TWAS Analysis of Diversity Outbred Strain RNAseq Data

Dave Bridges

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Contents

1 Purpose

2 Experimental Details

The RNA expression data was downloaded from GSE72759 as a matrix file, and compared with genotypes from the Svenson-183 dataset.

3 Raw Data

```
library(readr) #loads the readr package
expression.filename <- "GSE72759_DO192_RNAseq_UpperQuartileNormalized_n21454genes_forGEOSubmission.txt"
expression.data <- read_tsv(expression.filename, show_col_types = F) %>%
  dplyr::rename(ENSEMBL.ID=1)
```

```
genotype.filename <- 'Svenson-183_Svenson_DO-MegaMUGA-calls.csv'
genotype.data <- read_csv(genotype.filename,
                          col_types = cols(
  .default = col_character(),
  chr = col_factor(levels=NULL),
  pos = col_double()
))

phenotype.filename <- 'Svenson-183_Svenson_DO-phenotypes.csv'
phenotype.data <- read_csv(phenotype.filename, na='-999999')

phenotype.data[phenotype.data=='-999999'] <- NA


mean.expression <-
  expression.data %>%
  dplyr::select(contains(phenotype.data$sample))%>%
  rowMeans()

expression.data <-
   expression.data %>%
   filter(mean.expression>10)
```

4 Analysis

Only evaluated gene expression for genes with >10 TPM

4.1 Cholesterol Levels

Regressed cholesterol levels by diet and sex

```
summary.data <-
  phenotype.data %>%
  group_by(sex,diet) %>%
  summarize_at(.vars=vars(chol1), .funs=list(mean=mean,
                                             se=se,
                                             sd=sd,
                                             n=length))

kable(summary.data,caption="Summary statistics for cholesterol levels at 8 weeks")
```

Table 1: Summary statistics for cholesterol levels at 8 weeks

sex	diet	mean	se	sd	n
F	chow	NA	1.16	NA	225
F	hf	108	1.79	25.3	200
M	chow	NA	1.47	NA	224
M	hf	NA	2.06	NA	197

```
library(broom)
lm(chol1~sex*diet, data=phenotype.data) %>%
  tidy %>%
  kable(caption="Global interactions between sex and diet")
```

Table 2: Global interactions between sex and diet

term	estimate	std.error	statistic	p.value
(Intercept)	80.0	1.68	47.63	0.000
sexM	16.4	2.38	6.91	0.000
diethf	28.0	2.38	11.81	0.000
sexM:diethf	3.9	3.37	1.16	0.248

```
chol.lm <- lm(chol1~sex+diet, data=phenotype.data)
chol.lm.ncd <- lm(chol1~sex, data=filter(phenotype.data, diet=='chow'))

cholesterol.data.ncd <-
  phenotype.data %>%
  filter(!is.na(chol1)) %>%
  filter(diet=='chow') %>%
  mutate(adj.chol.ncd=residuals(chol.lm.ncd)+coefficients(chol.lm.ncd)['(Intercept)'])
```

```
summary.data <-
  phenotype.data %>%
  group_by(sex,diet) %>%
  summarize_at(.vars=vars(chol1), .funs=list(mean=mean,
                                             se=se,
                                             sd=sd,
                                             n=length))

kable(summary.data,caption="Summary statistics for cholesterol levels at 8 weeks")
```

Table 3: Summary statistics for cholesterol levels at 8 weeks

sex	diet	mean	se	sd	n
F	chow	NA	1.16	NA	225
F	hf	108	1.79	25.3	200
M	chow	NA	1.47	NA	224
M	hf	NA	2.06	NA	197

```
library(broom)
lm(chol1~sex*diet, data=phenotype.data) %>%
  tidy %>%
  kable(caption="Global interactions between sex and diet")
```

Table 4: Global interactions between sex and diet

term	estimate	std.error	statistic	p.value
(Intercept)	80.0	1.68	47.63	0.000
sexM	16.4	2.38	6.91	0.000
diethf	28.0	2.38	11.81	0.000

term	estimate	std.error	statistic	p.value
sexM:diethf	3.9	3.37	1.16	0.248

```
chol.lm <- lm(chol1~sex+diet, data=phenotype.data)
chol.lm.hf <- lm(chol1~sex, data=filter(phenotype.data, diet=='hf'))

cholesterol.data.hfd <-
  phenotype.data %>%
    filter(!is.na(chol1)) %>%
  filter(diet=='hf') %>%
  mutate(adj.chol.hf=residuals(chol.lm.hf)+coefficients(chol.lm.hf)['(Intercept)'])
```

5 TWAS with Cholesterol Levels for NCD

```
library("org.Mm.eg.db")

library(purrr)
possible.lm <- possibly(.f = lm, otherwise=NULL) # to catch errors when we only have one sex and a cont

twas.data.ncd <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.ncd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.ncd$sample),
               names_to='sample',
               values_to='expression') %>%
  full_join(cholesterol.data.ncd,by='sample') %>%
  dplyr::select(chol1,sex,sample,ENSEMBL.ID,expression) %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  filter(term=='chol1') %>%
  arrange(p.value)%>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.ncd.all <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.ncd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.ncd$sample),
               names_to='sample',
               values_to='expression') %>%
  full_join(cholesterol.data.ncd,by='sample') %>%
  dplyr::select(chol1,sex,sample,ENSEMBL.ID,expression) %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  filter(term %in% c('chol1', '(Intercept)')) %>%
  dplyr::select(ENSEMBL.ID,term,estimate,std.error) %>%
  pivot_wider(id_cols=ENSEMBL.ID,
              names_from = 'term',
              values_from = c(estimate,std.error)) %>%
  mutate(estimate.rel = estimate_chol1/`estimate_(Intercept)`,
         std.error.rel = std.error_chol1/`estimate_(Intercept)`)
```

```
twas.data.ncd.r2 <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.ncd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.ncd$sample),
               names_to='sample',
               values_to='expression') %>%
  full_join(cholesterol.data.ncd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::glance(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  arrange(p.value)%>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.ncd.int <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.ncd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.ncd$sample),
               names_to='sample',
               values_to='expression') %>%
  full_join(cholesterol.data.ncd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1*sex, data = .x))) %>%
  filter(term=='chol1:sexM') %>%
  arrange(p.value) %>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.ncd$symbol <- mapIds(org.Mm.eg.db,
                           keys=twas.data.ncd$ENSEMBL.ID,
                           column="SYMBOL",
                           keytype="ENSEMBL",
                           multiVals="first")

twas.data.ncd.int$symbol <- mapIds(org.Mm.eg.db,
                           keys=twas.data.ncd.int$ENSEMBL.ID,
                           column="SYMBOL",
                           keytype="ENSEMBL",
                           multiVals="first")

twas.data.ncd.all$symbol <- mapIds(org.Mm.eg.db,
                           keys=twas.data.ncd.all$ENSEMBL.ID,
                           column="SYMBOL",
                           keytype="ENSEMBL",
                           multiVals="first")


twas.data.ncd %>%
  head(10) %>%
  kable(caption="Top 10 liver TWAS assocations with cholesterol levels")
```

Table 5: Top 10 liver TWAS assocations with cholesterol levels

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000069805	chol1	-0.600	0.150	-3.99	0.000	0.000	Fbp1
ENSMUSG00000026895	chol1	-0.026	0.007	-3.89	0.000	0.000	Ndufa8

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000026814	chol1	-0.045	0.012	-3.71	0.000	0.000	Eng
ENSMUSG00000000088	chol1	-0.058	0.016	-3.71	0.000	0.000	Cox5a
ENSMUSG00000040048	chol1	-0.041	0.011	-3.70	0.000	0.000	Ndufb10
ENSMUSG00000049422	chol1	-0.207	0.057	-3.63	0.000	0.000	Chchd10
ENSMUSG00000038462	chol1	-0.074	0.020	-3.60	0.001	0.001	Uqcrfs1
ENSMUSG00000083863	chol1	2.112	0.591	3.58	0.001	0.001	NA
ENSMUSG00000026238	chol1	-0.086	0.025	-3.41	0.001	0.001	Ptma
ENSMUSG00000025481	chol1	-0.071	0.021	-3.37	0.001	0.001	Urah

```
twas.data.ncd.int %>%
  head(10) %>%
  kable(caption="Top 10 liver TWAS assocations with cholesterol levels that are modified by sex")
```

Table 6: Top 10 liver TWAS assocations with cholesterol levels that are modified by sex

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000071644	chol1:sexM	-0.123	0.032	-3.90	0.000	0.000	Eef1g
ENSMUSG00000063001	chol1:sexM	-0.098	0.028	-3.44	0.001	0.001	Gm9701
ENSMUSG00000028798	chol1:sexM	-0.039	0.012	-3.38	0.001	0.001	Eif3i
ENSMUSG00000025794	chol1:sexM	-0.099	0.029	-3.37	0.001	0.001	Rpl14
ENSMUSG00000003546	chol1:sexM	-0.065	0.019	-3.33	0.001	0.001	Klc4
ENSMUSG00000074227	chol1:sexM	-0.062	0.019	-3.23	0.002	0.002	Spint2
ENSMUSG00000040715	chol1:sexM	0.187	0.059	3.18	0.002	0.002	Rsc1a1
ENSMUSG00000024038	chol1:sexM	-0.053	0.017	-3.15	0.002	0.002	Ndufv3
ENSMUSG00000061477	chol1:sexM	-0.240	0.076	-3.13	0.002	0.002	Rps7
ENSMUSG00000040048	chol1:sexM	-0.068	0.022	-3.13	0.002	0.002	Ndufb10

```
write_csv(twas.data.ncd,
          file="NCD TWAS Results.csv")
write_csv(twas.data.ncd.int,
          file="NCD TWAS Interaction Results.csv")

twas.data.ncd.combined <-
  left_join(twas.data.ncd,
            filter(twas.data.ncd.int, p.value<0.05), #only append interaction values when significant
             by=c('ENSEMBL.ID','symbol'),
             suffix = c('_main','_int'))

write_csv(twas.data.ncd.combined,
          file="NCD TWAS Results - Combined.csv")
```

6 TWAS with Cholesterol Levels for HFD

```
twas.data.hf <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.hfd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.hfd$sample),
```

```
              names_to='sample',
              values_to='expression') %>%
  full_join(cholesterol.data.hfd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  filter(term=='chol1') %>%
  arrange(p.value)%>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.hf.all<-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.hfd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.hfd$sample),
              names_to='sample',
              values_to='expression') %>%
  full_join(cholesterol.data.hfd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  filter(term %in% c('chol1', '(Intercept)')) %>%
  dplyr::select(ENSEMBL.ID,term,estimate,std.error) %>%
  pivot_wider(id_cols=ENSEMBL.ID,
              names_from = 'term',
              values_from = c(estimate,std.error)) %>%
  mutate(estimate.rel = estimate_chol1/`estimate_(Intercept)`*100,
         std.error.rel = std.error_chol1/`estimate_(Intercept)`*100)


twas.data.hf.r2 <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.hfd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.hfd$sample),
              names_to='sample',
              values_to='expression') %>%
  full_join(cholesterol.data.hfd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::glance(possible.lm(expression ~ chol1+sex, data = .x))) %>%
  arrange(p.value)%>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.int.hf <-
  expression.data %>%
  dplyr::select(ENSEMBL.ID,one_of(cholesterol.data.hfd$sample))%>%
  pivot_longer(cols=one_of(cholesterol.data.hfd$sample),
              names_to='sample',
              values_to='expression') %>%
  full_join(cholesterol.data.hfd,by='sample') %>%
  group_by(ENSEMBL.ID) %>%
  group_modify(~ broom::tidy(possible.lm(expression ~ chol1*sex, data = .x))) %>%
  filter(term=='chol1:sexM') %>%
  arrange(p.value) %>%
  mutate(p.adj=p.adjust(p.value,method="BH"))

twas.data.hf$symbol <- mapIds(org.Mm.eg.db,
```

```
                            keys=twas.data.hf$ENSEMBL.ID,
                            column="SYMBOL",
                            keytype="ENSEMBL",
                            multiVals="first")

twas.data.int.hf$symbol <- mapIds(org.Mm.eg.db,
                            keys=twas.data.int.hf$ENSEMBL.ID,
                            column="SYMBOL",
                            keytype="ENSEMBL",
                            multiVals="first")

twas.data.hf.all$symbol <- mapIds(org.Mm.eg.db,
                            keys=twas.data.hf.all$ENSEMBL.ID,
                            column="SYMBOL",
                            keytype="ENSEMBL",
                            multiVals="first")


twas.data.hf %>%
  head(10) %>%
  kable(caption="Top 10 liver TWAS a ssocations with cholesterol levels for HFD")
```

