GSM3409160_33_day_21_screening_no" "GSM3409124_36_day_0_no"
## [45] "GSM3409125_36_day_1_no" "GSM3409126_36_day_3_no"
## [47] "GSM3409127_36_day_7_no"
"GSM3409123_36_day_21_screening_no"
## [49] "GSM3409135_38_day_0_no" "GSM3409136_38_day_1_no"
## [51] "GSM3409137_38_day_3_no" "GSM3409138_38_day_7_no"
## [53] "GSM3409134_38_day_21_screening_no"
```

The iron toxicity study put the males and females into three groups of the genotypes associated with hemochromatosis or iron toxicity. It also included healthy males and females to compare to. The fold change values are for the groups each compared to the healthy groups by gender by all three groups 'overall' and by each of the 3 groups separately. The iron toxicity column names are:

```
colnames(iron_toxicity)
```

```
## [1] "X" "G1M_Mean" "G2M_Mean"
## [4] "G3M_Mean" "G1F_Mean" "G2F_Mean"
## [7] "G3F_Mean" "healthyFemale_Mean" "healthyMale_Mean"
## [10] "hemoFemale_Mean" "hemoMale_Mean" "FC_1m"
## [13] "FC_2m" "FC_3m" "FC_1F"
## [16] "FC_2F" "FC_3F" "FC_malesOverall"
## [19] "FC_femalesOverall" "GSM3440208" "GSM3440209"
## [22] "GSM3440210" "GSM3440211" "GSM3440212"
## [25] "GSM3440213" "GSM3440214" "GSM3440215"
## [28] "GSM3440216" "GSM3440217" "GSM3440218"
## [31] "GSM3440219" "GSM3440220" "GSM3440221"
## [34] "GSM3440222" "GSM3440223" "GSM3440224"
## [37] "GSM3440225" "GSM3440226" "GSM3440227"
```



```
## [40] "GSM3440228"          "GSM3440229"          "GSM3440230"
## [43] "GSM3440231"
```

In the interest of being interesting, these tables have all the original samples in each respective table for that disease group. There are some healthy samples in some such as healthy overweight females before taking EGCG, non-Alzheimer's males and females, healthy Russian males without heart disease, and healthy females and males who do not have iron toxicity relative to the iron toxicity study. There are some studies that do not have healthy controls nor separation by gender such as the tetanis immunity and flu vaccinated samples.

Since the tetanis immunized samples don't have a control or healthy group to compare to which haven't been immunized, let us compare it to the samples of healthy overweight females, healthy Russian males, healthy females and males without iron toxicity, and flu vaccinated not treated with antibiotics and flu vaccinated treated with antibiotics. The values in the tetanis immunized samples have a very high range for some genes and doesn't get lower than 0.08 for any gene. We will compare the fields of each table that has the **mean** values across sample for each **gene** in that group by creating a table with those means.

```
tet_mean <- TetanisImmunity1[,c(7,8)] #gene and tetanis immunized mean
iron_mean <- iron_toxicity[,c(1,8:11)]#gene, healthy female, healthy male
# iron toxic females, iron toxic males
flu_7day <- flu_Vaccinated[,c(1,22,17)] #gene, not treated, treated
EGCG_females <- Overweight_Females_EGCG[,c(1,6:8)]#gene, pre,post EGCG,
# post EGCG+vitamin C+Fish Oil
MI_healthy_mean <- Heart_Disease_Males[,c(1,4,5)]#gene, healthy males,
# MI males
alz_means <- Alzheimer[,c(1:5)]#gene, female AD, female control, male AD,
# male control
```

Let us first compare the tetanis immunized to the healthy females no iron toxicity, healthy males no iron toxicity, overweight females EGCG, healthy males no MI, healthy AD females, and healthy AD males.

Gather only the healthy females and males of those studies.

```
healthy_iron <- iron_mean[,c(1:3)]
Healthy_EGCG_females <- EGCG_females #all are healthy
healthy_MI_males <- MI_healthy_mean[,1:2]
healthy_AD <- alz_means[,c(1,3,5)]

dim(healthy_iron)

## [1] 28433      3

colnames(healthy_iron)[2:3] <-
gsub('healthy','iron_healthy_',colnames(healthy_iron)[2:3])
colnames(healthy_iron)
```

```

## [1] "X"                                "iron_healthy_Female_Mean"
## [3] "iron_healthy_Male_Mean"

dim(Healthy_EGCG_females)

## [1] 22831      4

colnames(Healthy_EGCG_females)[2:4] <-
paste('overweight_females_', colnames(Healthy_EGCG_females)[2:4], sep='')
colnames(Healthy_EGCG_females)

## [1] "X"
## [2] "overweight_females_Pre_Means"
## [3] "overweight_females_Post_EGCG_Means"
## [4] "overweight_females_Post_EGCG_Quercentin_Means"

dim(healthy_MImales)

## [1] 30905      2

colnames(healthy_MImales)[2] <-
paste('MI_study_', colnames(healthy_MImales)[2], sep='')
colnames(healthy_MImales)

## [1] "X"                                "MI_study_HealthyMale_Means"

dim(healthy_AD)

## [1] 31700      3

colnames(healthy_AD)[2:3] <-
gsub('_control1_', '_no_AD_', colnames(healthy_AD)[2:3])
colnames(healthy_AD)

## [1] "X"                                "Females_no_AD_Mean" "males_no_AD_Mean"

dim(tet_mean)

## [1] 19749      2

colnames(tet_mean)

## [1] "Tetanis_Means" "GENE_SYMBOL"

```

The above tables all have a differing amount of genes in each table, so we need to merge the tables by genes in common to compare. The gene field is the 'X' field in these tables listed immediately above. We need to merge them all to the tetanis table, tet\_mean, to compare with tetanis immunity gene expression values over 6 days.

