Differential Gene Expression Analysis of LGRC Data

J Fah Sathirapongsasuti

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1 Introduction

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death in the United States. Since the year 2000 the number of females dying from COPD has surpassed the number of males, and there is an increasing body of research suggesting females may be biologically more susceptible to COPD. The goal of the study is to explore which molecular pathways might be associated with sexual dimorphism in COPD. This vignette uses gene expression data from the Lung Genomics Research Consortium to identify 959 genes with sexually-dimorphic differential expression in the presence of COPD ("sexually dimorphic and COPD differential" or "SDCD" genes).

2 Preprocessing

Load the necessary packages and datasets.

```
> library(COPDSexualDimorphism)
> `%+%` <- function(x,y) paste(x,y,sep="")
> p.cutoff = 0.01
> data(lgrc.expr)
> data(lgrc.expr.meta)
> data(lgrc.genes)
```

3 Sexually Dimorphic and COPD Differential Gene Expression Analysis

Sexually Dimorphic and COPD Differential (SDCD) analysis comprises of two stratifications: by sex and by COPD status. These tratified analysese are multivariate linear model performed by `limma`. In each of the analyses, the function `sdcd` contrasts the linear models from the two trata and outputs a list of genes with SDCD expression. The results from the two stratification analyses are combined at the end.

3.1 Model 1: expression = COPD + Age + pkyrs

Stratified by sex, then compare the betas.

```
> design.mtx = cbind(ctrl=1,
+                 copd=as.integer(grepl("COPD",colnames(expr))),
+                 age=expr.meta$age,
+                 pkyr=expr.meta$pkyrs)
> good.idx = apply(design.mtx,1,function(x){!any(is.na(x))}) & (expr.meta$gender == "1-Male")
> male.fit = lmFit(log(expr)[,good.idx], design.mtx[good.idx,])
> male.fit = eBayes(male.fit)
> good.idx = apply(design.mtx,1,function(x){!any(is.na(x))}) & (expr.meta$gender == "2-Female")
> female.fit = lmFit(log(expr)[,good.idx], design.mtx[good.idx,])
```

```
> female.fit = eBayes(female.fit)
> male.female.copd.beta.diff.genes = sdcd(male.fit, female.fit, "copd", lgrc.genes, fdr.cutoff=0.25, fil

[1] "Number of probes with sexual dimorphic differential expression: 1551"
```

3.2 Model 2: expression = Gender + Age + pkyrs

Male vs female analysis for COPD cases only.

```
> design.mtx = cbind(ctrl=1,
+                 gender=expr.meta$gender,
+                 age=expr.meta$age,
+                 pkyr=expr.meta$pkyrs)
> good.idx = apply(design.mtx,1,function(x){!any(is.na(x))}) & grepl("COPD",colnames(expr))
> copd.fit = lmFit(log(expr)[,good.idx], design.mtx[good.idx,])
> copd.fit = eBayes(copd.fit)
> good.idx = apply(design.mtx,1,function(x){!any(is.na(x))}) & grepl("CTRL",colnames(expr))
> ctrl.fit = lmFit(log(expr)[,good.idx], design.mtx[good.idx,])
> ctrl.fit = eBayes(ctrl.fit)
> copd.ctrl.gender.beta.diff.genes = sdcd(copd.fit, ctrl.fit, "gender", lgrc.genes, fdr.cutoff=0.25, fil

[1] "Number of probes with sexual dimorphic differential expression: 1656"
```