Table 7: Top 10 liver TWAS a ssocations with cholesterol levels for HFD

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000022615	chol1	-0.073	0.022	-3.29	0.001	0.001	Tymp
ENSMUSG00000052914	chol1	0.043	0.015	2.92	0.004	0.004	Cyp2j6
ENSMUSG00000028760	chol1	-0.028	0.010	-2.84	0.006	0.006	Eif4g3
ENSMUSG00000024913	chol1	-0.032	0.012	-2.76	0.007	0.007	Lrp5
ENSMUSG00000028656	chol1	0.014	0.005	2.71	0.008	0.008	Cap1
ENSMUSG00000025003	chol1	0.319	0.118	2.70	0.008	0.008	Cyp2c39
ENSMUSG00000052520	chol1	0.352	0.131	2.69	0.009	0.009	Cyp2j5
ENSMUSG00000057530	chol1	-0.106	0.040	-2.68	0.009	0.009	Ece1
ENSMUSG00000074063	chol1	-0.140	0.052	-2.68	0.009	0.009	Osgin1
ENSMUSG00000024456	chol1	-0.018	0.007	-2.68	0.009	0.009	Diaph1

```
twas.data.int.hf %>%
  head(10) %>%
  kable(caption="Top 10 liver TWAS assocations with cholesterol levels that are modified by sex for HFD
```

Table 8: Top 10 liver TWAS assocations with cholesterol levels that are modified by sex for HFD

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000090231	chol1:sexM	-1.177	0.316	-3.72	0.000	0.000	Cfb
ENSMUSG00000040855	chol1:sexM	-0.044	0.012	-3.57	0.001	0.001	Reps2
ENSMUSG00000052520	chol1:sexM	-0.886	0.249	-3.56	0.001	0.001	Cyp2j5
ENSMUSG00000031138	chol1:sexM	-0.214	0.065	-3.28	0.001	0.001	F9
ENSMUSG00000016534	chol1:sexM	-0.345	0.105	-3.27	0.002	0.002	Lamp2
ENSMUSG00000021091	chol1:sexM	-0.802	0.246	-3.26	0.002	0.002	Serpina3n
ENSMUSG00000039197	chol1:sexM	-0.380	0.119	-3.19	0.002	0.002	Adk

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000023175	chol1:sexM	-0.198	0.063	-3.15	0.002	0.002	Bsg
ENSMUSG00000068036	chol1:sexM	-0.042	0.013	-3.12	0.002	0.002	Afdn
ENSMUSG00000021794	chol1:sexM	-0.739	0.238	-3.11	0.003	0.003	Glud1

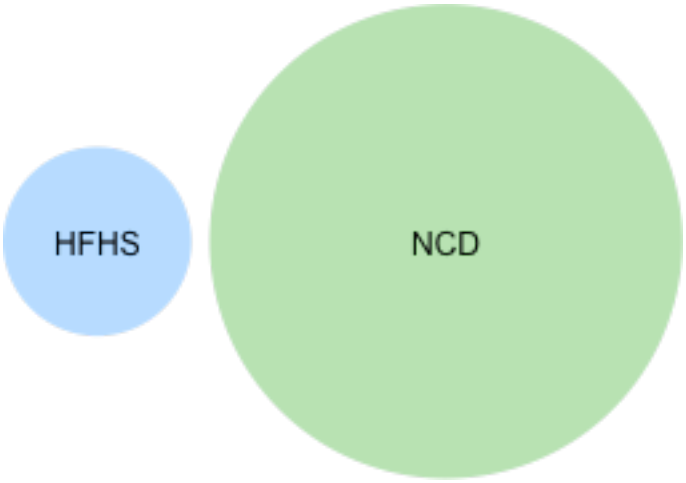
```
write_csv(twas.data.hf,
          file="HFD TWAS Results.csv")
write_csv(twas.data.int.hf,
          file="HFD TWAS Interaction Results.csv")

twas.data.combined.hf <-
  left_join(twas.data.hf,
            filter(twas.data.int.hf, p.value<0.05), #only append interaction values when significant
             by=c('ENSEMBL.ID','symbol'),
             suffix = c('_main','_int'))

write_csv(twas.data.combined.hf,
          file="HFD TWAS Results - Combined.csv")

sig.twas.data.hf <-
    twas.data.hf %>%
  filter(p.adj<0.05)

sig.twas.data.ncd <-
  twas.data.ncd %>%
  filter(p.adj<0.05)

sig.twas.data.ncd$symbol %in% sig.twas.data.hf$symbol %>% table
```

```
## .
## FALSE  TRUE
##   208     8
```

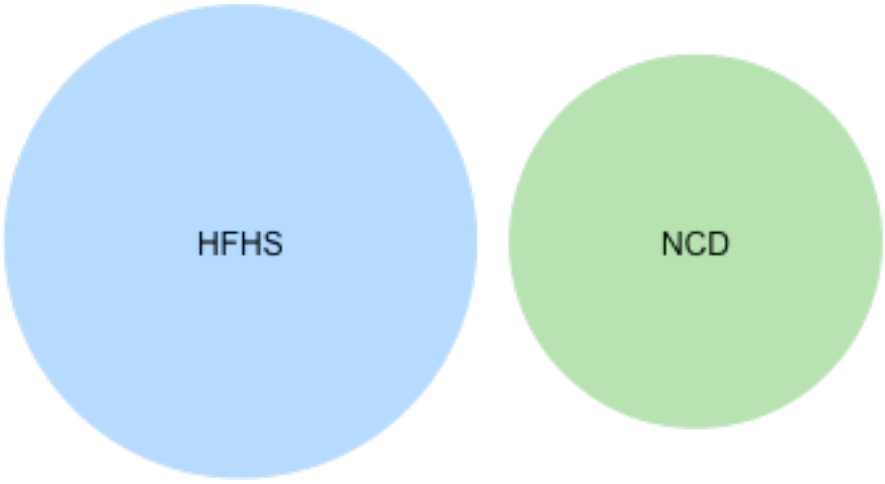
```
sig.twas.data.ncd$genes %in% sig.twas.data.hf$genes %>% table
```

```
## < table of extent 0 >
```

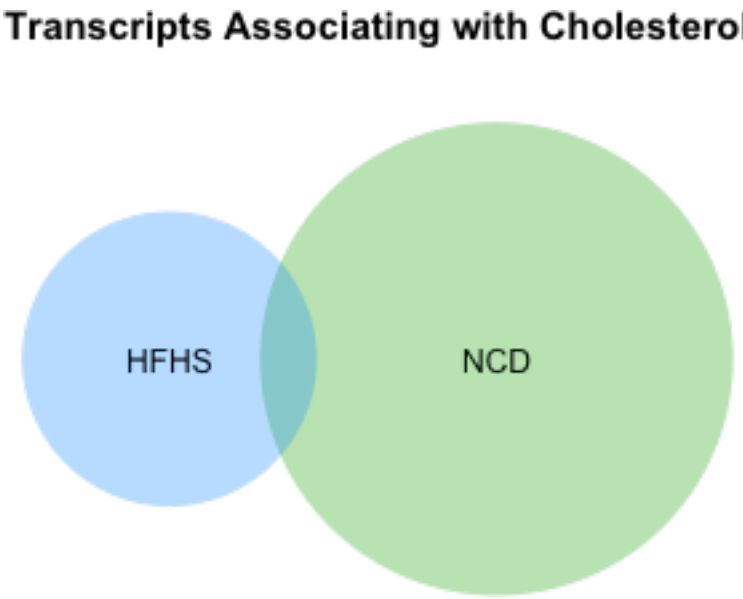
```
library(venneuler)
vd.down.up <- venneuler(c("NCD"=170, "HFHS"=27, "NCD&HFHS"=0))
vd.down.down <- venneuler(c("NCD"=43, "HFHS"=69, "NCD&HFHS"=0))
vd.all <- venneuler(c("NCD"=dim(sig.twas.data.ncd)[1],
                      "HFHS"=dim(sig.twas.data.hf)[1],
                      "NCD&HFHS"=intersect(sig.twas.data.hf$symbol,sig.twas.data.ncd$symbol) %>% length
plot(vd.down.up)
```




```
plot(vd.down.down)
```




```
plot(vd.all, main="Transcripts Associating with Cholesterol")
```



Our analysis identified **216** nominally significant associations between expression of genes and adjusted cholesterol levels on a normal chow diet. Among those, 44 were positively correlated and 44 were negatively correlated with cholesterol levels.

6.1 TWAS Modification by Sex

By modeling the interactions between sex and expression on cholesterol levels, we identified **205** genes where the cholesterol/expression relationship was modified by sex in a nominally significant manner. This included 19 genes where the relationship was stronger in males, and 186 where it was stronger in females.

7 Comparason with mouse GWAS

```
chr11.genes <- c('Znrf3', 'Xbp1', 'Ccdc117', 'Ankrd36', 'Mrps24', 'Urgcp', 'Dbnl', 'Pgam2', 'Polm', 'Ael
genes.of.interest <- c('Cyp7a1','Fasn','Ldlr','Hmgcr')
twas.data.ncd %>%
  filter(symbol %in% c(chr11.genes,genes.of.interest)) %>%
  kable(caption="Genes in the chromosome 11 interval with liver expression")
```

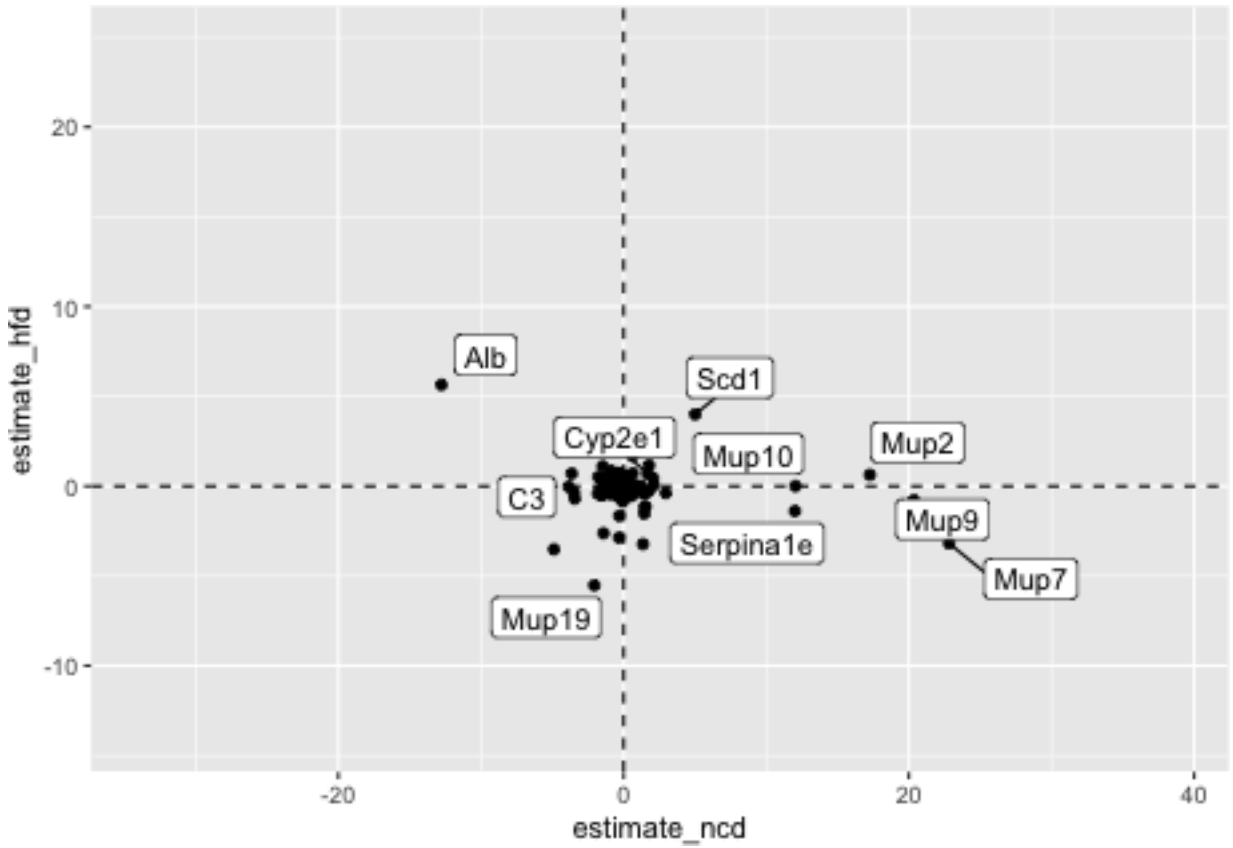
Table 9: Genes in the chromosome 11 interval with liver expression