```

healthy0 <- merge(tet_mean, healthy_iron, by.x='GENE_SYMBOL', by.y='X')
dim(healthy0)

## [1] 15059      4

```

```

healthy1 <- merge(healthy0, Healthy_EGCG_females, by.x='GENE_SYMBOL',
by.y='X')
dim(healthy1)

## [1] 14639      7

healthy2 <- merge(healthy1, healthy_MImales, by.x='GENE_SYMBOL', by.y='X')
dim(healthy2)

## [1] 14475      8

healthy3 <- merge(healthy2, healthy_AD, by.x='GENE_SYMBOL', by.y='X')
dim(healthy3)

## [1] 13193     10

colnames(healthy3)

## [1] "GENE_SYMBOL"
## [2] "Tetanis_Means"
## [3] "iron_healthy_Female_Mean"
## [4] "iron_healthy_Male_Mean"
## [5] "overweight_females_Pre_Means"
## [6] "overweight_females_Post_EGCG_Means"
## [7] "overweight_females_Post_EGCG_Quercentin_Means"
## [8] "MI_study_HealthyMale_Means"
## [9] "Females_no_AD_Mean"
## [10] "males_no_AD_Mean"

head(healthy3)

##   GENE_SYMBOL Tetanis_Means iron_healthy_Female_Mean
iron_healthy_Male_Mean
## 1      A1BG      22.5144403                4.816690
4.706227
## 2      A1CF      22.9031540                2.546950
2.229808
## 3      A2M       0.2789759                3.998705
4.323837
## 4     A2ML1     21.1270650                3.357760
3.048290
## 5      AAAS     24.6740221                5.287245
5.522265
## 6      AACS     21.2603715                5.471870
5.673802
##   overweight_females_Pre_Means overweight_females_Post_EGCG_Means
## 1                5.789                5.703
## 2                4.959                5.043
## 3                5.221                5.215
## 4                4.443                4.516
## 5                8.117                8.109
## 6                6.799                6.835

```

```
## overweight_females_Post_EGCG_Quercentin_Means MI_study_HealthyMale_Means
## 1 5.697 5.653239
## 2 4.914 2.616675
## 3 5.236 3.568283
## 4 4.483 3.014349
## 5 8.128 7.043090
## 6 6.787 5.423620
## Females_no_AD_Mean males_no_AD_Mean
## 1 6.647 6.619
## 2 6.495 6.506
## 3 10.231 10.377
## 4 6.571 6.568
## 5 6.875 6.828
## 6 7.946 7.907
```

We need to use dplyr to analyze this data as is for the healthy samples compared to the tetanis samples.

```
library(dplyr)
```

Get the fold change 'FC' values for the between samples of genes using the healthy3 table.

```
FC0 <- healthy3 %>% mutate(FC_iron_fem =
Tetanis_Means/iron_healthy_Female_Mean)

FC1 <- FC0 %>% mutate(FC_iron_mal = Tetanis_Means/iron_healthy_Male_Mean)

FC2 <- FC1 %>% mutate(FC_heavy_fems_no_EGCG =
Tetanis_Means/overweight_females_Pre_Means)

FC3 <- FC2 %>% mutate(FC_heavy_fems_EGCG =
Tetanis_Means/overweight_females_Post_EGCG_Means)

FC4 <- FC3 %>% mutate(FC_heavy_fems_EGCG_plus =
Tetanis_Means/overweight_females_Post_EGCG_Quercentin_Means)

FC5 <- FC4 %>% mutate(FC_healthyMI_males =
Tetanis_Means/MI_study_HealthyMale_Means)

FC6 <- FC5 %>% mutate(FC_fems_noAD = Tetanis_Means/Females_no_AD_Mean)
FC7 <- FC6 %>% mutate(FC_mals_noAD = Tetanis_Means/males_no_AD_Mean)

healthy_tetanis_FCs <- FC7
dim(healthy_tetanis_FCs)

## [1] 13193 18

colnames(healthy_tetanis_FCs)

## [1] "GENE_SYMBOL"
## [2] "Tetanis_Means"
```

```
## [3] "iron_healthy_Female_Mean"
## [4] "iron_healthy_Male_Mean"
## [5] "overweight_females_Pre_Means"
## [6] "overweight_females_Post_EGCG_Means"
## [7] "overweight_females_Post_EGCG_Quercetin_Means"
## [8] "MI_study_HealthyMale_Means"
## [9] "Females_no_AD_Mean"
## [10] "males_no_AD_Mean"
## [11] "FC_iron_fem"
## [12] "FC_iron_mal"
## [13] "FC_heavy_fems_no_EGCG"
## [14] "FC_heavy_fems_EGCG"
## [15] "FC_heavy_fems_EGCG_plus"
## [16] "FC_healthyMI_males"
## [17] "FC_fems_noAD"
## [18] "FC_mals_noAD"
```

There are 13,193 genes to compare fold change values of tetanis immunized samples to the healthy control samples from the combined studies.

```
write.csv(healthy_tetanis_FCs, 'healthy_tetanis_FCs.csv', row.names=FALSE)
```

Now, compare the tetanis immunized to the flu vaccinated samples treated and not treated with antibiotics. The flu gene samples seemed to have the most changes in values after 7 days of receiving the flu immunization and the antibiotic or no antibiotic addition. This is why the flu immunizations for day 7 means were selected. Only the tet\_mean and flu\_7day tables will be used for genes in common as neither has the gender information attached.

```
dim(tet_mean);colnames(tet_mean)
## [1] 19749      2
## [1] "Tetanis_Means" "GENE_SYMBOL"
dim(flu_7day);colnames(flu_7day)
## [1] 20633      3
## [1] "Gene"      "NT7_Mean" "T7_Mean"
```

Merge these two tables of the tetanis and flu not treated (NT7 prefix) or treated (T7 prefix) means.

