GSReg: A Package for Gene Set Variability Analysis

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1 Introduction

The `GSReg` package allows to analyze pathways based on the variability of the expression of sets of genes that are targets of those pathways. Basing this set statistic on variability enables inference of dysregulated pathways in diseases, including notably cancers. The first set statistic for gene variability was in the work of Eddy and his colleagues (see [1]) which used a ranked based methodology called *DIRAC*. *DIRAC* calculates a measure of variability of the ordering of the expression of genes in a pathway for specific phenotype. The basic idea behind *DIRAC* is to generate a template for the pair-wise comparisons of gene expressions

of a pathway within a phenotype. DIRAC calculates a measure of the variability of the ordering within the phenotype, i.e. the expected distance of a sample from the phenotype and the template of the phenotype. In mathematical terms, if we denote denote two i.i.d. samples from the same phenotypes by X and X' and D Kendall-τ-distance on the specific pathways, then the EVA statistic is $E(D(X, X'))$. It identifies significantly dysregulated pathways by estimating p-values from a permutation test. Eddy et al. found that more pathological phenotypes usually have more pathways with higher variability compared to less pathological phenotypes.

However, the permutation test in DIRAC is computationally intensive and reaching low p-values may be impractical since they require a huge number of permutations. Low p-values are required for multiple hypothesis correction. A similar measure of variability of the orderings of gene sets was proposed in [2]. This method approximates the p-value theoretically, without a permutation test. This method is based on Kendall-τ distance [3] and the theory of U-Statistics, thus we call this method Gene Set Expression Variation Analysis (or in short EVA). Specifically, Kendall-τ distance between two expression profiles counts the number of disagreeing pairwise comparisons between two profiles. The EVA measures the variability of the gene expression of pathway genes from a phenotype by calculating the expectation of Kendall-τ distance between two random samples from the phenotype. EVA then identifies if the variability is significantly different across two phenotypes. To approximate this p-value EVA applies a U-Statistic Theory approach.

The `GReg` package contains two following utilities:

1. Identifying the dysregulated pathways with *DIRAC* measure of variability. The significance is calculated using permutation test. This is the first time that DIRAC analysis has been implemented in *R*. It also is more adaptable to new datasets than the original Matlab code in [1].

2. Identifying the dysregulated pathways with *EVA* measure of variability. The significance is approximated through applying U-statistics theory. This is very time efficient and consistent with both *DIRAC* and applying permutation test on EVA.

2 Input Data

2.1 Data structure

In short, the `GSReg` package requires the following data in the following format:

1. Gene Expression Data

(a) The expression be in the form of a matrix where rows represent genes (or probes) and columns represent samples.

(b) The expression matrix cannot have NAs.

(c) The expression matrix rows must have names of genes or the probes.

2. Pathways

   (a) The list of pathways must contain character vectors. Only the elements of the vectors which appear in rownames of the expression matrix are considered for analysis.

   (b) The list of the pathways must have names for each vectors.

3. Phenotypes

   (a) A factor with binary levels.

We used the data provided in the `GSBenchMark` package to reproduce the results in Eddy et al. [1]. The `GSBenchMark` contains data for the pathways as well as the gene expression and phenotype data from twelve studies. We load the information about the pathways from `GSBenchMark`:

```
> library(GSBenchMark)
> data(diracpathways)
> class(diracpathways)

[1] "list"

> names(diracpathways)[1:5]

[1] "DEATHPATHWAY"       "TCAPOPTOSISPATHWAY" "CCR3PATHWAY"
[4] "NEUTROPHILPATHWAY"  "ALTERNATIVEPATHWAY"

> class(diracpathways[[1]])

[1] "character"
```

AS mentioned `GSReg` package requires the information of the pathways to be as a list of character vectors. Also, `GSReg` requires the pathways to have names. The variable `diracpathways` contains gene pathways. It is a list. Each element represents a pathway with its name. Each elements contains a list of characters which represent the genes in the pathway. e.g. `diracpathways[["DEATHPATHWAY"]]`.