ENSEMBL.ID	term	estimate	std.error	statistic	p.value	p.adj	symbol
ENSMUSG00000004394	chol1	-0.015	0.006	-2.495	0.014	0.014	Tmed4
ENSMUSG00000002741	chol1	-0.012	0.008	-1.433	0.155	0.155	Ykt6
ENSMUSG00000025153	chol1	-0.903	0.657	-1.374	0.173	0.173	Fasn
ENSMUSG00000021670	chol1	0.106	0.094	1.121	0.265	0.265	Hmgcr
ENSMUSG00000028240	chol1	0.102	0.144	0.712	0.478	0.478	Cyp7a1
ENSMUSG00000032193	chol1	-0.017	0.045	-0.387	0.700	0.700	Ldlr
ENSMUSG00000020484	chol1	0.022	0.083	0.272	0.786	0.786	Xbp1
ENSMUSG00000041798	chol1	-0.015	0.056	-0.262	0.794	0.794	Gck

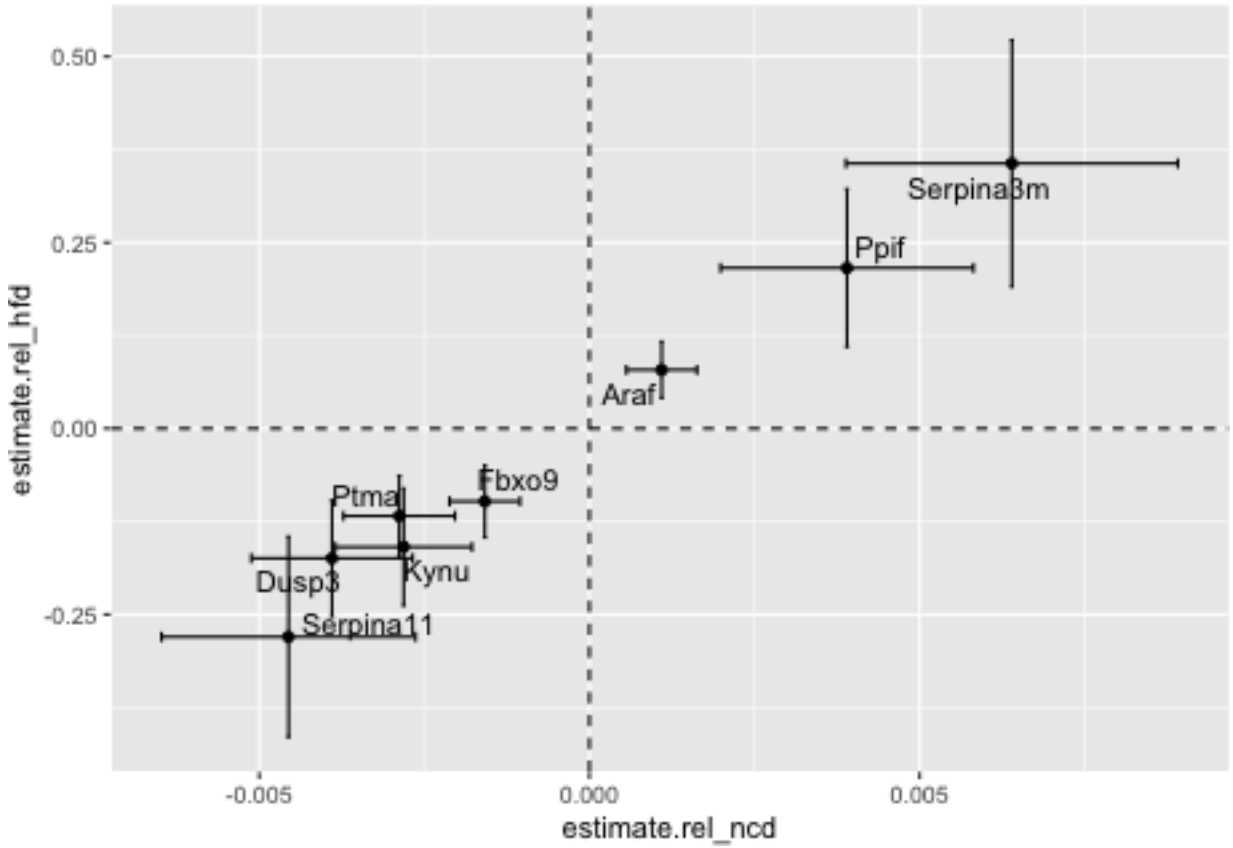
8 Integrated TWAS Analysis

```
combined.twas.data <-
  full_join(twas.data.ncd, twas.data.hf, by=c('ENSEMBL.ID','term','symbol'), suffix=c('_ncd','_hfd'))

combined.twas.data.all <-
  full_join(twas.data.ncd.all, twas.data.hf.all, by=c('ENSEMBL.ID','symbol'), suffix=c('_ncd','_hfd'))

library(ggplot2)
library(ggrepel)
ggplot(combined.twas.data,
       aes(y=estimate_hfd, x=estimate_ncd,
           xmin=estimate_ncd-std.error_ncd, xmax=estimate_ncd+std.error_ncd,
           ymin=estimate_hfd-std.error_hfd, ymax=estimate_hfd+std.error_hfd )) +
  geom_point() +
  #geom_errorbar() +
  #geom_errorbarh() +
  geom_hline(yintercept = 0, lty=2) +
```

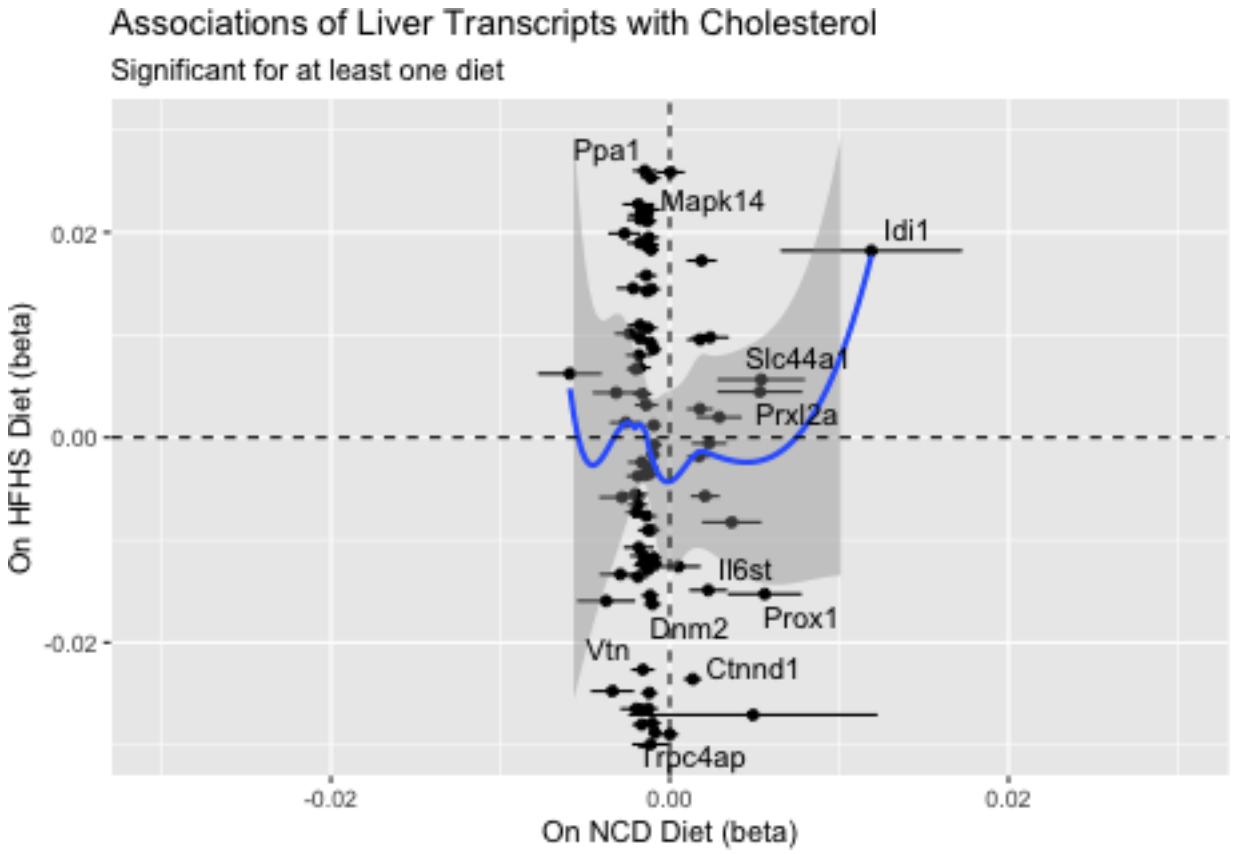
```
  geom_vline(xintercept = 0, lty=2) +
  geom_label_repel(aes(label=symbol),
                   data = subset(combined.twas.data, abs(estimate_hfd) > 2|abs(estimate_ncd) > 2))
```