```
tet_flu_immune <- merge(tet_mean, flu_7day, by.x='GENE_SYMBOL', by.y='Gene')
dim(tet_flu_immune)
## [1] 17447      4
colnames(tet_flu_immune)
## [1] "GENE_SYMBOL" "Tetanis_Means" "NT7_Mean"      "T7_Mean"
```

Now for the fold change values between the Tetanis\_Means and the flu immunization means with/without added antibiotics treatment, dplyr will be used to get that information.

```
tetanis_flu_FCs <- tet_flu_immune %>% mutate(FC_tet_flu_nt7 =
Tetanis_Means/NT7_Mean)

tetanis_flu_FCs1 <- tetanis_flu_FCs %>% mutate(FC_tet_flu_t7 =
Tetanis_Means/T7_Mean)

dim(tetanis_flu_FCs1)

## [1] 17447      6

colnames(tetanis_flu_FCs1)

## [1] "GENE_SYMBOL"      "Tetanis_Means"    "NT7_Mean"        "T7_Mean"
## [5] "FC_tet_flu_nt7"   "FC_tet_flu_t7"

head(tetanis_flu_FCs1)

##   GENE_SYMBOL Tetanis_Means NT7_Mean  T7_Mean FC_tet_flu_nt7 FC_tet_flu_t7
## 1      A1BG      22.5144403 4.237321 4.283943      5.31336641      5.25554149
## 2      A1CF      22.9031540 3.081198 3.188847      7.43319700      7.18226799
## 3      A2BP1       0.3968902 2.905718 3.187804      0.13658936      0.12450270
## 4      A2LD1       0.1132560 3.233051 4.178542      0.03503068      0.02710418
## 5       A2M       0.2789759 2.411421 3.146397      0.11568944      0.08866520
## 6      A2ML1      21.1270650 2.327324 2.872788      9.07783517      7.35420306

write.csv(tetanis_flu_FCs1, 'tetanis_flu_FCs.csv', row.names=FALSE)
```

Going to genecards.org I searched for Immune genes and found some genes that I want to analyze in this data set, tetanis\_flu\_FCs1, to see how immune cells being treated with the tetanis antibodies after six days and the separate study of flu immunized cells being treated with antibiotics after seven days, and not treated with antibiotics after 7 days. These genes are: \* **CD4** (big autoimmune indicator in lupus) \* **IL2** (produced by Produced by T-cells in response to antigenic or mitogenic...and other activities crucial to regulation of the immune response. Can stimulate B-cells, monocytes, lymphokine-activated... (UniProtKB/Swiss-Prot)) \* **IFNG** (Produced by lymphocytes activated by specific antigens or mitogens. IFN-gamma, in addition to having antiviral activity, has important immunoregulatory functions. It is a potent activator of macrophages, it has antiproliferative effects on transformed cells and it can potentiate the antiviral and antitumor effects of the type I interferons.) \* **IL10** (Major immune regulatory cytokine that acts on many cells of the immune system where it has profound anti-inflammatory functions, limiting excessive tissue disruption caused by inflammation.) \* **IL6** (Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. Plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. Acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. Required for the generation of T(H)17 cells. Also acts as a myokine. It is discharged into the bloodstream

after muscle contraction and acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation.)

```
CD4 <- grep('^CD4$', tetanis_flu_FCs1$GENE_SYMBOL)
IL2 <- grep('^IL2$', tetanis_flu_FCs1$GENE_SYMBOL)
IFNG <- grep('^IFNG$', tetanis_flu_FCs1$GENE_SYMBOL)
IL10 <- grep('^IL10$', tetanis_flu_FCs1$GENE_SYMBOL)
IL6 <- grep('^IL6$', tetanis_flu_FCs1$GENE_SYMBOL)

tet_flu_immune_genes <- tetanis_flu_FCs1[c(CD4,IL2,IFNG,IL10,IL6),]
head(tet_flu_immune_genes)
```

	GENE_SYMBOL	Tetanis_Means	NT7_Mean	T7_Mean	FC_tet_flu_nt7
## 2945	CD4	26.2162826	4.443672	5.086368	5.8996883
					5.15422438
## 7354	IL2	0.1720607	2.324467	3.035514	0.0740216
					0.05668258
## 7237	IFNG	21.2490376	4.843903	4.516635	4.3867596
					4.70461734
## 7308	IL10	18.5088908	2.852249	3.788690	6.4892270
					4.88530134
## 7387	IL6	22.2122479	11.282219	3.585461	1.9687837
					6.19508788

We need to tidy this data set for plotting with the tidyr and ggplot2 libraries.