Now, we load the datasets' names:

```
> data(GSBenchMarkDatasets)
> print(GSBenchMark.Dataset.names)
```

```
 [1] "leukemia_GSEA"         "marfan_GDS2960"        "melanoma_GDS2735"
 [4] "parkinsons_GDS2519"    "prostate_GDS2545_m_nf" "prostate_GDS2545_m_p"
 [7] "prostate_GDS2545_p_nf" "sarcoma_data"          "squamous_GDS2520"
[10] "breast_GDS807"         "bipolar_GDS2190"
```

The remaining examples in this vignette rely on one of the datasets, i.e. "squamous GDS2520." Similar analyses may be reproduces for other datasets by selecting a different element of "GSBenchMark.Dataset.names."

```
> DataSetStudy = GSBenchMark.Dataset.names[[9]]
> print(DataSetStudy)
```

```
[1] "squamous_GDS2520"
```

```
> data(list=DataSetStudy)
```

The data consists of two variables: `exprsdata` and `phenotypes`. `exprsdata` consists of a gene expression matrix where the rows and columns represent genes and the samples respectively. `GSReg` requires the rownames of gene expression variable represent the gene names, *i.e.* they are represented in the pathway information variable.

The `GSReg` does not allow any missing data. To comply with the requirements we remove genes with NAs. The user may use any imputation to resolve this issue:

```
> if(sum(apply(is.nan(exprsdata),1,sum)>0))
   exprsdata = exprsdata[-which(apply(is.nan(exprsdata),1,sum)>0),];
```

One can extract the gene names by:

```
> genenames = rownames(exprsdata);
> genenames[1:10]
```

```
 [1] "MAPK3"   "TIE1"    "CYP2C19" "CXCR5"   "CXCR5"   "DUSP1"   "MMP10"   "DDR1"
 [9] "EIF2AK2" "HINT1"
```

3 Analysis of the pathways

Here, we demonstrate how to use the GSReg package to compute DIRAC and EVA statistics.

3.1 DIRAC Analysis

First, we load the library:

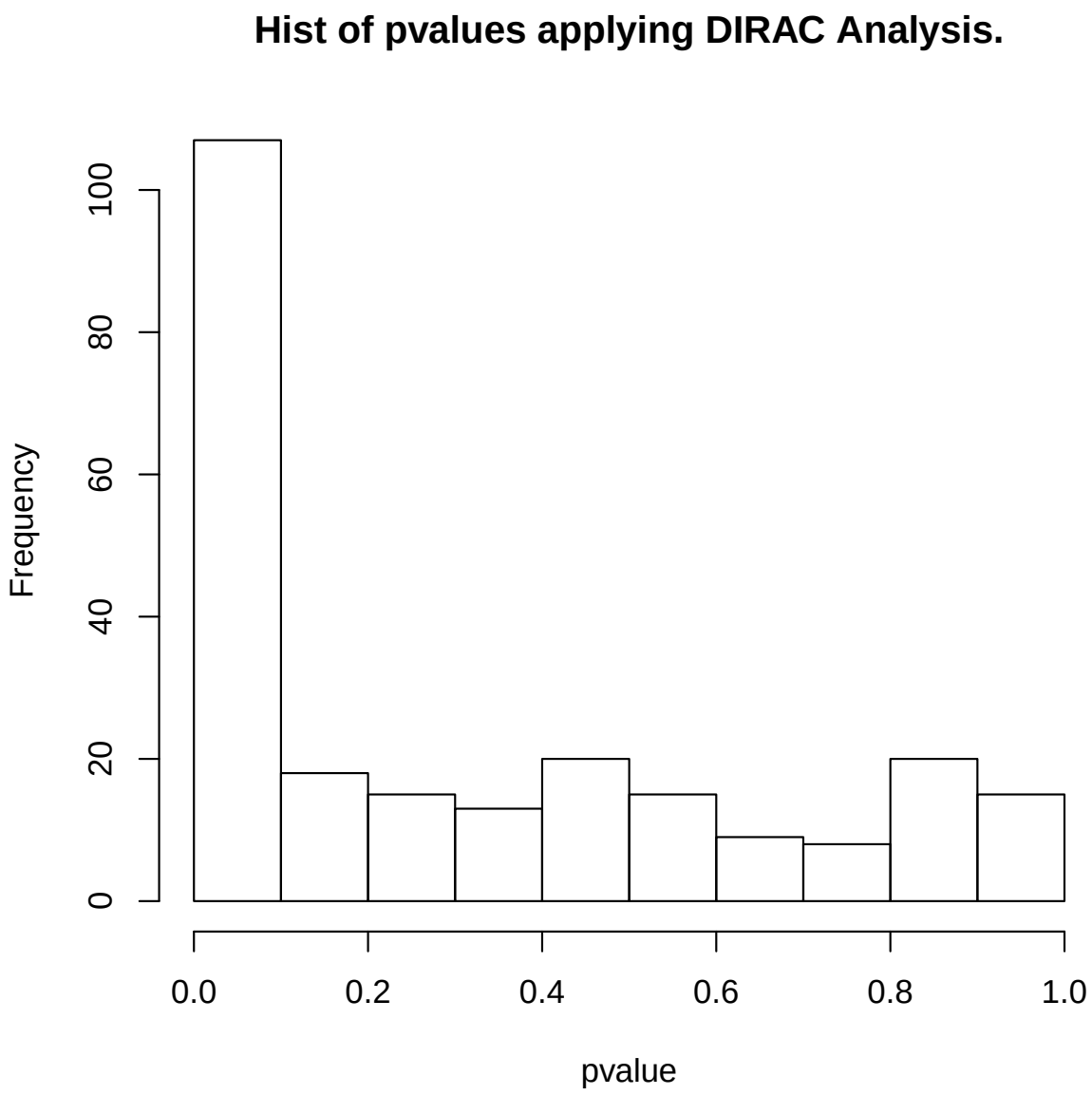
```
> library(GSReg)
```

The package also implements the alternative EVA statistic in the function `GSReg.GeneSets.DIRAC`. This function receives gene expression as `geneexpres`, the pathway information as `pathways` and phenotypes of samples as a factor with two levels and length equal to column number of `geneexpres`. *DIRAC* uses a permutation test for p-value calculation; so, `GSReg.GeneSets.DIRAC` receives the number of permutations through (`Nperm`) with default value equal to 1000.

```
> Nperm = 10
> system.time({DIRACAn =GSReg.GeneSets.DIRAC(exprsdata,diracpathways,phenotypes,Nperm=Nperm)})
   user  system elapsed
  3.084   0.000   3.087
```

Here is the histogram of the DIRAC p-values:

```
> hist(DIRACAn$pvalues,xlab="pvalue",main="Hist of pvalues applying DIRAC Analysis.")
```



3.2 EVA

The package also implements the alternative EVA statistic in the function `GSReg.GeneSets.EVA`. The function requires the similar inputs as `GSReg.GeneSets.DIRAC` (i.e. `geneexpres`, `pathways`, `phenotypes`) except it does not need `Nperm` since the p-value is not calculated through permutation test but through the mentioned U-statistic theory approach.

```
> #Calculating the variance for the pathways
> #Calculate how much it takes to calculate the statistics and their p-value for all pathways
```

```
>
> system.time({VarAnKendallV = GSReg.GeneSets.EVA(geneexpres=exprsdata,
   pathways=diracpathways, phenotypes=as.factor(phenotypes)) })

   user  system elapsed
  0.371   0.000   0.371

> names(VarAnKendallV)[[1]]

[1] "DEATHPATHWAY"

> VarAnKendallV[[1]]

$E1
[1] 0.09441352

$E2
[1] 0.1310383

$zscore
[1] -3.711215

$VarEta1
[1] 3.480369e-05

$VarEta2
[1] 6.248694e-05

$sdtotal
[1] 0.009863601

$pvalue
[1] 0.0002062669
```

The output consists of a list. Each element of the list corresponds to a pathway. The element itself is a list. `E1` and `E2` are two fields which contain the measure of variability for phenotype `levels(phenotypes)[1]` and `levels(phenotypes)[2]` respectively. Other list elements are `pvalue` and `zscore` which are calculated through the theory of U-statistics and indicate the statistical significance of the difference between $E1$ and $E2$.