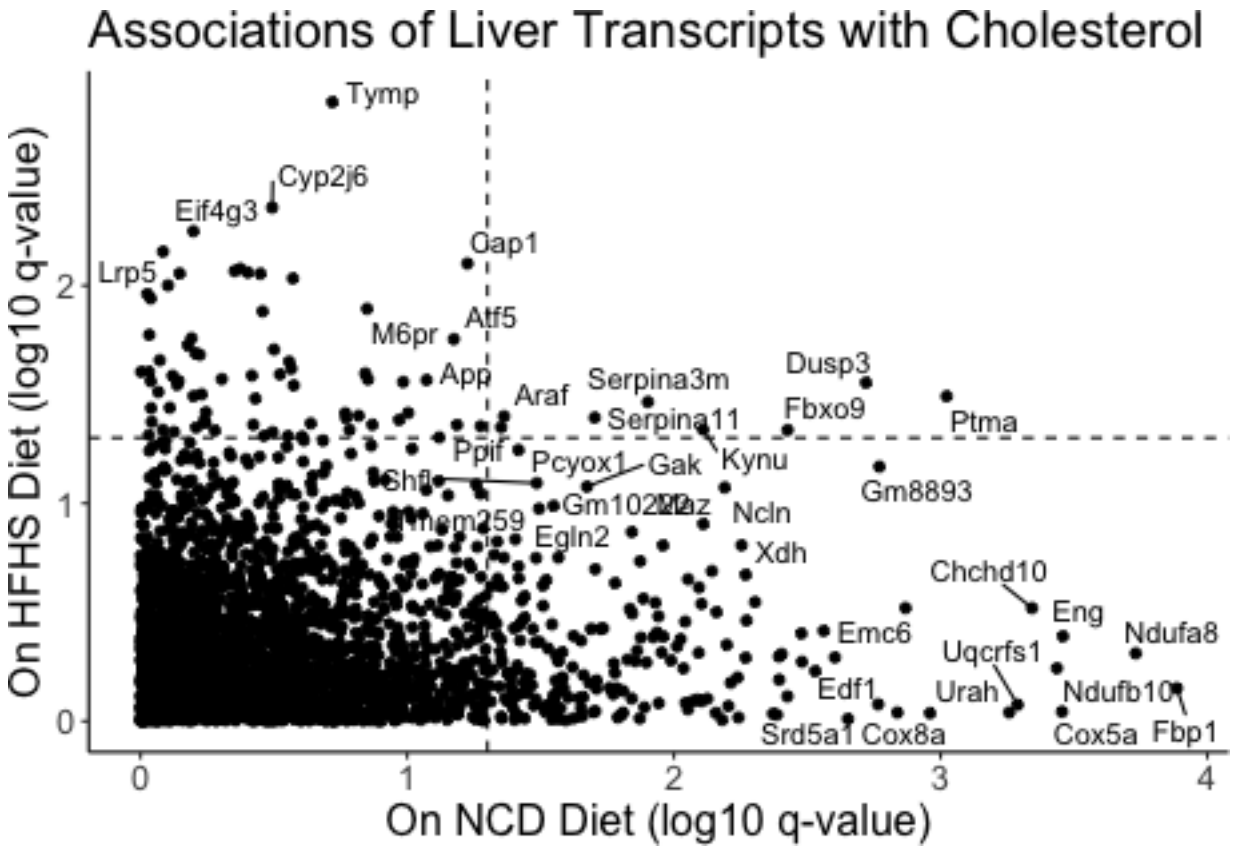

```
sig.genes <- filter(combined.twas.data, p.adj_ncd<0.05&p.adj_hfd<0.05) %>% pull(symbol)
ggplot(combined.twas.data.all %>% filter(symbol %in% sig.genes),
       aes(y=estimate.rel_hfd, x=estimate.rel_ncd,
           xmin=estimate.rel_ncd-std.error.rel_ncd,
           xmax=estimate.rel_ncd+std.error.rel_ncd,
           ymin=estimate.rel_hfd-std.error.rel_hfd,
           ymax=estimate.rel_hfd+std.error.rel_hfd )) +
  geom_point() +
  geom_errorbar() +
  geom_errorbarh() +
  geom_hline(yintercept = 0, lty=2) +
  geom_vline(xintercept = 0, lty=2) +
  geom_text_repel(aes(label=symbol))
```




```
sig.genes <- filter(combined.twas.data, p.adj_ncd<0.05|p.adj_hfd<0.05) %>% pull(symbol)
ggplot(combined.twas.data.all %>% filter(symbol %in% sig.genes),
       aes(y=estimate.rel_hfd, x=estimate.rel_ncd,
           xmin=estimate.rel_ncd-std.error.rel_ncd,
           xmax=estimate.rel_ncd+std.error.rel_ncd,
           ymin=estimate.rel_hfd-std.error.rel_hfd,
           ymax=estimate.rel_hfd+std.error.rel_hfd )) +
  geom_point() +
  geom_errorbar() +
  geom_errorbarh() +
  geom_smooth() +
  geom_hline(yintercept = 0, lty=2) +
  geom_vline(xintercept = 0, lty=2) +
  xlim(-0.03,0.03) +
  ylim(-0.03,0.03) +
  geom_text_repel(aes(label=symbol)) +
  labs(y="On HFHS Diet (beta)",
       x="On NCD Diet (beta)",
       title="Associations of Liver Transcripts with Cholesterol",
       subtitle="Significant for at least one diet")
```




```
cutoff <- -log10(0.05)
ggplot(combined.twas.data,
       aes(y=-log10(p.adj_hfd), x=-log10(p.adj_ncd) )) +
  geom_point() +
  #geom_errorbar() +
  #geom_errorbarh() +
  geom_hline(yintercept = -log10(0.05), lty=2) +
  geom_vline(xintercept = -log10(0.05), lty=2) +
  geom_text_repel(aes(label=symbol),
                  data = subset(combined.twas.data, -log10(p.adj_hfd) > cutoff|-log10(p.adj_ncd) > cut
  labs(y="On HFHS Diet (log10 q-value)",
       x="On NCD Diet (log10 q-value)",
       title="Associations of Liver Transcripts with Cholesterol") +
  theme_classic() +
  theme(text=element_text(size=16))
```



Comparason with human GWAS

Downloaded human cholesterol associated alleles from https://t2d.hugeamp.org/phenotype.html?phenotype=CHOL

"'{ chol-human-gwas} gwas.filename <- 'cholesterol-associations.csv' gwas.data <- read_csv(gwas.filename) %>% mutate(Symbol=gsub('.{2}$', '', nearest)) %>% mutate(Symbol=gsub("^.{0,2}", "",Symbol)) #removes first and last characters

library(biomaRt) human = useMart("ensembl", dataset = "hsapiens_gene_ensembl") mouse = useMart("ensembl", dataset = "mmusculus_gene_ensembl")

mapping.data <- getLDS(attributes = c("hgnc_symbol"), filters = "hgnc_symbol", values = gwas.data$Symbol , mart = human, attributesL = c("mgi_symbol"), martL = mouse, uniqueRows=T)

gwas.data <- full_join(gwas.data, mapping.data, by=c('Symbol'='HGNC.symbol')) %>% dplyr::filter(!(is.na(MGI.symbol)))

#are gwas alleles enriched in correlation analyses

sig.twas.data <- filter(twas.data, p.value<0.05) #sig.twas.data*symbol*MGI.symbol %>% table

combined.twas.data %>% filter(symbol %in% gwas.data$MGI.symbol) %>% arrange(p.value) %>% head %>% kable(caption="Most significant TWAS association hits that are also nearby GWAS hits")

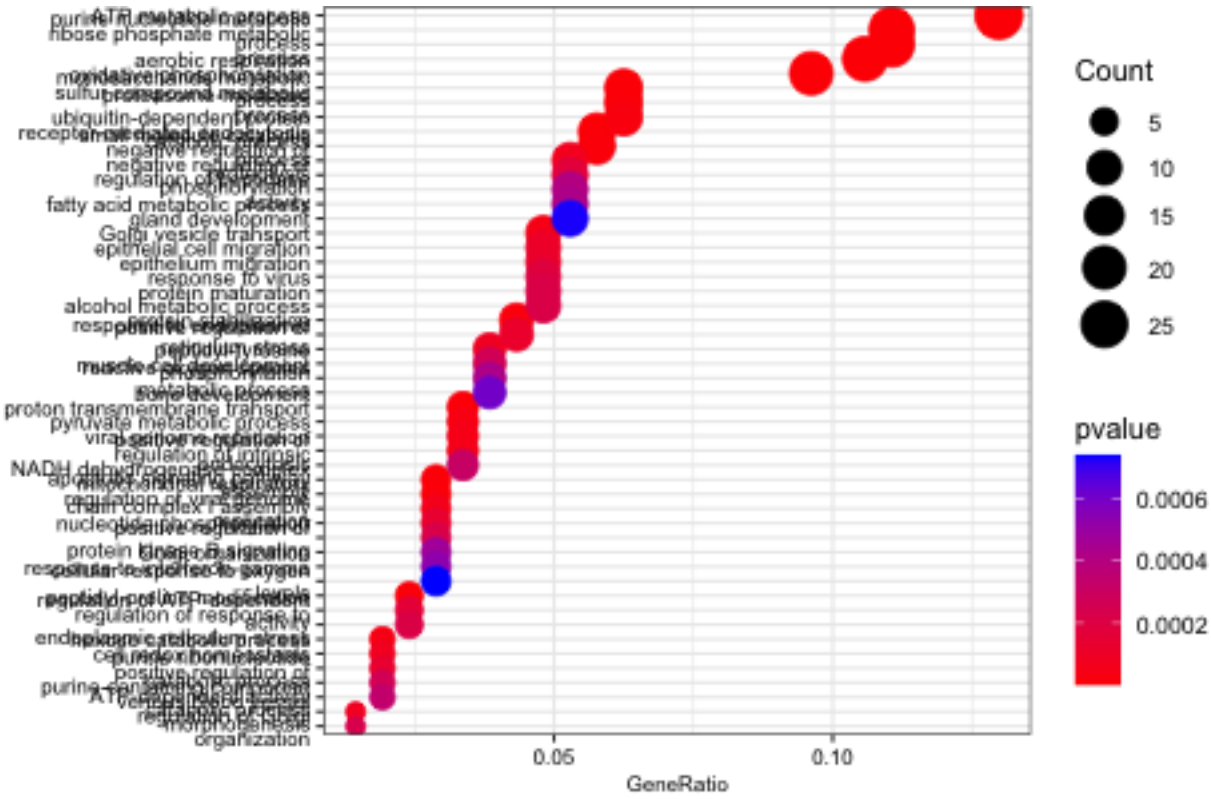
#checked if a TWAS was a GWAS hit twas.data.matched <- combined.twas.data %>% mutate(hGWAS.match=symbol %in% gwas.data$MGI.symbol)

with(twas.data.matched, table(hGWAS.match,p.value<0.05)) %>% fisher.test() %>% tidy %>% kable(caption='Fisher test for enrichment of GWAS hits in liver TWAS genes')

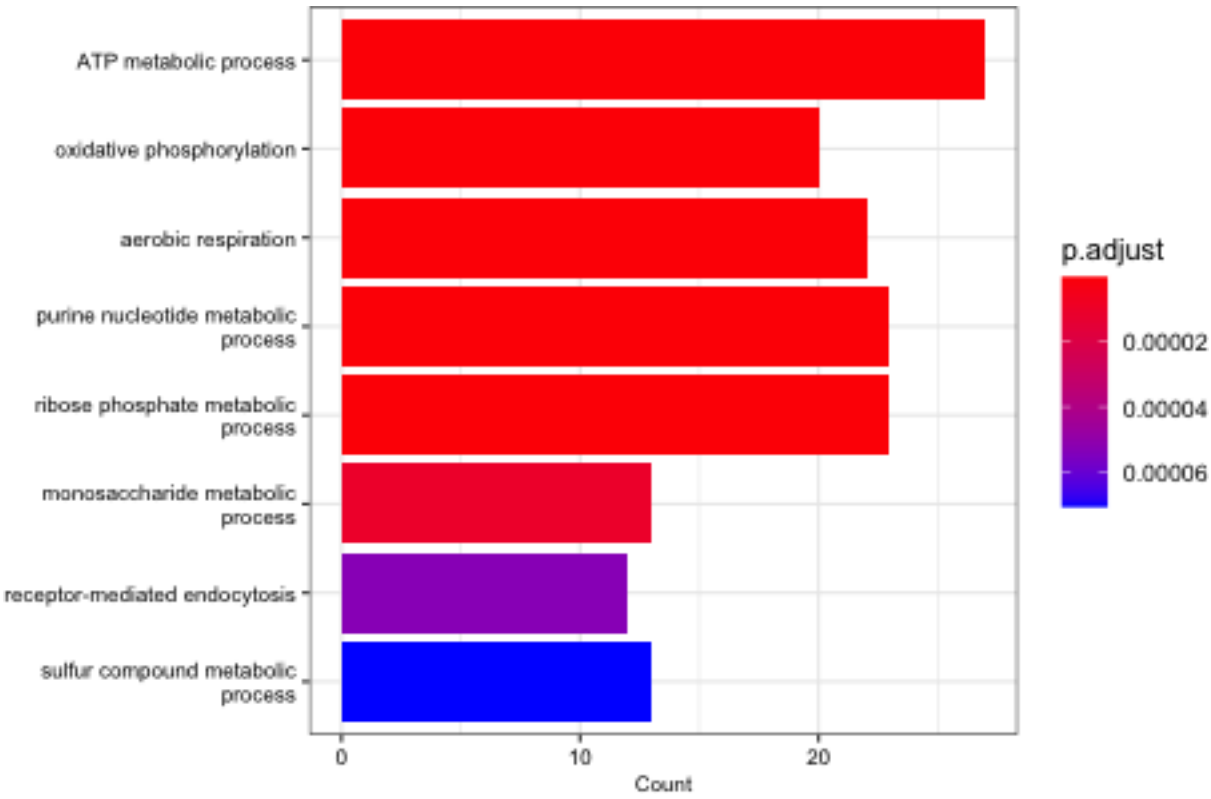
glm(hGWAS.match~p.value, data=twas.data.matched, family='binomial') %>% tidy %>% kable(caption="Logistic regression of TWAS values against likilihood of a GWAS hit.")