```
library(tidyr)
library(ggplot2)
```

Tidy the table into a tibble

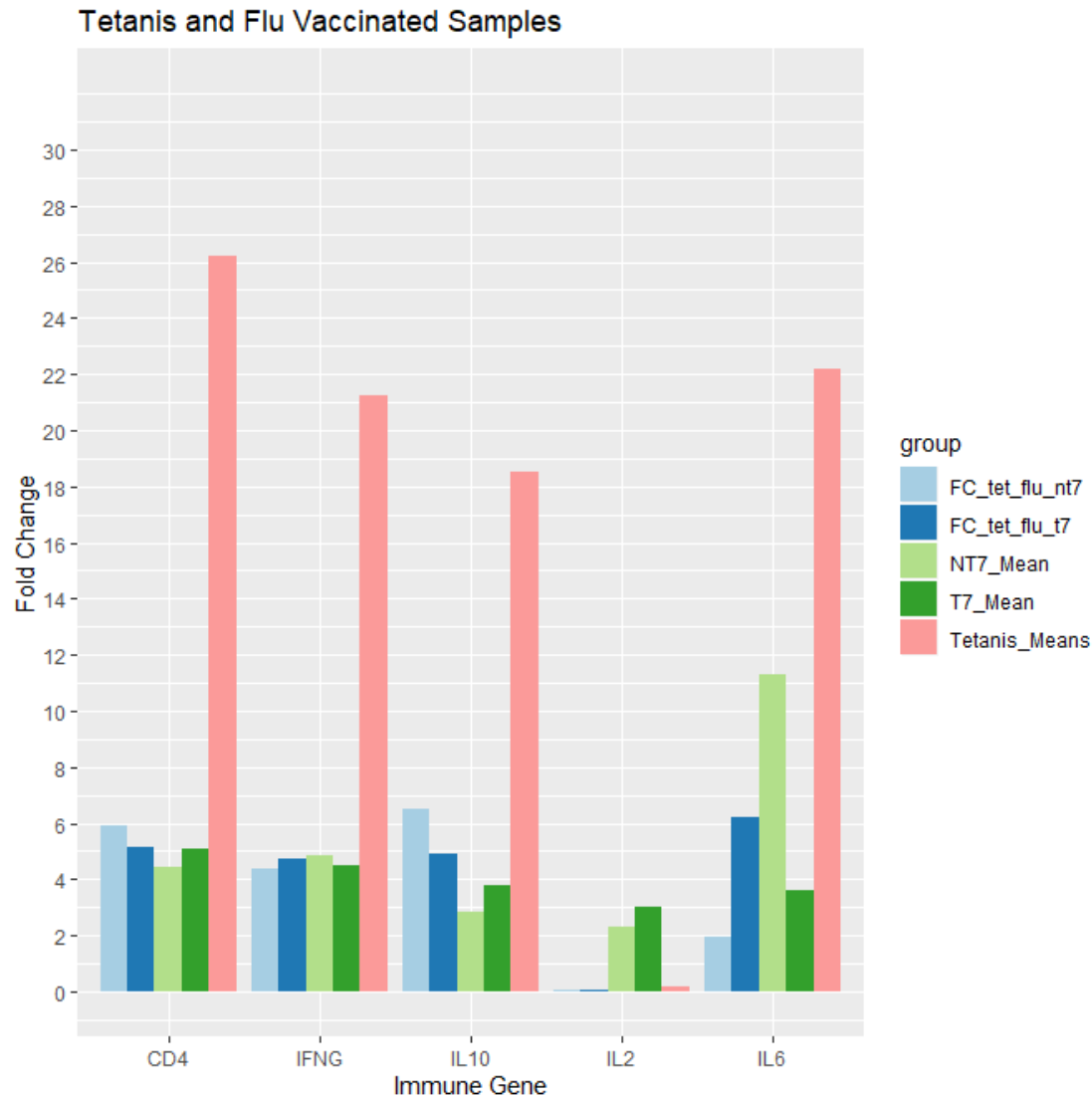
```
tet_flu_tidy <- gather(tet_flu_immune_genes, 'group', 'foldChange', 2:6)
tet_flu_tidy$foldChange <- round(tet_flu_tidy$foldChange, 3)
head(tet_flu_tidy)
```

	GENE_SYMBOL	group	foldChange
## 1	CD4	Tetanis_Means	26.216
## 2	IL2	Tetanis_Means	0.172
## 3	IFNG	Tetanis_Means	21.249
## 4	IL10	Tetanis_Means	18.509
## 5	IL6	Tetanis_Means	22.212
## 6	CD4	NT7_Mean	4.444

Plot the table or tibble of data above using bar plot to see how the gene expression values of immune genes compare.

```
ggplot(data = tet_flu_tidy, aes(x=GENE_SYMBOL, y=foldChange, fill=group)) +
  geom_bar(stat='identity', position=position_dodge()) +
  scale_y_continuous(breaks = seq(0, 30, by=2), limits=c(0,32)) +
```