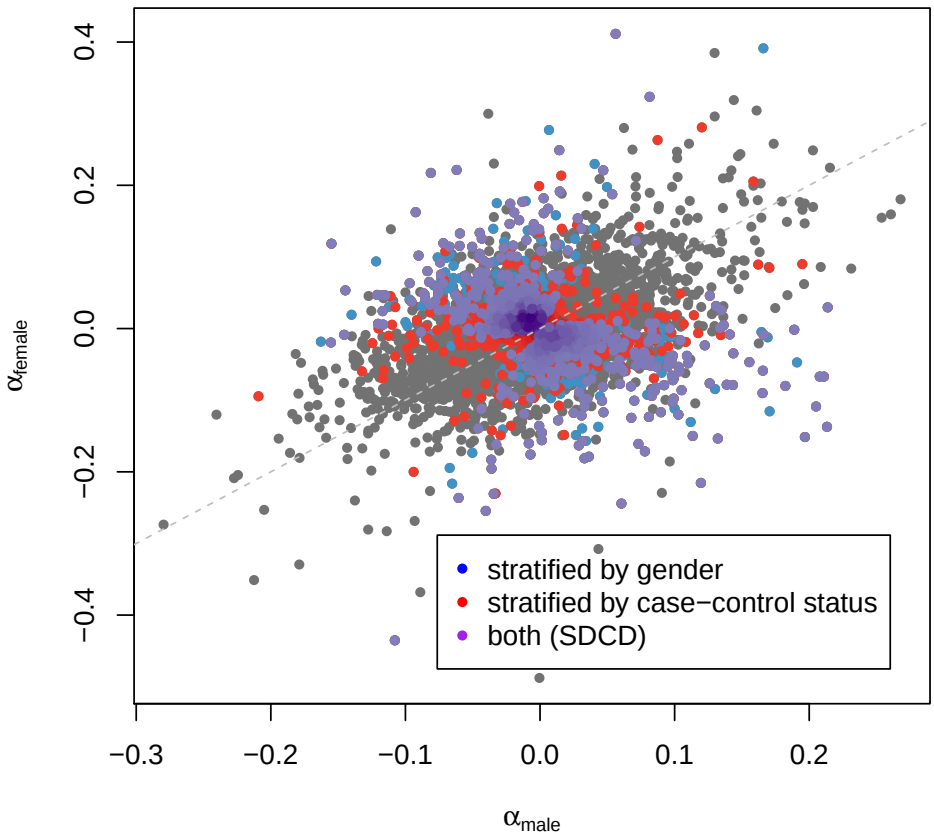
4 Combine the Results

We use set intersection to combine the results from the two stratification analyses.

```
> male.female.copd.beta.diff.genes.all = sdcd(male.fit, female.fit, "copd", lgrc.genes, fdr.cutoff=10, f

[1] "Number of probes with sexual dimorphic differential expression: 13870"

> copd.ctrl.gender.beta.diff.genes.all = sdcd(copd.fit, ctrl.fit, "gender", lgrc.genes, fdr.cutoff=10, f

[1] "Number of probes with sexual dimorphic differential expression: 13870"

> all.beta.diff.genes = cbind(copd.ctrl.gender.beta.diff.genes.all, male.female.copd.beta.diff.genes.all
> rename.col = grep("beta.diff", names(all.beta.diff.genes))
> names(all.beta.diff.genes)[rename.col[1:2]] = names(all.beta.diff.genes)[rename.col[1:2]] %+% ".copd.c
> names(all.beta.diff.genes)[rename.col[3:4]] = names(all.beta.diff.genes)[rename.col[3:4]] %+% ".male.f

> sdcd.genes = merge(copd.ctrl.gender.beta.diff.genes, male.female.copd.beta.diff.genes, by=setdiff(inte
> sdcd.genes = unique(sdcd.genes)

> data(lgrc.sdcd.genes)
> print("There are " %+% nrow(sdcd.genes) %+% " SDCD genes")

[1] "There are 959 SDCD genes"
```

Then we can plot the results:

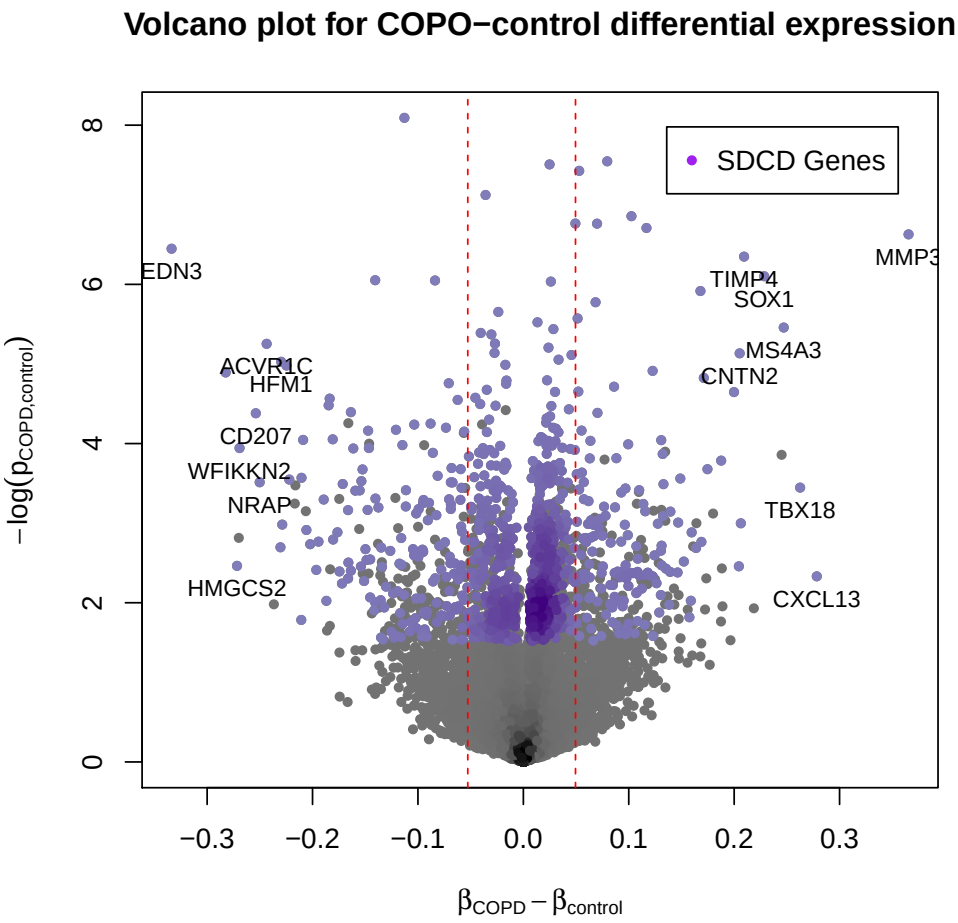
```
> # FIGURE 1B
> my.smart.plot(male.fit$coefficients[,"copd"], female.fit$coefficients[,"copd"], main="Coefficients of
> my.smart.plot(male.fit$coefficients[male.female.copd.beta.diff.genes$ensembl_gene_id,"copd"], female.f
> my.smart.plot(male.fit$coefficients[copd.ctrl.gender.beta.diff.genes$ensembl_gene_id,"copd"], female.f
> my.smart.plot(male.fit$coefficients[sdcd.genes$ensembl_gene_id,"copd"], female.fit$coefficients[sdcd.g
> abline(0,1,lty=2,col="gray")
> abline(h=c(qnorm(0.025),qnorm(0.975)),v=c(qnorm(0.025),qnorm(0.975)),lty=3,col="gray")
> smartlegend("right","bottom",c("stratified by gender","stratified by case-control status","both (SDCD)
```




```
> # FIGURE 1C
> all.beta.diff.genes$copd.ctrl.beta.diff = all.beta.diff.genes$copd.beta - all.beta.diff.genes$ctrl.bet
> this.pch = 20
> my.smart.plot(all.beta.diff.genes$copd.ctrl.beta.diff, -log10(all.beta.diff.genes$copd.ctrl.p), main='
> my.smart.plot(all.beta.diff.genes[sdcd.genes$ensembl_gene_id,"copd.ctrl.beta.diff"], -log10(all.beta.d
> smartlegend("right","top",c("SDCD Genes"),pch=this.pch,col=c("purple"))
> CIpercent = 0.9
> abline(v=quantile(all.beta.diff.genes$beta.diff.copd.ctrl, c((1-CIpercent)/2, (1+CIpercent)/2)), col='
> extreme.betas.idx = abs(sdcd.genes$beta.diff.x) > 0.25 | (abs(sdcd.genes$beta.diff.x) > 0.2 & sdcd.gen
> extreme.betas = cbind(sdcd.genes[extreme.betas.idx, c("hgnc_symbol","beta.diff.x","copd.ctrl.p","male.
+ n.log.p=-log10(sdcd.genes[extreme.betas.idx, c("copd.ctrl.p")]))
> print("Extreme beta_diff points are: ")

[1] "Extreme beta_diff points are: "

> print(extreme.betas)

    hgnc_symbol beta.diff.x  copd.ctrl.p male.female.p.adj copd.ctrl.p.adj
244        TBX18   0.2625562 3.576277e-04       0.028464545    0.0280109784
260        CD207  -0.2537996 4.160540e-05       0.125745744    0.0098877620
298       ACVR1C  -0.2435254 5.584837e-06       0.010899375    0.0028915492
304         EDN3  -0.3337424 3.564346e-07       0.006448865    0.0004697484
377      HMGCS2  -0.2716475 3.437951e-03       0.102258238    0.0839056712
```

```
511       MS4A3   0.2470051 3.487462e-06       0.014957343    0.0022980789
515        MMP3   0.3654424 2.353935e-07       0.221377877    0.0003412500
546      CXCL13   0.2783933 4.655509e-03       0.063294179    0.0984226964
551       TIMP4   0.2093865 4.481220e-07       0.001322135    0.0005413688