Pathway Analyses for NCD

```r
twas.list <- twas.data.ncd %>% arrange(-estimate) %>% pull(estimate)
names(twas.list) <- twas.data.ncd %>% arrange(-estimate) %>% pull(symbol)
twas.list <- sort(twas.list, decreasing = TRUE)
#twas.list <- twas.list[!(is.na(names(twas.list)))]


library(clusterProfiler)
go.twas.bp <- gseGO(geneList=twas.list,
               ont="BP",
               keyType='SYMBOL',
               OrgDb=org.Mm.eg.db,
               pvalueCutoff=0.25,
               verbose=T,
               by='fgsea',
               eps=1E-25)

#enrichement
twas.data.ncd %>%
  filter(p.value<0.05) %>%
  pull(symbol) ->
  twas.sig

go.twas.bp.enrich <- enrichGO(gene=twas.sig,
               ont="BP",
               keyType='SYMBOL',
               OrgDb=org.Mm.eg.db,
               pvalueCutoff=0.05)

go.twas.bp.enrich.simpl <- simplify(go.twas.bp.enrich,
                            cutoff=0.7,
                            by="p.adjust",
                            select_fun=min)

library(enrichplot)

dotplot(go.twas.bp.enrich.simpl,
        showCategory=50,
        color='pvalue',
        font.size=8)
```




```
barplot(go.twas.bp.enrich.simpl,font.size=8)
```

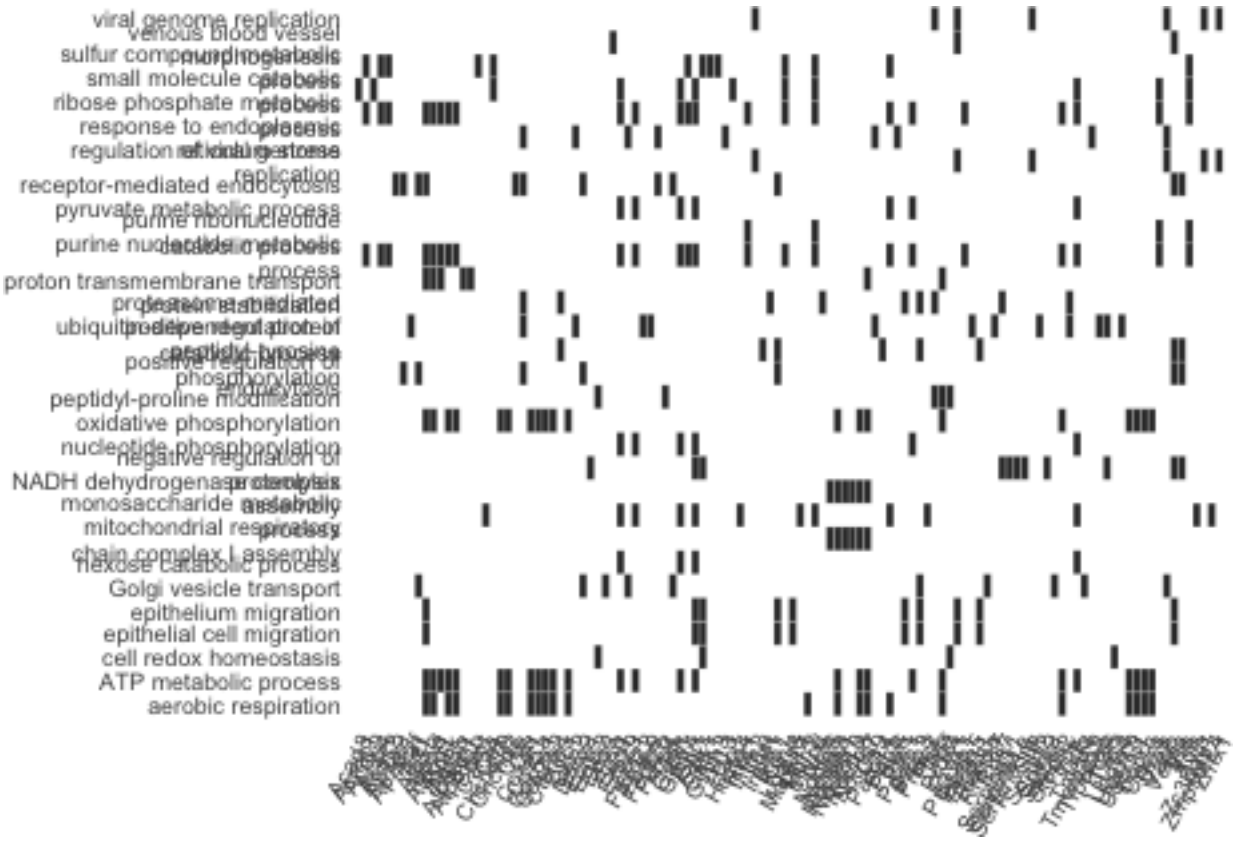



```
cnetplot(go.twas.bp.enrich.simpl,
```

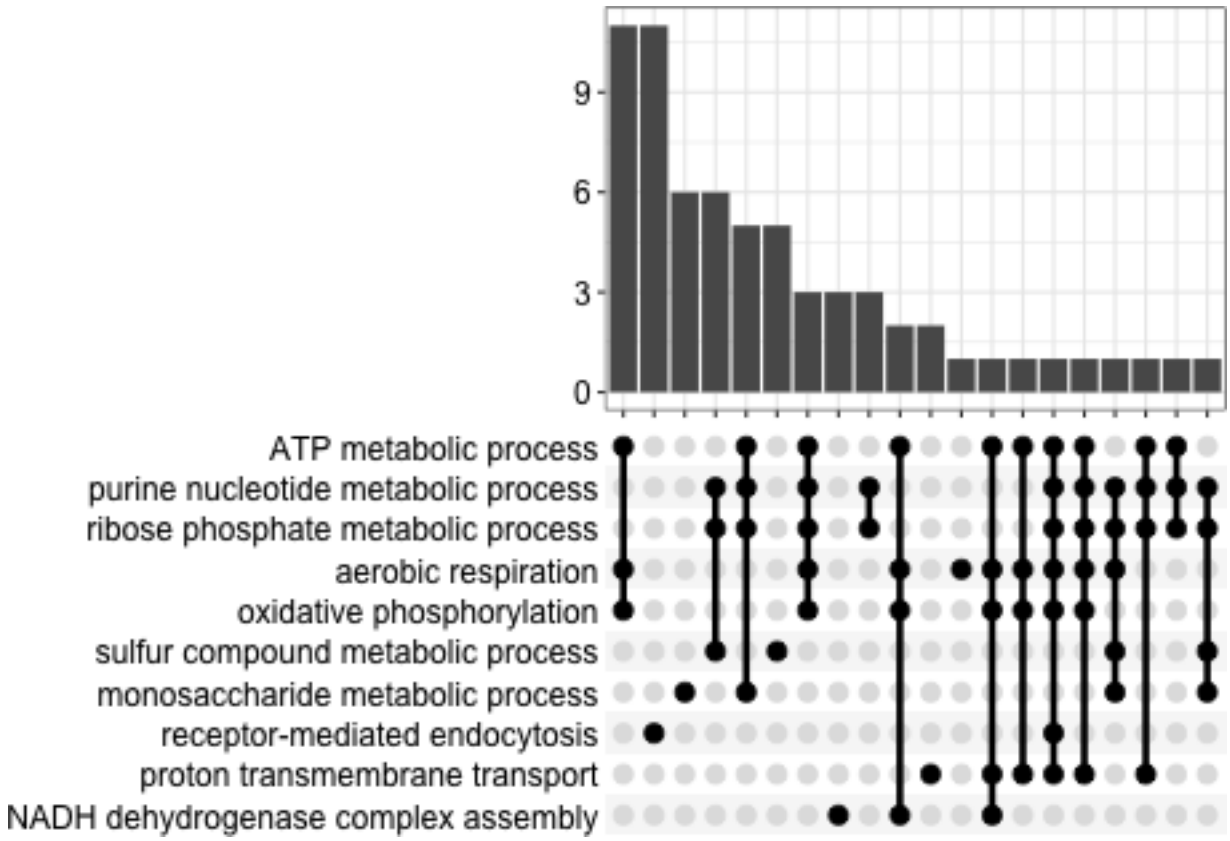
```
        showCategory=100,
        node_label="gene",
        cex_label_gene=0.5)
```


```
cnetplot(go.twas.bp.enrich.simpl,
        showCategory=100,
        node_label="category",
        cex_label_category=0.5)
```

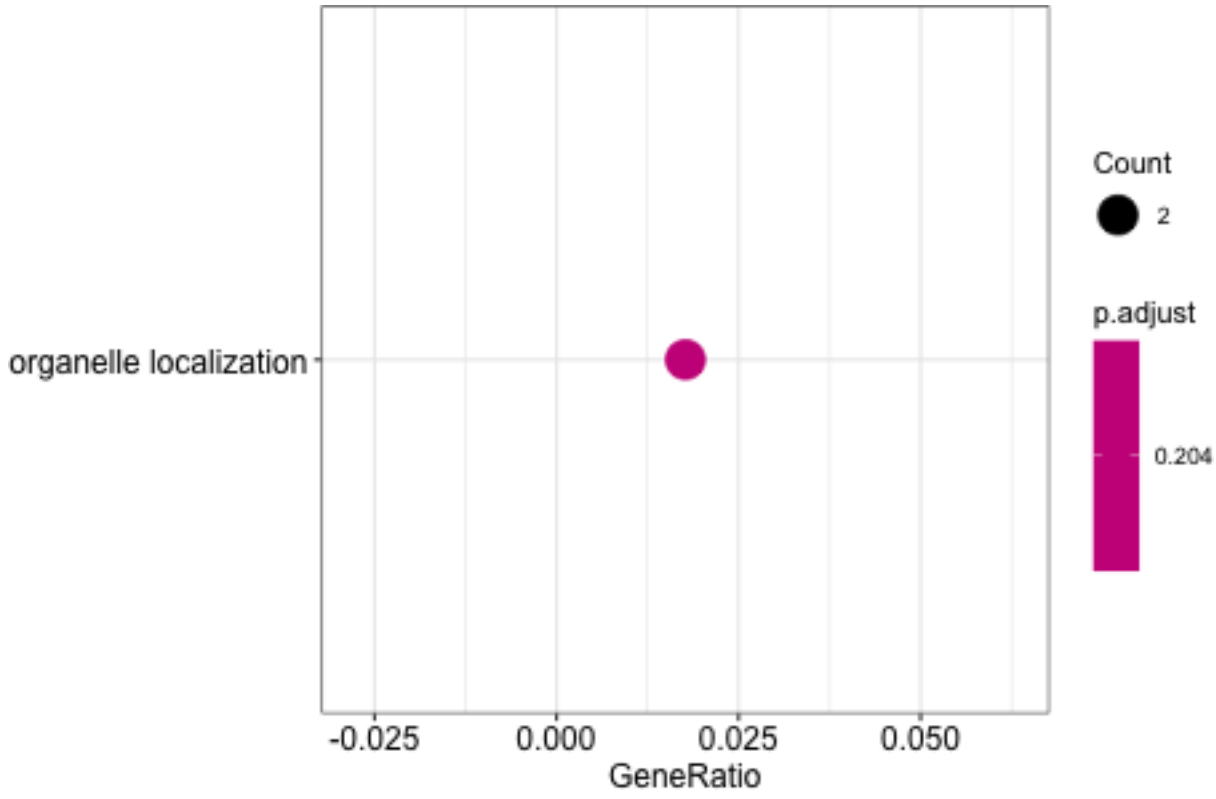

```
heatplot(go.twas.bp.enrich.simpl)
```