```
scale_fill_brewer(palette='Paired') +
ggtitle('Tetanus and Flu Vaccinated Samples') +
ylab('Fold Change') +
xlab('Immune Gene')
```



Now that we have a couple bar charts of these immune genes selected to compare across the fold change values and mean values of the tetanus and flu vaccinated samples that were and weren't treated with antibiotics, let's identify some conditions that stand out for gene expression values of the fold change values of tetanus to flu vaccinated.

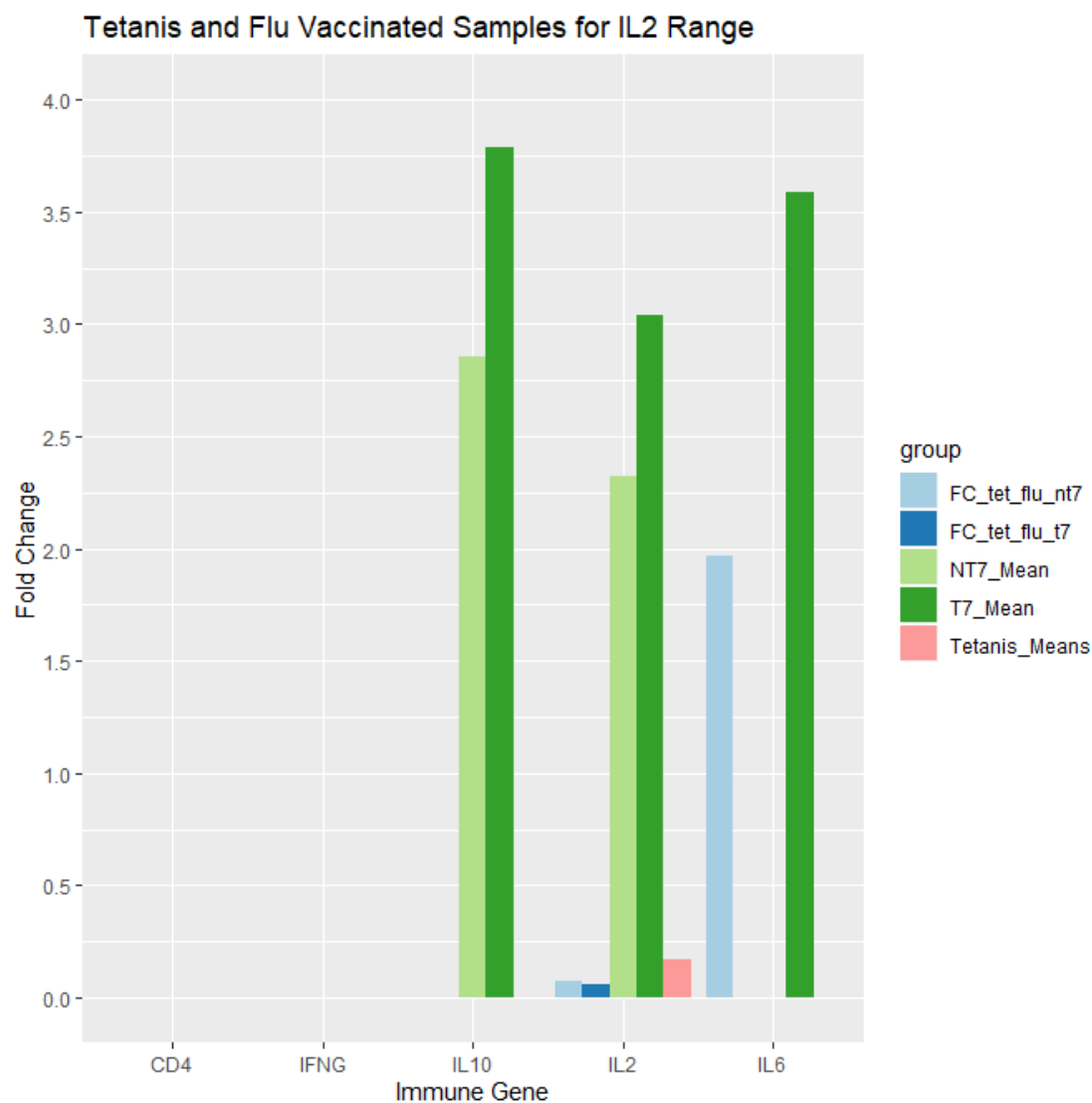
It is apparently clear that the tetanus vaccinated mean values are highest in almost every immune gene above except for the IL2 gene which is produced by the T-cells and crucial to the immune response. There is a higher fold change in tetanus to flu without antibiotics than the fold change of tetanus to flu treated with antibiotics in the CD4, IL10, and IL2



genes, but the IFNG (antiviral properties) and IL6 genes have a lower fold change with the IL6 (increases break down of fats and insulin resistance) gene dramatically decreased.

From the above sample the IL2 gene is not visible, so we should scale it down knowing the other genes will be missing from the next plot.

```
ggplot(data = tet_flu_tidy, aes(x=GENE_SYMBOL, y=foldChange, fill=group)) +  
  geom_bar(stat='identity', position=position_dodge()) +  
  scale_y_continuous(breaks = seq(0, 4, by=.5), limits=c(0,4)) +  
  scale_fill_brewer(palette='Paired') +  
  ggtitle('Tetanus and Flu Vaccinated Samples for IL2 Range') +  
  ylab('Fold Change') +  
  xlab('Immune Gene')
```



What about comparing all the healthy female and male samples to the tetanis vaccinated samples and looking at those same immune genes, and doing the same for the flu samples? Would this show some interesting data? This table is the healthy\_tetanis\_FCs table.

```
colnames(healthy_tetanis_FCs)

## [1] "GENE_SYMBOL"
## [2] "Tetanis_Means"
## [3] "iron_healthy_Female_Mean"
## [4] "iron_healthy_Male_Mean"
## [5] "overweight_females_Pre_Means"
## [6] "overweight_females_Post_EGCG_Means"
## [7] "overweight_females_Post_EGCG_Quercentin_Means"
## [8] "MI_study_HealthyMale_Means"
## [9] "Females_no_AD_Mean"
## [10] "males_no_AD_Mean"
## [11] "FC_iron_fem"
## [12] "FC_iron_mal"
## [13] "FC_heavy_fems_no_EGCG"
## [14] "FC_heavy_fems_EGCG"
## [15] "FC_heavy_fems_EGCG_plus"
## [16] "FC_healthyMI_males"
## [17] "FC_fems_noAD"
## [18] "FC_mals_noAD"
```

Use dplyr to get gene means of all the sample means of healthy patients except the tetanis means. This will combine the mixed genders and ages of the healthy blood samples to compare with the tetanis vaccinated samples.

```
healthy <- healthy_tetanis_FCs[,c(1:10)]

CD4 <- grep('^CD4$', healthy$GENE_SYMBOL)
IL2 <- grep('^IL2$', healthy$GENE_SYMBOL)
IFNG <- grep('^IFNG$', healthy$GENE_SYMBOL)
IL10 <- grep('^IL10$', healthy$GENE_SYMBOL)
IL6 <- grep('^IL6$', healthy$GENE_SYMBOL)

healthy_ig <- healthy[,c(CD4,IL2,IFNG,IL10,IL6),]

healthy_ig$all_healthy_Means <- rowMeans(healthy_ig[3:10])
healthy_FC <- healthy_ig %>% mutate(FC_tet_healthy =
Tetanis_Means/all_healthy_Means)