590        HFM1  -0.2297657 9.393241e-06       0.128370483    0.0040051121
744     WFIKKN2  -0.2692408 1.139020e-04       0.065013255    0.0159592624
820        SOX1   0.2284689 7.902981e-07       0.068103876    0.0008357564
830       CNTN2   0.2052041 7.347437e-06       0.001349746    0.0034359932
861              -0.2824065 1.279387e-05       0.063908211    0.0048808618
892        NRAP  -0.2501514 3.063447e-04       0.014957343    0.0262491592
    chromosome_name  n.log.p
244               6 3.446569
260               2 4.380850
298               2 5.252990
304              20 6.448020
377               1 2.463700
511              11 5.457491
515              11 6.628206
546               4 2.332033
551               3 6.348604
590               1 5.027185
744              17 3.943468
820              13 6.102209
830               1 5.133864
861               2 4.892998
892              10 3.513790
```

```
> text(extreme.betas$beta.diff.x, extreme.betas$n.log.p, extreme.betas$hgnc_symbol, pos=1, cex=0.8)
```




```
> # Figure S2
> all.beta.diff.genes$male.female.beta.diff = all.beta.diff.genes$male.beta - all.beta.diff.genes$female
> this.pch = 20
> my.smart.plot(all.beta.diff.genes$male.female.beta.diff, -log10(all.beta.diff.genes$male.female.p), ma
> my.smart.plot(all.beta.diff.genes[sdcd.genes$ensembl_gene_id,"male.female.beta.diff"], -log10(all.beta
> smartlegend("right","top",c("SDCD Genes"),pch=this.pch,col=c("purple"))
> CIpercent = 0.9
> abline(v=quantile(all.beta.diff.genes$beta.diff.male.female, c((1-CIpercent)/2, (1+CIpercent)/2)), col
```

5 Session Information

```
> sessionInfo()

R Under development (unstable) (2014-10-07 r66723)
Platform: x86_64-unknown-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
```

```
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] stats4    parallel  stats     graphics  grDevices utils     datasets
[8] methods   base

other attached packages:
 [1] COPDSexualDimorphism_1.3.0      gtools_3.4.1
 [3] gplots_2.14.2                   GenomicRanges_1.19.0
 [5] GenomeInfoDb_1.3.0              IRanges_2.1.0
 [7] S4Vectors_0.5.0                 BiocGenerics_0.13.0
 [9] limma_3.23.0                    beeswarm_0.1.6
[11] RColorBrewer_1.0-5              NCBI2R_1.4.6
[13] COPDSexualDimorphism.data_1.1.0

loaded via a namespace (and not attached):
[1] KernSmooth_2.23-13 XVector_0.7.0      bitops_1.0-6       caTools_1.17.1
[5] gdata_2.13.3       tools_3.2.0
```