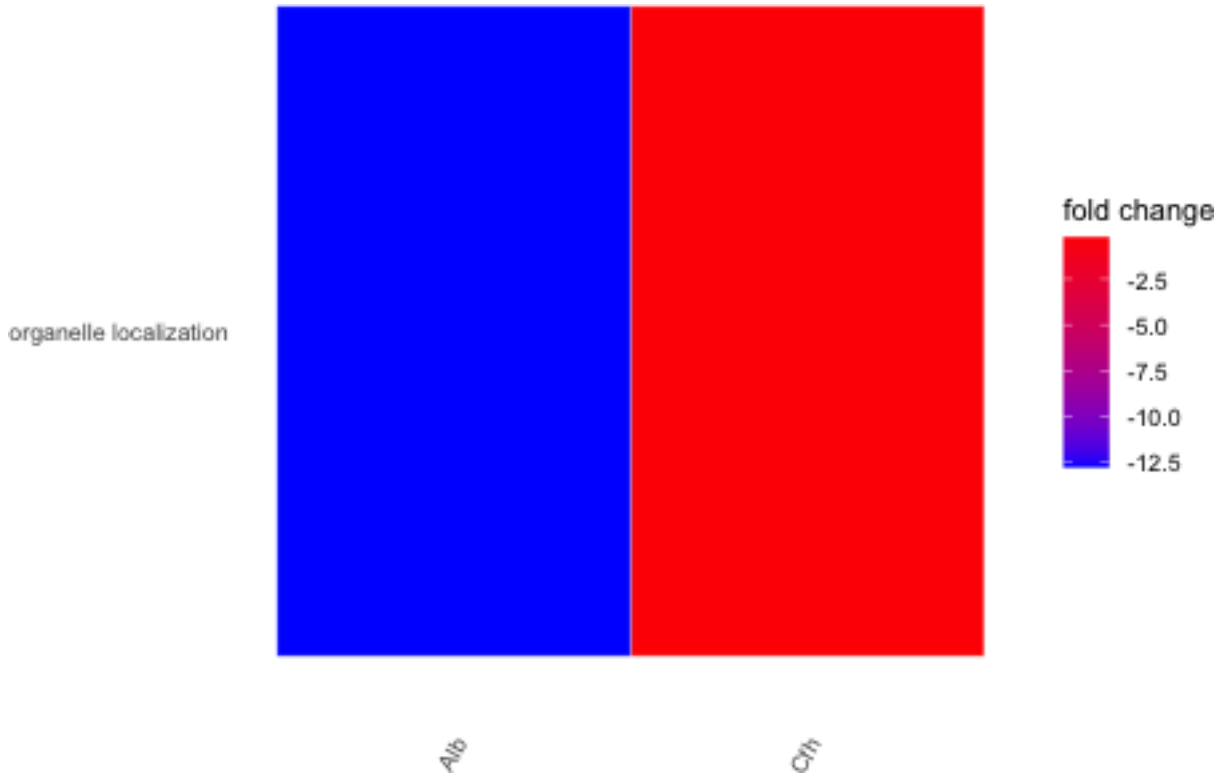

```
library(ggupset)
upsetplot(go.twas.bp.enrich.simpl)
```



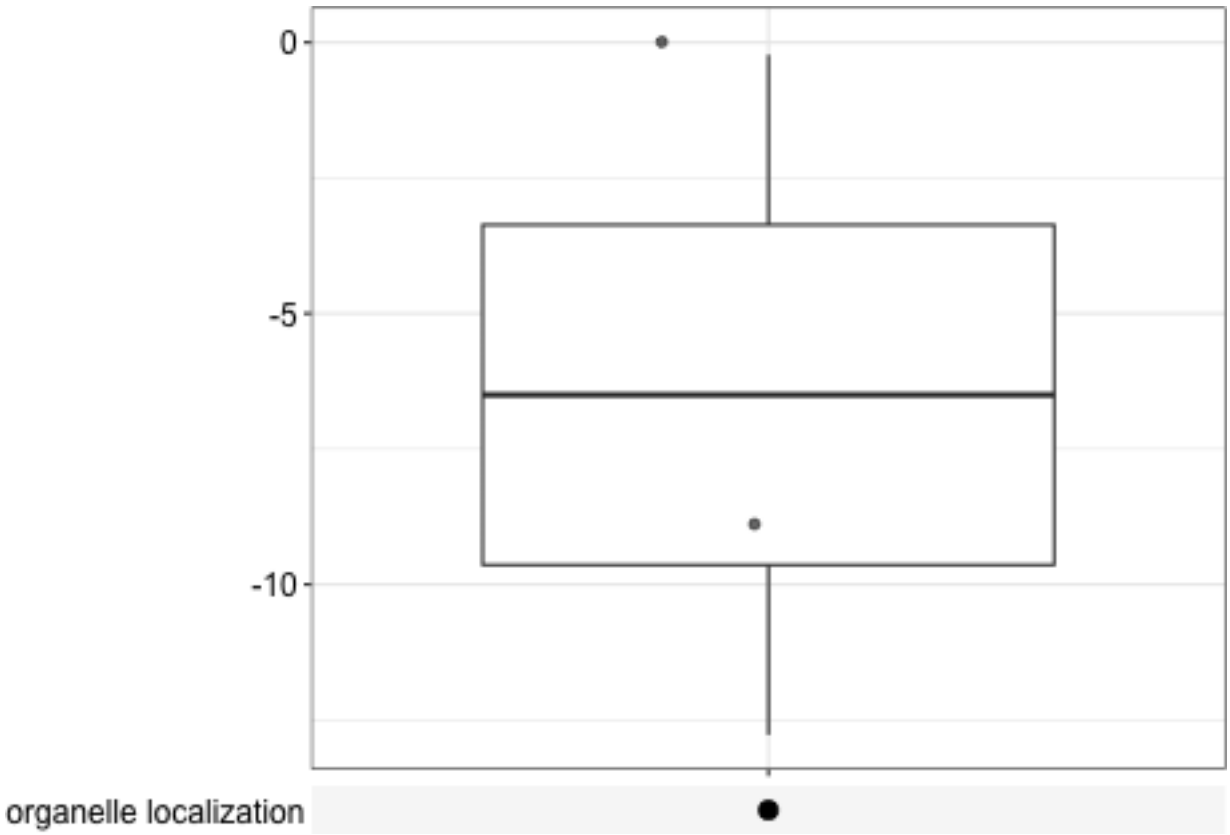

```
dotplot(go.twas.bp, showCategory=30)
```



```
heatplot(go.twas.bp, foldChange=twas.list)
```




```
upsetplot(go.twas.bp)
```




```
as.data.frame(go.twas.bp) %>% select("Description","setSize","enrichmentScore","NES","pvalue","p.adjust
  kable(caption="NCD GO BP Analysis of TWAS Associations from GSEA")
```

Table 10: NCD GO BP Analysis of TWAS Associations from GSEA

	Description	setSize	enrichmentScore	NES	pvalue	p.adjust
GO:0051640	organelle localization	113	-0.874	-1.79	0	0.204

```
as.data.frame(go.twas.bp) %>%
  write_csv(file="NCD TWAS GO-BP GSEA.csv")

as.data.frame(go.twas.bp.enrich.simpl) %>% select("Description","GeneRatio","Count","pvalue","p.adjust"
  kable(caption="NCD GO BP Analysis of TWAS Associations from Enrichment")
```

Table 11: NCD GO BP Analysis of TWAS Associations from Enrichment

	Description	GeneRatio	Count	pvalue	p.adjust
GO: 0046034	ATP metabolic process	27/208	27	0.000	0.000
GO: 0006119	oxidative phosphorylation	20/208	20	0.000	0.000
GO: 0009060	aerobic respiration	22/208	22	0.000	0.000
GO: 0006163	purine nucleotide metabolic process	23/208	23	0.000	0.000

	Description	GeneRatio	Count	pvalue	p.adjust
GO: 0019693	ribose phosphate metabolic process	23/208	23	0.000	0.000
GO: 0005996	monosaccharide metabolic process	13/208	13	0.000	0.000
GO: 0006898	receptor-mediated endocytosis	12/208	12	0.000	0.000
GO: 0006790	sulfur compound metabolic process	13/208	13	0.000	0.000
GO: 1902600	proton transmembrane transport	7/208	7	0.000	0.000
GO: 0010257	NADH dehydrogenase complex assembly	6/208	6	0.000	0.000
GO: 0032981	mitochondrial respiratory chain complex I assembly	6/208	6	0.000	0.000
GO: 0044282	small molecule catabolic process	12/208	12	0.000	0.000
GO: 0018208	peptidyl-proline modification	5/208	5	0.000	0.000
GO: 0050821	protein stabilization	9/208	9	0.000	0.001
GO: 0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	13/208	13	0.000	0.001
GO: 0006090	pyruvate metabolic process	7/208	7	0.000	0.001
GO: 0019320	hexose catabolic process	4/208	4	0.000	0.002
GO: 0048193	Golgi vesicle transport	10/208	10	0.000	0.002
GO: 0019079	viral genome replication	7/208	7	0.000	0.002
GO: 0045807	positive regulation of endocytosis	7/208	7	0.000	0.002
GO: 0045069	regulation of viral genome replication	6/208	6	0.000	0.002
GO: 0045861	negative regulation of proteolysis	11/208	11	0.000	0.003
GO: 0045454	cell redox homeostasis	4/208	4	0.000	0.003
GO: 0048845	venous blood vessel morphogenesis	3/208	3	0.000	0.003
GO: 0050731	positive regulation of peptidyl-tyrosine phosphorylation	8/208	8	0.000	0.003
GO: 0010631	epithelial cell migration	10/208	10	0.000	0.004
GO: 0090132	epithelium migration	10/208	10	0.000	0.004
GO: 0034976	response to endoplasmic reticulum stress	9/208	9	0.000	0.004
GO: 0009154	purine ribonucleotide catabolic process	4/208	4	0.000	0.004
GO: 0046939	nucleotide phosphorylation	6/208	6	0.000	0.004

	Description	GeneRatio	Count	pvalue	p.adjust
GO: 0043462	regulation of ATP-dependent activity	5/208	5	0.000	0.005
GO: 0042326	negative regulation of phosphorylation	11/208	11	0.000	0.005
GO: 0009615	response to virus	10/208	10	0.000	0.005
GO: 1905897	regulation of response to endoplasmic reticulum stress	5/208	5	0.000	0.006
GO: 0051604	protein maturation	10/208	10	0.000	0.006
GO: 0006066	alcohol metabolic process	10/208	10	0.000	0.006
GO: 0051897	positive regulation of protein kinase B signaling	6/208	6	0.000	0.006
GO: 1903358	regulation of Golgi organization	3/208	3	0.000	0.006
GO: 0032781	positive regulation of ATP-dependent activity	4/208	4	0.000	0.007
GO: 0055001	muscle cell development	8/208	8	0.000	0.007
GO: 2001242	regulation of intrinsic apoptotic signaling pathway	7/208	7	0.000	0.008
GO: 0072523	purine-containing compound catabolic process	4/208	4	0.000	0.008
GO: 0052547	regulation of peptidase activity	11/208	11	0.000	0.010
GO: 0006631	fatty acid metabolic process	11/208	11	0.000	0.010
GO: 0072593	reactive oxygen species metabolic process	8/208	8	0.000	0.010
GO: 0007030	Golgi organization	6/208	6	0.000	0.011
GO: 0034341	response to interferon-gamma	6/208	6	0.001	0.012
GO: 0060348	bone development	8/208	8	0.001	0.013
GO: 0048732	gland development	11/208	11	0.001	0.015
GO: 0071453	cellular response to oxygen levels	6/208	6	0.001	0.015
GO: 0006979	response to oxidative stress	10/208	10	0.001	0.017
GO: 0030970	retrograde protein transport, ER to cytosol	3/208	3	0.001	0.017
GO: 1903513	endoplasmic reticulum to cytosol transport	3/208	3	0.001	0.017
GO: 0010634	positive regulation of epithelial cell migration	6/208	6	0.001	0.019
GO: 0001666	response to hypoxia	7/208	7	0.001	0.020
GO: 0001933	negative regulation of protein phosphorylation	9/208	9	0.001	0.021