healthy_tet_fc <- healthy_FC[,c(1,2,11,12)]
```

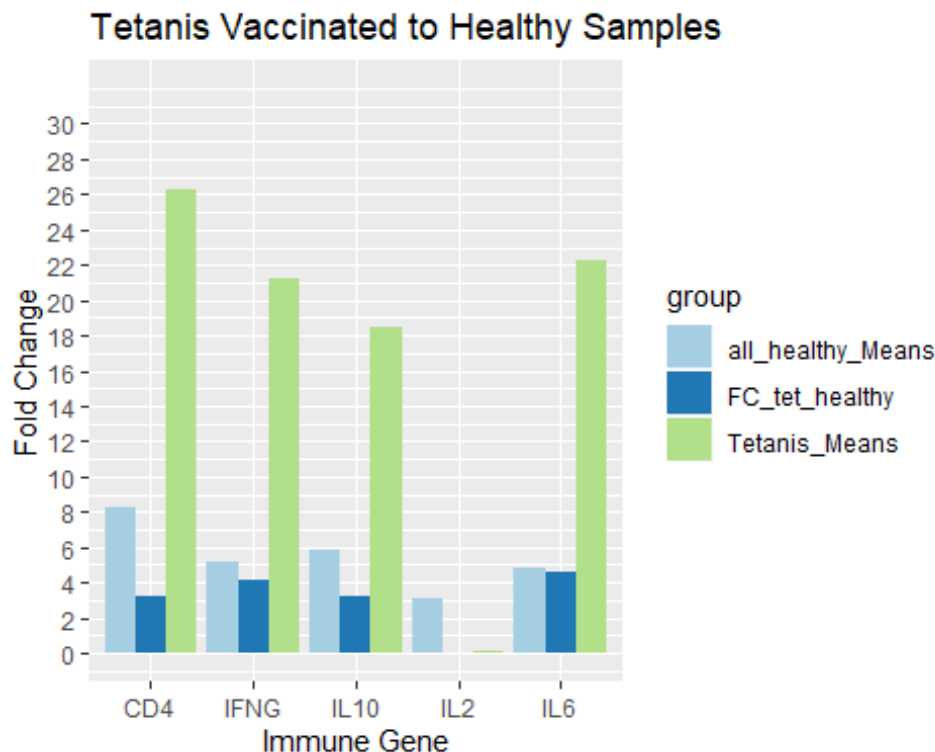
Tidy this table, healthy\_tet\_fc, into a tibble to plot a bar chart of the values.

```
healthy_tet_fc_tidy <- gather(healthy_tet_fc, 'group', 'foldChange', 2:4)
healthy_tet_fc_tidy$foldChange <- round(healthy_tet_fc_tidy$foldChange, 3)
head(healthy_tet_fc_tidy)
```

```
##  GENE_SYMBOL      group foldChange
##  1          CD4      Tetanis_Means    26.216
##  2          IL2      Tetanis_Means     0.172
##  3          IFNG      Tetanis_Means    21.249
##  4          IL10      Tetanis_Means    18.509
##  5          IL6      Tetanis_Means    22.212
##  6          CD4 all_healthy_Means     8.253
```

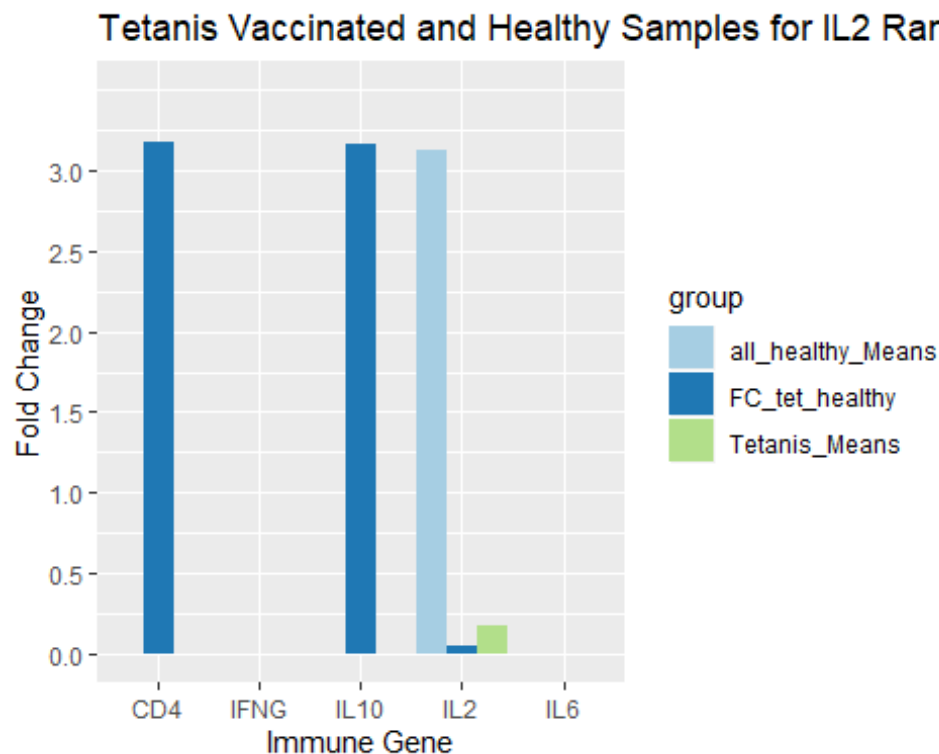
Now plot the results.

```
ggplot(data = healthy_tet_fc_tidy, aes(x=GENE_SYMBOL, y=foldChange,
fill=group)) +
  geom_bar(stat='identity', position=position_dodge())+
  scale_y_continuous(breaks = seq(0, 30, by=2), limits=c(0,32))+
  scale_fill_brewer(palette='Paired') +
  ggtitle('Tetanis Vaccinated to Healthy Samples')+
  ylab('Fold Change')+
  xlab('Immune Gene')
```



From the bar chart above, the Tetanis mean values are greatest (dramatically) in all immune genes selected except for the IL2 gene, as was the same for the tetanis to flu immunized samples. The fold change for tetanis vaccinated compared to healthy non-vaccinated samples show that Tetanis fold change values are all more than a 100% increase for these specific immune genes, except for the IL2 gene that will be shown in the plot below.