3.3 Comparison of DIRAC and EVA

We ran the following code to compare statistics from DIRAC and from EVA.

```
> Nperm = 10;
> VarAnPerm = vector(mode="list",length=Nperm)
> for( i in seq_len(Nperm))
 {
```

```
   VarAnPerm[[i]] = GSReg.GeneSets.EVA(geneexpres=exprsdata, pathways=diracpathways,
                                          phenotypes=sample(phenotypes))
 }
> pvaluesperm = vector(mode="numeric",length=length(VarAnPerm[[1]]))
> for( i in seq_along(VarAnPerm[[1]]))
 {
   z = sapply(VarAnPerm,function(x) x[[i]]$E1 - x[[i]]$E2)
   pvaluesperm[i] = mean(abs(VarAnKendallV[[i]]$E1-VarAnKendallV[[i]]$E2)<abs(z))
 }
>  zscore = sapply(VarAnKendallV,function(x) x$zscore);
>  pvalustat = sapply(VarAnKendallV,function(x) x$pvalue);
```

The figure represents that the theoretical p-value and p-value calculated from permutation test in EVA are very similar and we can use the theoretical p-value as a surrogate for p-value. Here is the histogram.

```
> hist(x=pvalustat,breaks=20,main="P-value Hist of U-Stat",xlim=c(0,1))
```

```
> plot(x=abs(zscore),y=pvaluesperm,xlab="|Z-score|",
  ylab="p-value",col="red1",main="p-value comparisons")
> zscorelin = seq(0,6,0.1);
> pvaltheoretic = (1-pnorm(zscorelin))*2
> lines(x=zscorelin,y=pvaltheoretic,type="l",pch=50,lty=5,col="darkblue")
>  legend("topright",legend=c("permutation test","U-Stat Estimation"),
       col=c("red","blue"),text.col=c("red","blue"),
  lty=c(NA,1),lwd=c(NA,2.5),pch=c(21,NA))
```