	Description	GeneRatio	Count	pvalue	p.adjust
GO: 0022411	cellular component disassembly	10/208	10	0.001	0.021
GO: 0043254	regulation of protein-containing complex assembly	10/208	10	0.001	0.022
GO: 0045862	positive regulation of proteolysis	9/208	9	0.001	0.022
GO: 0021549	cerebellum development	5/208	5	0.001	0.023
GO: 0006641	triglyceride metabolic process	5/208	5	0.001	0.024
GO: 0001936	regulation of endothelial cell proliferation	6/208	6	0.001	0.024
GO: 0051346	negative regulation of hydrolase activity	9/208	9	0.001	0.025
GO: 0007565	female pregnancy	6/208	6	0.002	0.026
GO: 0006734	NADH metabolic process	3/208	3	0.002	0.027
GO: 0045446	endothelial cell differentiation	5/208	5	0.002	0.028
GO: 0002064	epithelial cell development	7/208	7	0.002	0.028
GO: 0150117	positive regulation of cell-substrate junction organization	3/208	3	0.002	0.029
GO: 0097193	intrinsic apoptotic signaling pathway	8/208	8	0.002	0.031
GO: 0035335	peptidyl-tyrosine dephosphorylation	3/208	3	0.002	0.031
GO: 0071466	cellular response to xenobiotic stimulus	6/208	6	0.002	0.033
GO: 0001935	endothelial cell proliferation	6/208	6	0.002	0.034
GO: 0006735	NADH regeneration	2/208	2	0.002	0.034
GO: 0061718	glucose catabolic process to pyruvate	2/208	2	0.002	0.034
GO: 0070862	negative regulation of protein exit from endoplasmic reticulum	2/208	2	0.002	0.034
GO: 0032456	endocytic recycling	4/208	4	0.002	0.034
GO: 0045927	positive regulation of growth	8/208	8	0.002	0.037
GO: 1901361	organic cyclic compound catabolic process	10/208	10	0.003	0.038
GO: 0009410	response to xenobiotic stimulus	8/208	8	0.003	0.038
GO: 0048638	regulation of developmental growth	9/208	9	0.003	0.038
GO: 0002176	male germ cell proliferation	2/208	2	0.003	0.038
GO: 0036302	atrioventricular canal development	2/208	2	0.003	0.038

	Description	GeneRatio	Count	pvalue	p.adjust
GO: 0045050	protein insertion into ER membrane by stop-transfer membrane-anchor sequence	2/208	2	0.003	0.038
GO: 0045602	negative regulation of endothelial cell differentiation	2/208	2	0.003	0.038
GO: 0060020	Bergmann glial cell differentiation	2/208	2	0.003	0.038
GO: 0061635	regulation of protein complex stability	2/208	2	0.003	0.038
GO: 0090160	Golgi to lysosome transport	2/208	2	0.003	0.038
GO: 0003158	endothelium development	5/208	5	0.003	0.038
GO: 0031623	receptor internalization	5/208	5	0.003	0.042
GO: 0008202	steroid metabolic process	8/208	8	0.003	0.043
GO: 0032527	protein exit from endoplasmic reticulum	3/208	3	0.003	0.044
GO: 0010811	positive regulation of cell-substrate adhesion	5/208	5	0.004	0.045
GO: 0044706	multi-multicellular organism process	6/208	6	0.004	0.046
GO: 0006575	cellular modified amino acid metabolic process	6/208	6	0.004	0.046
GO: 0048675	axon extension	5/208	5	0.004	0.047
GO: 0009120	deoxyribonucleoside metabolic process	2/208	2	0.004	0.047
GO: 0033627	cell adhesion mediated by integrin	4/208	4	0.004	0.049
GO: 0044270	cellular nitrogen compound catabolic process	9/208	9	0.004	0.050

```
as.data.frame(go.twas.bp.enrich.simpl) %>%
  write_csv(file="NCD TWAS GO-BP Enrichment.csv")

steroid.enrichment <- as.data.frame(go.twas.bp) %>% filter(ID=='GO:0016126')
```

The pathway () was enriched with significant TWAS associations (p=). The genes that were part of the core enrichment of this pathway were .

8.1 Strongest Associations

“‘{ strongest-associations} library(ggplot2) genes <- twas.data.ncd.combined %>% arrange(p.value_main) %>% filter(is.na(p.value_main)) %>% filter(!(is.na(symbol))) %>% head(6) %>% pull(symbol) p <- list()

for(i in 1:6){ gene <- genes[i] gene.ens <- filter(twas.data.ncd.int, symbol==genes[i]) %>% pull(ENSEMBL.ID)

p[[i]] <- expression.data %>% filter(ENSEMBL.ID == gene.ens) %>% pivot_longer(cols=c(starts_with(‘F’), starts_with(‘M’)), names_to=‘sample’, values_to=‘expression’) %>% full_join(phenotype.data,by=‘sample’) %>% filter(!is.na(diet)) %>% ggplot(aes(y=chol1,expression,col=sex)) + geom_point(size=0.1) +

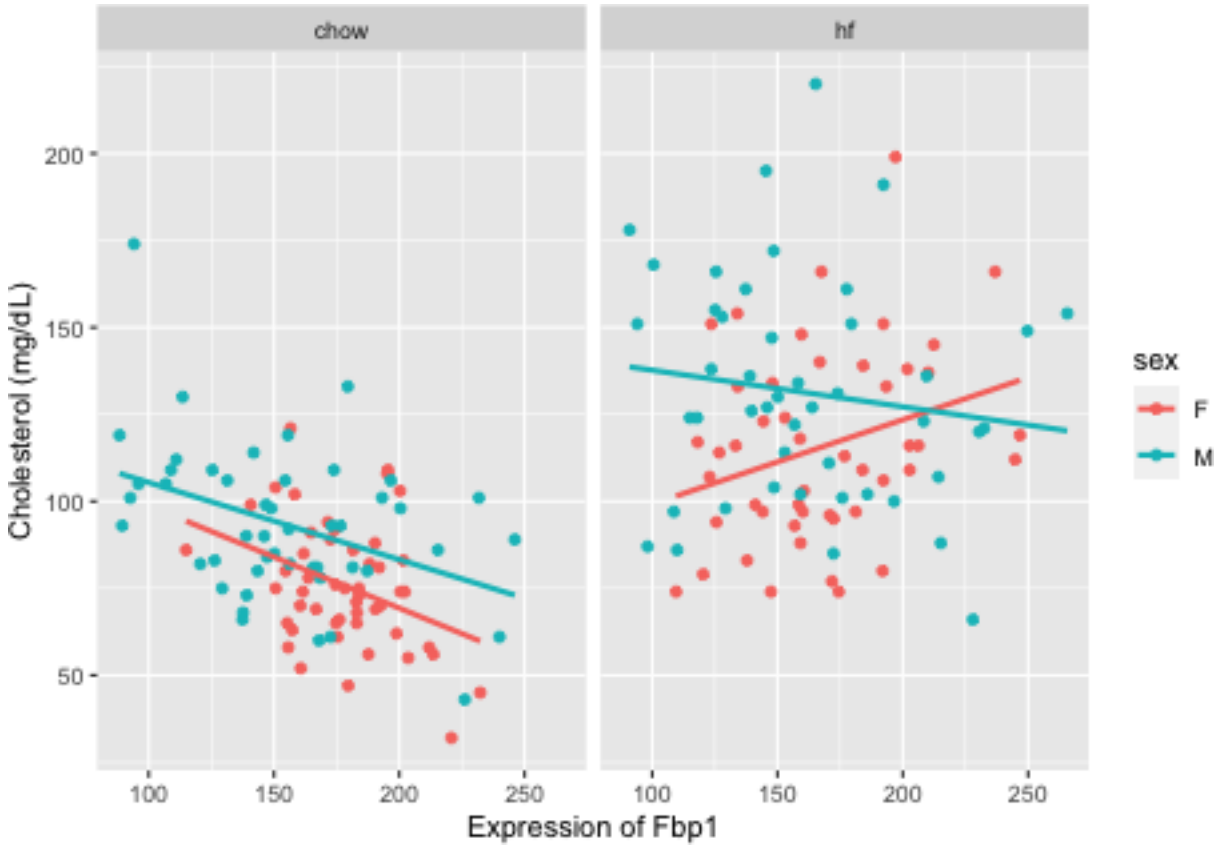
geom_smooth(method='lm',se=F) + facet_grid(~diet) + labs(y="Cholesterol (mg/dL)", x=paste('Expression of', gene, sep="")) + theme(text=element_text(size=8), legend.position ="none") } library(gridExtra) do.call(grid.arrange,p)

Strongest Interactions Between Expression and Sex

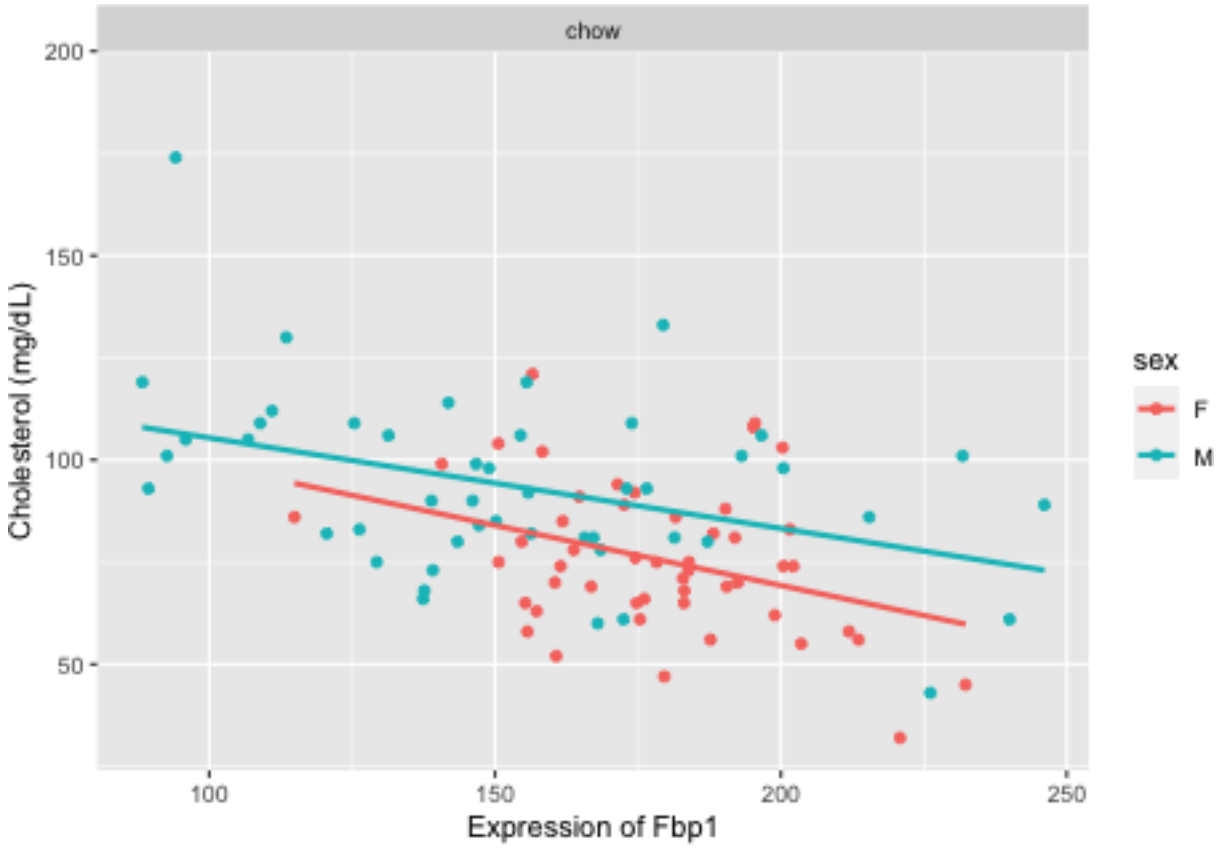
```{ strongest-interactions}
genes <- twas.data.ncd.combined %>%
  arrange(p.value_int) %>%
  filter(!(is.na(symbol))) %>%
  head(6) %>%
  pull(symbol)
p <- list()

for(i in 1:6){
  gene <- genes[i]
  gene.ens <- filter(twas.data.ncd.int, symbol==genes[i]) %>% pull(ENSEMBL.ID)

p[[i]] <- expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  ggplot(aes(y=chol1,expression,col=sex)) +
  geom_point(size=0.1) +
  geom_smooth(method='lm',se=F) +
  facet_grid(~diet) +
  labs(y="Cholesterol (mg/dL)",
       x=paste('Expression of ', gene, sep="")) +
  theme(text=element_text(size=8),
        legend.position = "none")
}
library(gridExtra)
do.call(grid.arrange,p)
```

```
gene <- 'Fbp1'
gene.ens <- filter(twas.data.ncd, symbol==gene) %>% pull(ENSEMBL.ID)
library(ggplot2)

expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  ggplot(aes(y=chol1,expression,col=sex)) +
  geom_point() +
  geom_smooth(method='lm',se=F) +
  facet_grid(~diet) +
```

```
  labs(y="Cholesterol (mg/dL)",
       x=paste('Expression of ', gene, sep=""))
```




```
expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  filter(diet=='chow') %>%
  ggplot(aes(y=chol1,expression,col=sex)) +
  geom_point() +
  geom_smooth(method='lm',se=F) +
  facet_grid(~diet) +
  labs(y="Cholesterol (mg/dL)",
       x=paste('Expression of ', gene, sep=""))
```