```
ggplot(data = healthy_tet_fc_tidy, aes(x=GENE_SYMBOL, y=foldChange,
fill=group)) +
  geom_bar(stat='identity', position=position_dodge())+
  scale_y_continuous(breaks = seq(0, 3, by=.5), limits=c(0,3.5))+
  scale_fill_brewer(palette='Paired') +
  ggtitle('Tetanus Vaccinated and Healthy Samples for IL2 Range')+
  ylab('Fold Change')+
  xlab('Immune Gene')
```



There are some more analytics or comparisons that can be done with this sort of data warehouse on gene expression values from blood in microarray medium. The flu vaccinated samples, flu\_7day, could then be compared to these healthy samples in the healthy table. The EGCG effects on overweight females, the hemochromatosis patients' gene values can be compared to find how these specific immune genes compare to immunized flu and tetanus samples are a few other comparisons that can be made.

```
colnames(flu_7day)[2:3] <- paste('flu_',colnames(flu_7day)[2:3], sep='')
colnames(flu_7day)

## [1] "Gene"          "flu_NT7_Mean" "flu_T7_Mean"

colnames(healthy)

## [1] "GENE_SYMBOL"
## [2] "Tetanus_Means"
```

```

## [3] "iron_healthy_Female_Mean"
## [4] "iron_healthy_Male_Mean"
## [5] "overweight_females_Pre_Means"
## [6] "overweight_females_Post_EGCG_Means"
## [7] "overweight_females_Post_EGCG_Quercentin_Means"
## [8] "MI_study_HealthyMale_Means"
## [9] "Females_no_AD_Mean"
## [10] "males_no_AD_Mean"

flu_healthy <- merge(flu_7day, healthy, by.x='Gene', by.y='GENE_SYMBOL')
colnames(flu_healthy)

## [1] "Gene"
## [2] "flu_NT7_Mean"
## [3] "flu_T7_Mean"
## [4] "Tetanis_Means"
## [5] "iron_healthy_Female_Mean"
## [6] "iron_healthy_Male_Mean"
## [7] "overweight_females_Pre_Means"
## [8] "overweight_females_Post_EGCG_Means"
## [9] "overweight_females_Post_EGCG_Quercentin_Means"
## [10] "MI_study_HealthyMale_Means"
## [11] "Females_no_AD_Mean"
## [12] "males_no_AD_Mean"

CD4 <- grep('^CD4$', flu_healthy$Gene)
IL2 <- grep('^IL2$', flu_healthy$Gene)
IFNG <- grep('^IFNG$', flu_healthy$Gene)
IL10 <- grep('^IL10$', flu_healthy$Gene)
IL6 <- grep('^IL6$', flu_healthy$Gene)

flu_healthy1 <- flu_healthy[c(CD4,IL2,IFNG,IL10,IL6),]
head(flu_healthy1)

##      Gene flu_NT7_Mean flu_T7_Mean Tetanis_Means iron_healthy_Female_Mean
## 1633  CD4      4.443672    5.086368    26.2162826      6.927265
## 5108  IL2      2.324467    3.035514     0.1720607      0.974014
## 5014 IFNG      4.843903    4.516635    21.2490376      3.724940
## 5068 IL10      2.852249    3.788690    18.5088908      3.144525
## 5137 IL6     11.282219    3.585461    22.2122479      3.760520
##      iron_healthy_Male_Mean overweight_females_Pre_Means
## 1633      7.269700      9.828
## 5108      1.253690      2.485
## 5014      3.830002      4.897
## 5068      2.871570      5.011
## 5137      3.863898      4.792
##      overweight_females_Post_EGCG_Means
## 1633      9.757
## 5108      2.549
## 5014      4.889
## 5068      5.043

```

```
## 5137                                4.671
##      overweight_females_Post_EGCG_Quercentin_Means
MI_study_HealthyMale_Means
## 1633                                9.771
8.863609
## 5108                                2.575
2.271007
## 5014                                5.077
5.672044
## 5068                                4.856
3.693453
## 5137                                4.799
3.667605
##      Females_no_AD_Mean males_no_AD_Mean
## 1633                6.821          6.785
## 5108                6.457          6.471
## 5014                6.446          6.472
## 5068               11.122         11.133
## 5137                6.731          6.699
```

These are the fold change value comparisons to healthy mixed ages and gender samples. Where NT7 is not treated with antibiotics and T7 was treated for the flu fold change values only.