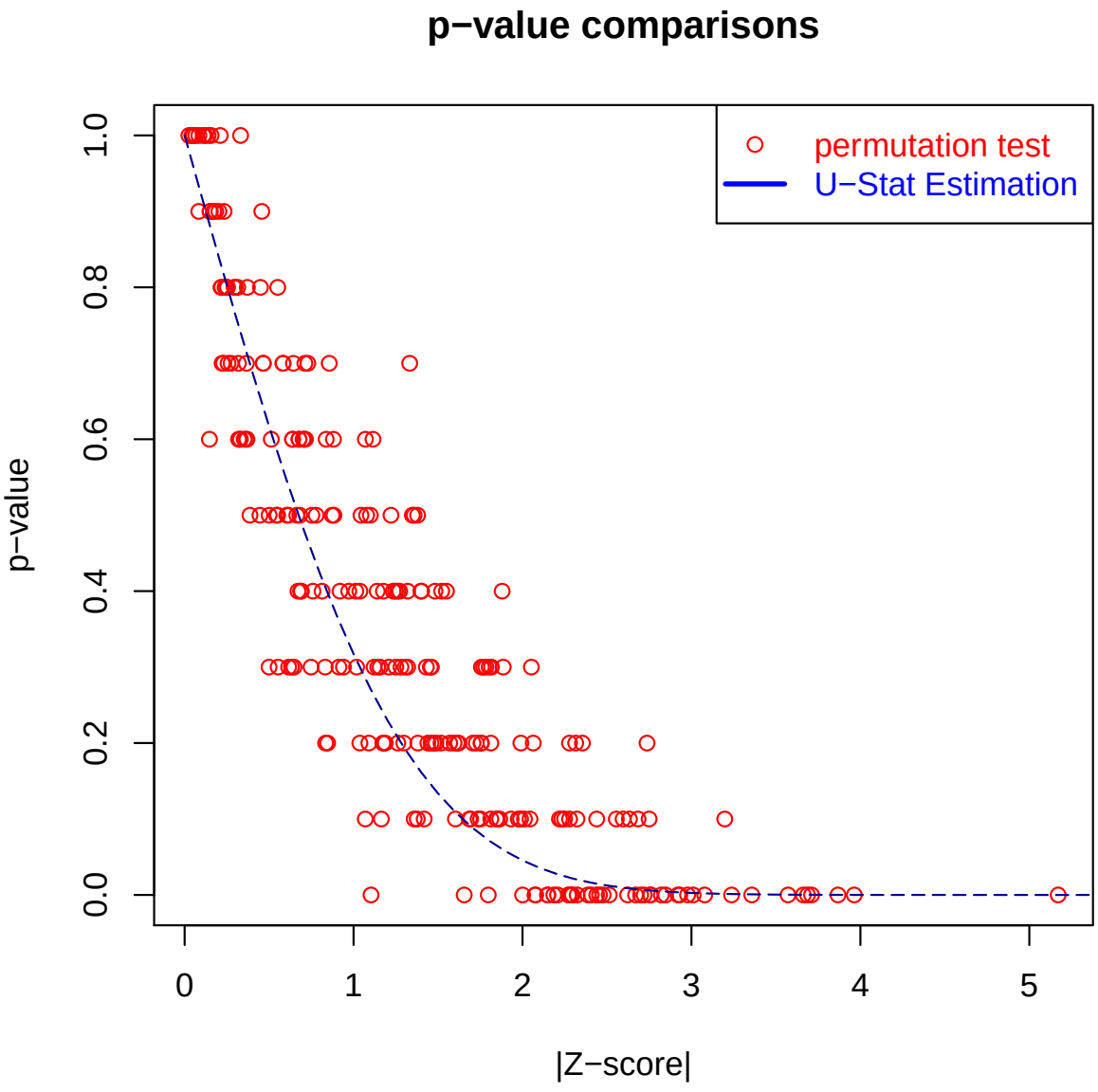


Figure 1: Comparing p-value from permutation test and U-statistic theory with only 10 permutations.