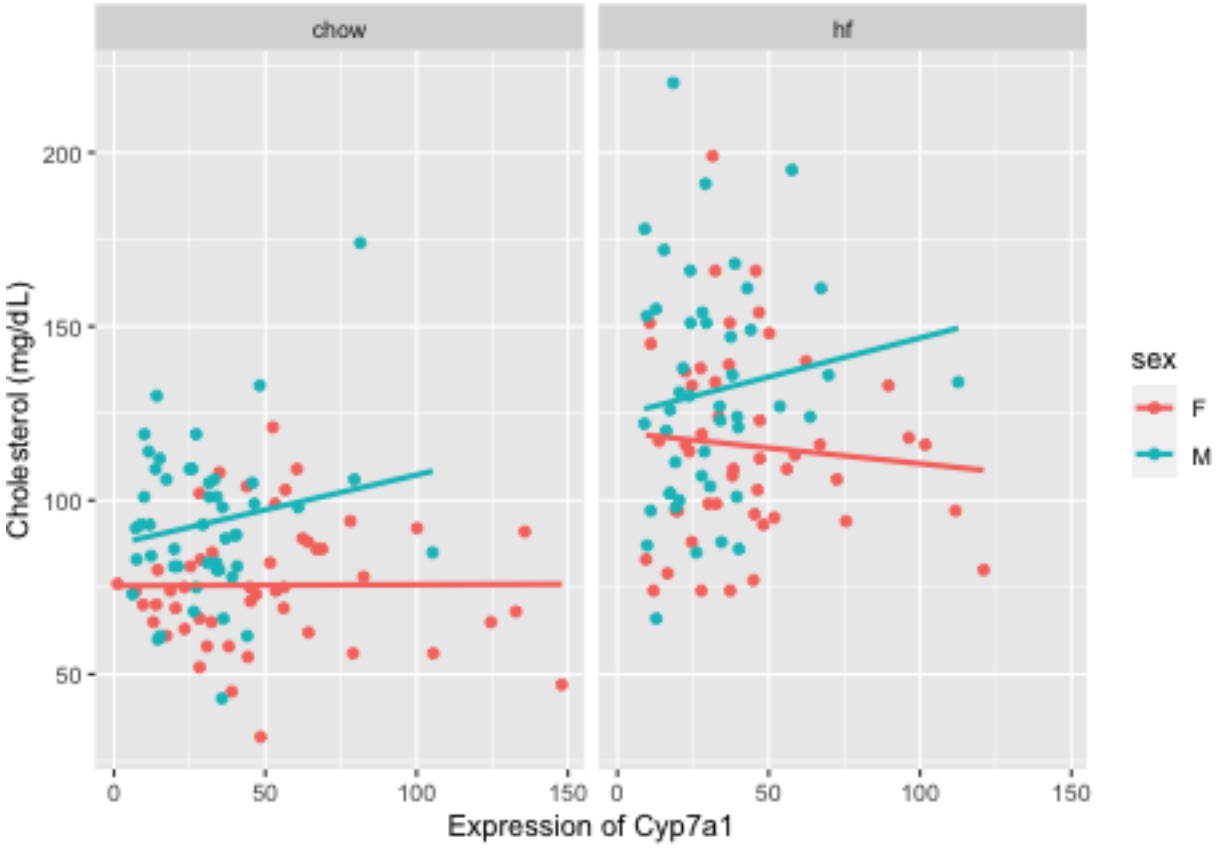

```
expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  filter(diet=='chow') -> fbp1.chow.data

lm(data=fbp1.chow.data, chol1 ~ expression + sex) %>%
  glance
```

```
## # A tibble: 1 x 12
##   r.squ~1 adj.r~2 sigma stati~3 p.value    df logLik   AIC   BIC devia~4 df.re~5
##     <dbl>   <dbl> <dbl>   <dbl>   <dbl> <dbl>  <dbl> <dbl> <dbl>   <dbl>   <int>
## 1   0.287   0.272  18.7    19.1 1.07e-7     2  -424.  857.  867.  33158.      95
## # ... with 1 more variable: nobs <int>, and abbreviated variable names
## #   1: r.squared, 2: adj.r.squared, 3: statistic, 4: deviance, 5: df.residual
```

```
expression.data %>%
  filter(ENSEMBL.ID == gene) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) -> fbp.hf.data
```

9 Bile Acid Metabolism

```
gene <- 'Cyp7a1'
gene.ens <- filter(twas.data.ncd, symbol==gene) %>% pull(ENSEMBL.ID)

expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  ggplot(aes(y=chol1,expression,col=sex)) +
  geom_point() +
  geom_smooth(method='lm',se=F) +
  facet_grid(~diet) +
  labs(y="Cholesterol (mg/dL)",
       x=paste('Expression of ', gene, sep=""))
```




```
expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
```

```
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) %>%
  filter(diet=='chow') -> gene.chow.data

lm(data=gene.chow.data, chol1 ~ expression + sex) %>%
  tidy %>%
  kable(caption="Summary associations of Cyp7a1 and cholesterol on chow")
```

Table 12: Summary associations of Cyp7a1 and cholesterol on chow

term	estimate	std.error	statistic	p.value
(Intercept)	72.968	4.666	15.637	0.000
expression	0.052	0.073	0.712	0.478
sexM	18.851	4.320	4.364	0.000

```
expression.data %>%
  filter(ENSEMBL.ID == gene.ens) %>%
  pivot_longer(cols=c(starts_with('F'),
                      starts_with('M')),
               names_to='sample',
               values_to='expression') %>%
  full_join(phenotype.data,by='sample') %>%
  filter(!is.na(diet)) -> gene.hf.data

lm(data=gene.hf.data, chol1 ~ expression + sex) %>%
  tidy %>%
  kable(caption="Summary associations of Cyp7a1 and cholesterol on HFD")
```

Table 13: Summary associations of Cyp7a1 and cholesterol on HFD

term	estimate	std.error	statistic	p.value
(Intercept)	95.946	5.33	18.003	0.000
expression	-0.015	0.09	-0.171	0.865
sexM	16.476	4.86	3.391	0.001

10 Session Information

```
sessionInfo()
```

```
## R version 4.2.2 (2022-10-31)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur ... 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.2/Resources/lib/libRlapack.dylib
```

```
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] ggupset_0.3.0         enrichplot_1.16.2     clusterProfiler_4.4.4
##  [4] ggrepel_0.9.2         ggplot2_3.4.0         venneuler_1.1-3
##  [7] rJava_1.0-6           purrr_1.0.1           org.Mm.eg.db_3.15.0
## [10] AnnotationDbi_1.58.0  IRanges_2.30.1        S4Vectors_0.34.0
## [13] Biobase_2.56.0        BiocGenerics_0.42.0   broom_1.0.2
## [16] readr_2.1.3           dplyr_1.0.10          tidyr_1.2.1
## [19] knitr_1.41
##
## loaded via a namespace (and not attached):
##   [1] fgsea_1.22.0           colorspace_2.0-3       ggtree_3.4.4
##   [4] ellipsis_0.3.2         qvalue_2.28.0          XVector_0.36.0
##   [7] aplot_0.1.9            rstudioapi_0.14        farver_2.1.1
##  [10] graphlayouts_0.8.4     bit64_4.0.5            scatterpie_0.1.8
##  [13] fansi_1.0.3            codetools_0.2-18       splines_4.2.2
##  [16] cachem_1.0.6           GOSemSim_2.22.0        polyclip_1.10-4
##  [19] jsonlite_1.8.4         GO.db_3.15.0           png_0.1-8
##  [22] ggforce_0.4.1          compiler_4.2.2         httr_1.4.4
##  [25] backports_1.4.1        lazyeval_0.2.2         assertthat_0.2.1
##  [28] Matrix_1.5-3           fastmap_1.1.0          cli_3.6.0
##  [31] tweenr_2.0.2           htmltools_0.5.4        tools_4.2.2
##  [34] igraph_1.3.5           gtable_0.3.1           glue_1.6.2
##  [37] GenomeInfoDbData_1.2.8 reshape2_1.4.4         DO.db_2.9
##  [40] fastmatch_1.1-3        Rcpp_1.0.9             vctrs_0.5.1
##  [43] Biostrings_2.64.1      ape_5.6-2              nlme_3.1-161
##  [46] ggraph_2.1.0           xfun_0.36              stringr_1.5.0
##  [49] lifecycle_1.0.3        DOSE_3.22.1            zlibbioc_1.42.0
##  [52] MASS_7.3-58.1          scales_1.2.1           tidygraph_1.2.2
##  [55] vroom_1.6.0            hms_1.1.2              parallel_4.2.2
##  [58] RColorBrewer_1.1-3     yaml_2.3.6             memoise_2.0.1
##  [61] gridExtra_2.3          downloader_0.4         ggfun_0.0.9
##  [64] yulab.utils_0.0.6      stringi_1.7.12         RSQLite_2.2.20
##  [67] highr_0.10             tidytree_0.4.2         BiocParallel_1.30.4
##  [70] GenomeInfoDb_1.32.4    rlang_1.0.6            pkgconfig_2.0.3
##  [73] bitops_1.0-7           evaluate_0.19          lattice_0.20-45
##  [76] treeio_1.20.2          patchwork_1.1.2        labeling_0.4.2
##  [79] shadowtext_0.1.2       bit_4.0.5              tidyselect_1.2.0
##  [82] plyr_1.8.8             magrittr_2.0.3         R6_2.5.1
##  [85] magick_2.7.3           generics_0.1.3         DBI_1.1.3
##  [88] pillar_1.8.1           withr_2.5.0            mgcv_1.8-41
##  [91] KEGGREST_1.36.3        RCurl_1.98-1.9         tibble_3.1.8
##  [94] crayon_1.5.2           utf8_1.2.2             tzdb_0.3.0
##  [97] rmarkdown_2.19         viridis_0.6.2          grid_4.2.2
## [100] data.table_1.14.6      blob_1.2.3             digest_0.6.31
## [103] gridGraphics_0.5-1     munsell_0.5.0          viridisLite_0.4.1
## [106] ggplotify_0.1.0
```