```
flu_healthy1$All_Healthy_Mean <- rowMeans(flu_healthy1[,5:12])
flu_healthy2 <- flu_healthy1 %>% mutate(FC_tet_healthy =
                                         Tetanis_Means/All_Healthy_Mean)
flu_healthy3 <- flu_healthy2 %>% mutate(FC_flu_NT7_healthy =
                                         flu_NT7_Mean/All_Healthy_Mean)
flu_healthy4 <- flu_healthy3 %>% mutate(FC_flu_T7_healthy =
                                         flu_T7_Mean/All_Healthy_Mean)
Flu_Healthy <- flu_healthy4[,c(1:4,13:16)]
```

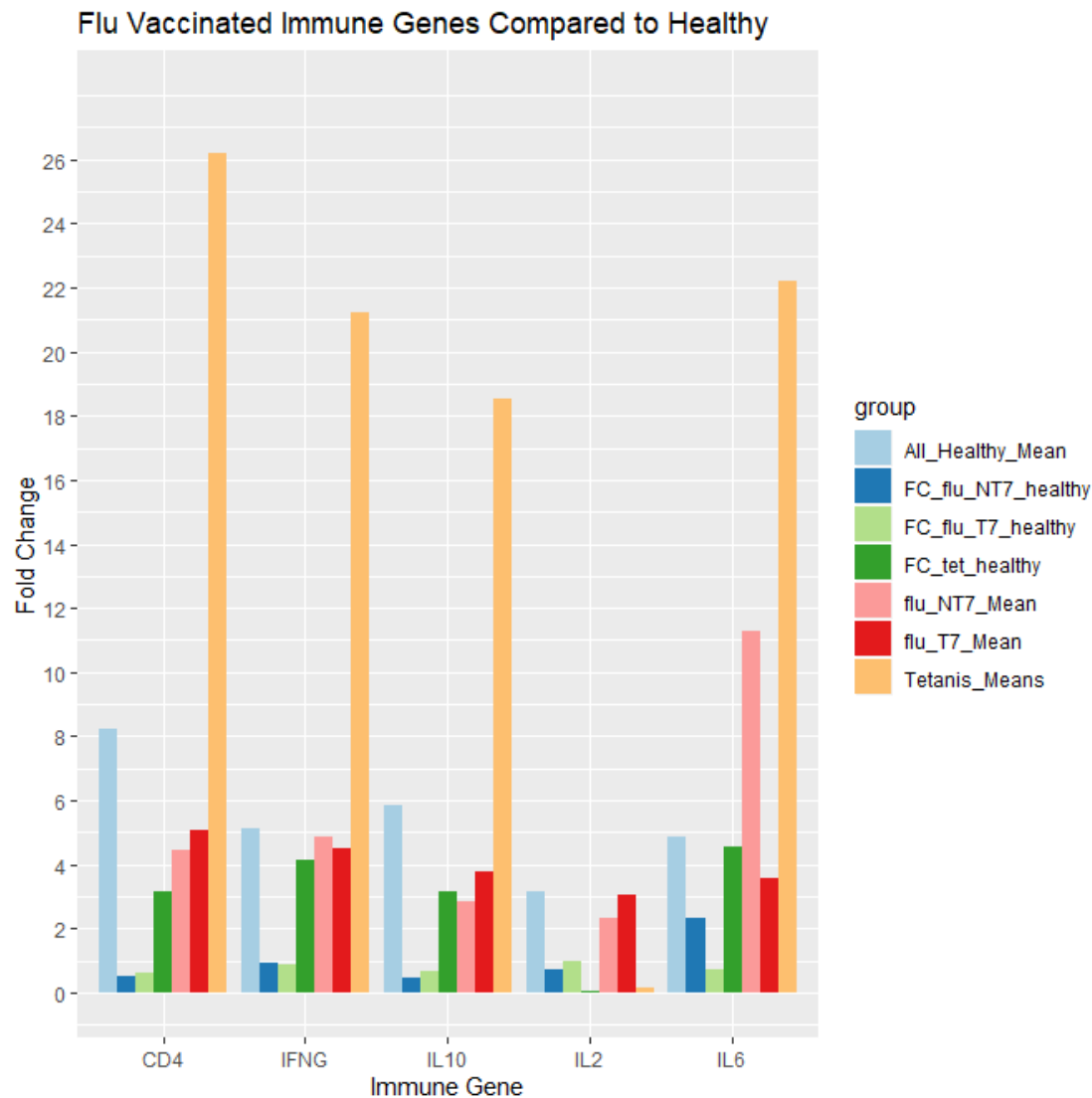
Use tidy on this data.

```
Flu_Healthy_tidy <- gather(Flu_Healthy, 'group', 'foldChange', 2:8)
Flu_Healthy_tidy$foldChange <- round(Flu_Healthy_tidy$foldChange, 3)
head(Flu_Healthy_tidy)

##   Gene      group foldChange
## 1  CD4 flu_NT7_Mean    4.444
## 2  IL2 flu_NT7_Mean    2.324
## 3 IFNG flu_NT7_Mean    4.844
## 4 IL10 flu_NT7_Mean    2.852
## 5  IL6 flu_NT7_Mean   11.282
## 6  CD4 flu_T7_Mean    5.086

ggplot(data = Flu_Healthy_tidy, aes(x=Gene, y=foldChange, fill=group)) +
  geom_bar(stat='identity', position=position_dodge()) +
  scale_y_continuous(breaks = seq(0, 27, by=2), limits=c(0,28)) +
  scale_fill_brewer(palette='Paired') +
```

```
ggtitle('Flu Vaccinated Immune Genes Compared to Healthy')+
ylab('Fold Change')+
xlab('Immune Gene')
```



The above bar chart shows the Mean values for Tetanis and Flu vaccinated samples for these immune genes are higher than the mean values for these immune genes in non-vaccinated healthy samples of mixed genders and ages 18-95 years old.

The fold change values compared to healthy for the flu vaccinated and treated with antibiotics show these immune genes to be higher than the treated in only the IL10 and IL2 immune genes.

Again, IL2 is the only gene that the tetanis mean and fold change in gene expression values is not greater than the healthy mean values or the flu vaccinated mean values and fold change values compared to healthy gene expression means.

In the IL6 gene, the fold change value of the flu vaccinated not treated with antibiotics is more than double the fold change value of the flu vaccinated that was treated with antibiotics. This could mean seven days after being treated with antibiotics when also being vaccinated for the flu, that the IL6 immune gene slows down expression values below the healthy levels.

---

It still remains to compare these samples of Alzheimer's Disease patients across samples of the healthy controls, and the same for the EGCG overweight female treatment, the heart disease males, iron toxicity males and females for the vaccinations. Since immune genes are a good way of telling how stressed a person's body is outside of what he or she can control this gives another option for analyzing health of a person to get vaccinated or when testing a new diet or discovering gene targets involved in an increased risk of death in heart disease or Alzheimer's disease.

What questions are you thinking about discovering given this data? Can you uncover what you know piled on top of what this data can explain for you to help answer the questions you have? If so, then play around with this data to answer those questions. If not, then go get more data or develop a different way to analyze this warehouse of data, then make note of any questions you have that you find interesting or curious and answer it with the data.

---