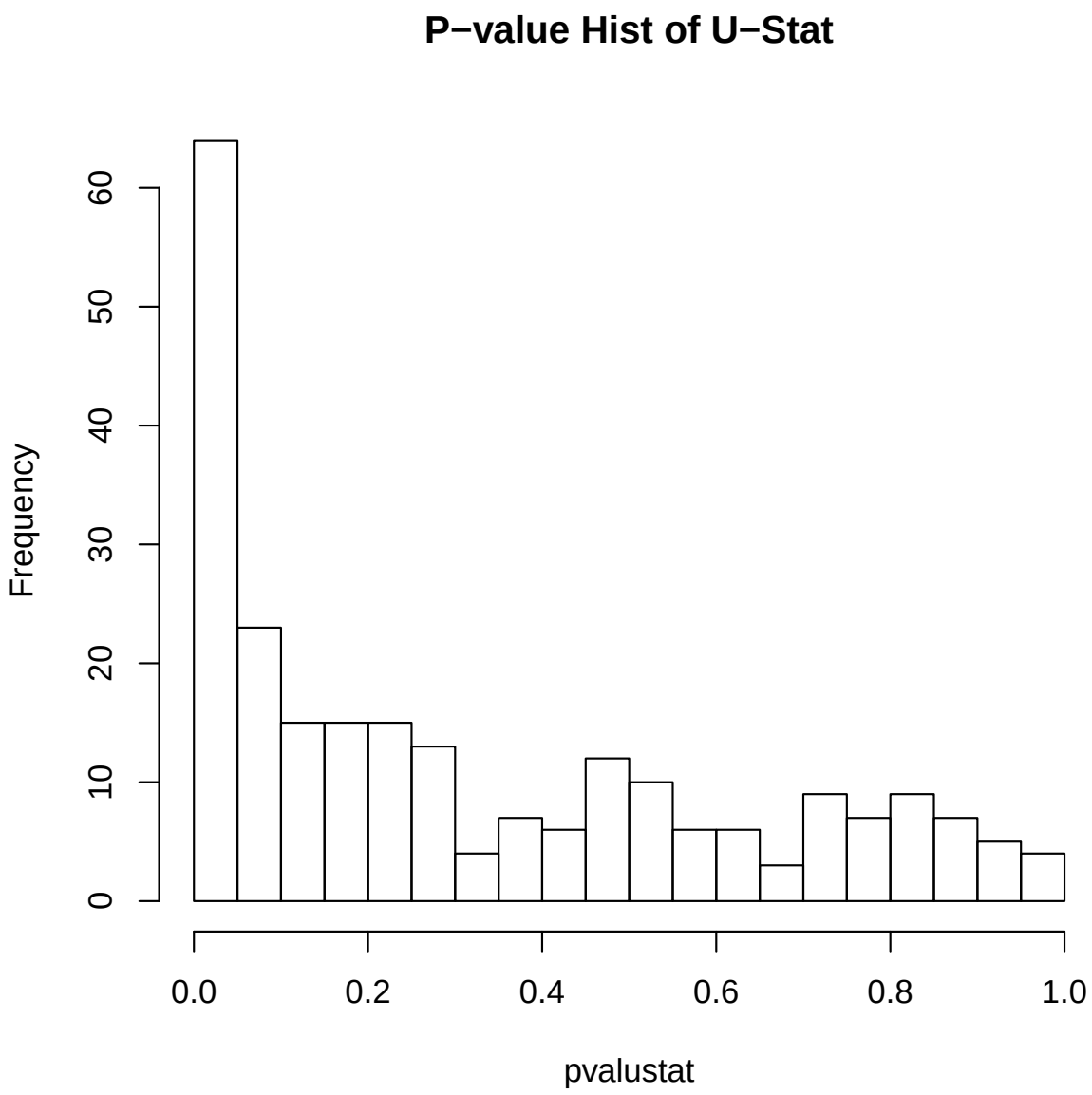


Figure 2 shows the result of comparing p-value EVA computing from 1000 permutation test and approximation using U-statistics theory (offline generated).

To compare with the p-value of the DIRAC analysis, we show the p-values of DIRAC versus U-Statistic methodology:

```
> plot(x=DIRACAn$pvalues,y=pvalustat,xlab ="DIRAC",
        ylab="EVA",main=sprintf("P-value Comparison corr=%2.2g",cor(x=DIRACAn$pvalues,y=pvalustat)))
> lmfit = lm(pvalustat~DIRACAn$pvalues-1)
> abline(lmfit)
> cor.test(x=DIRACAn$pvalues,y=pvalustat)
```

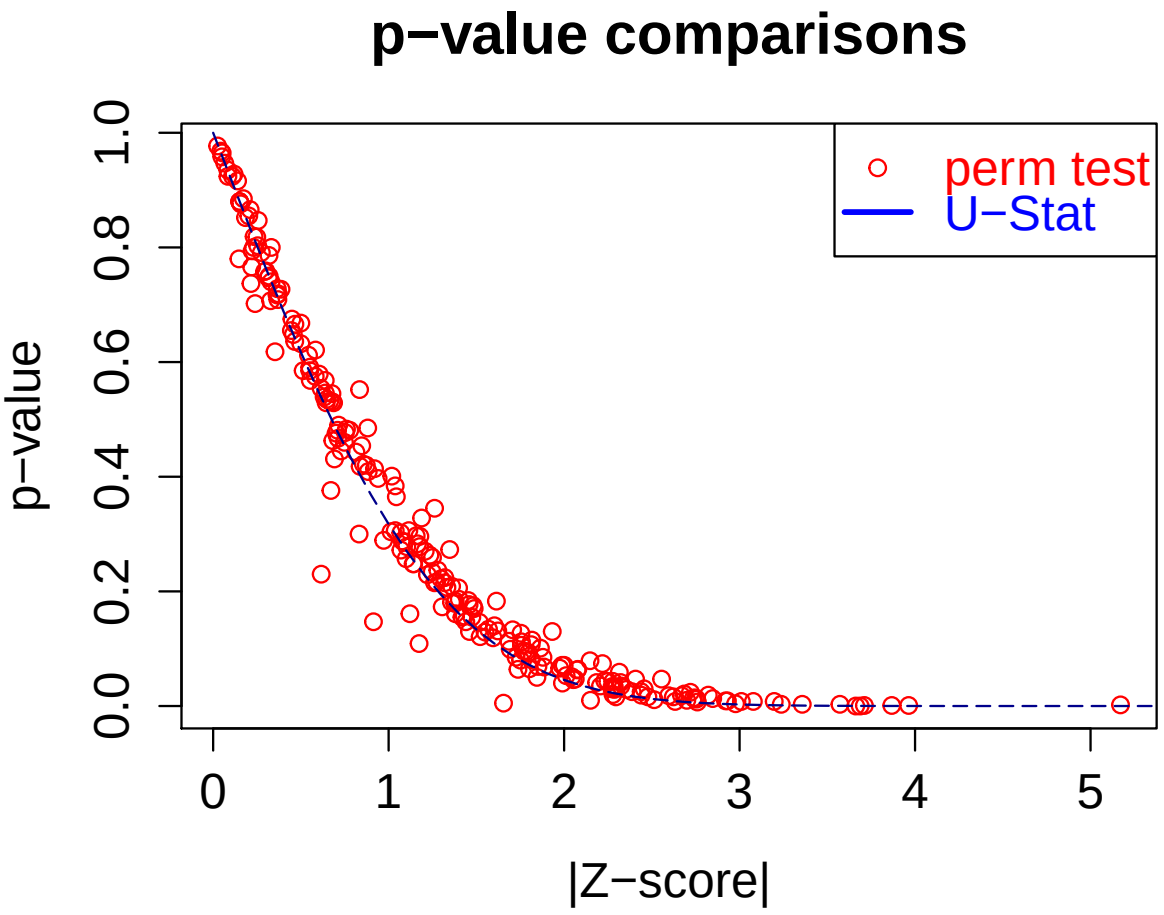
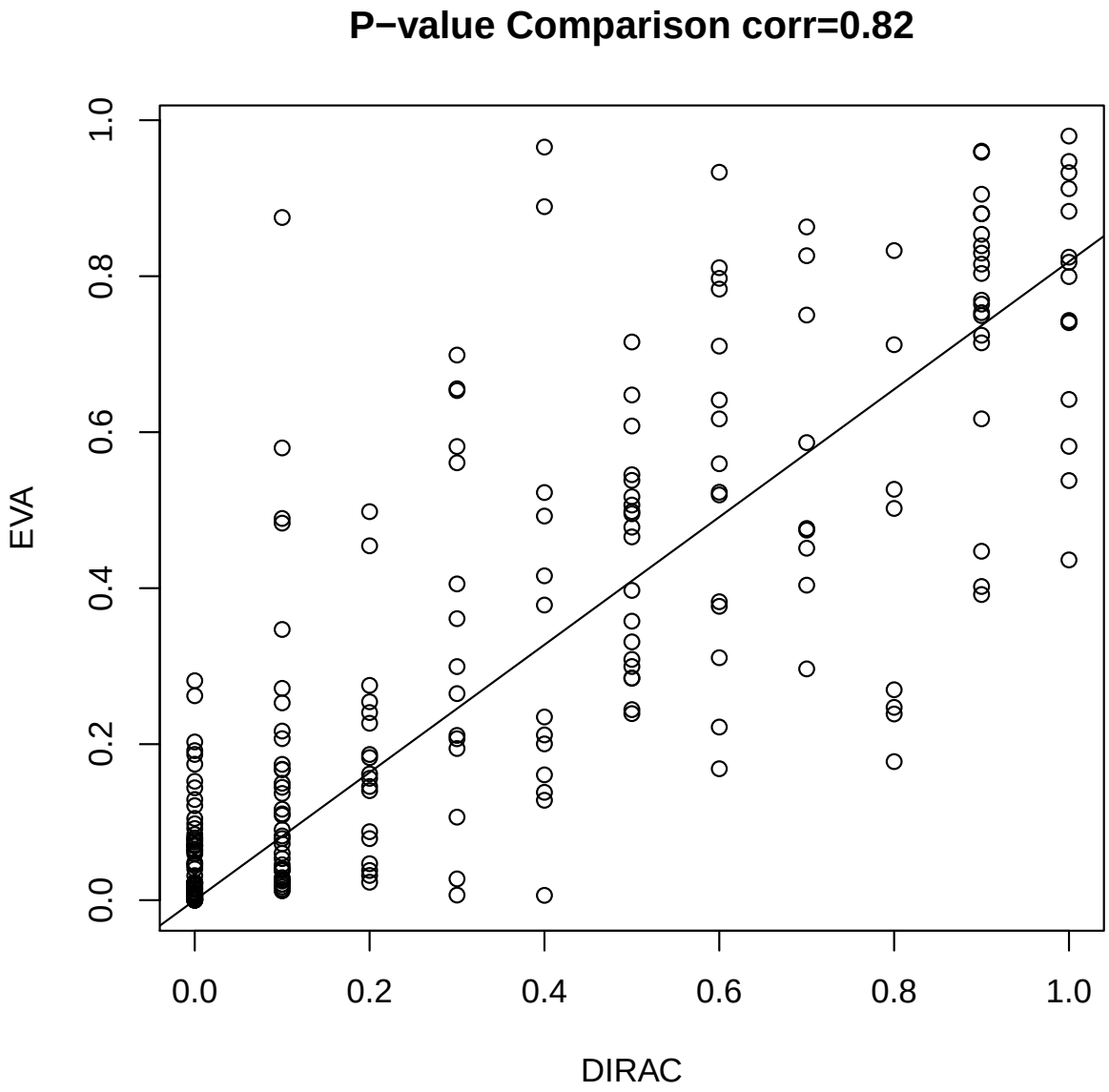


Figure 2: Theoretical p-value versus empirical p-value using 1000 permutations.

```
        Pearson's product-moment correlation

data:  DIRACAn$pvalues and pvalustat
t = 22.301, df = 238, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.7766437 0.8595145
sample estimates:
      cor
0.8223942
```



Also, the correlation of the p-values of DIRAC and U-Statistics is very high:

```
> cor(x=DIRACAn$pvalues,y=pvalustat)
[1] 0.8223942
```

If we use 1000 permutations instead of 10 permutations, we can see that the correlation is higher (0.88) as seen in Figure (3). The dysregulated pathways identified by *DIRAC* are the following pathways:

```
 [1] "DEATHPATHWAY"          "NEUTROPHILPATHWAY"     "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"         "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"       "TGFBPATHWAY"           "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"           "PYK2PATHWAY"           "MYOSINPATHWAY"
[16] "BETAOXIDATIONPATHWAY" "IL7PATHWAY"            "FMLPPATHWAY"
[19] "VITCBPATHWAY"          "CD40PATHWAY"           "CDC25PATHWAY"
[22] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
[28] "ALKPATHWAY"            "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"           "RELAPATHWAY"           "METPATHWAY"
[34] "TNFR2PATHWAY"          "AT1RPATHWAY"           "FREEPATHWAY"
[37] "ARAPPATHWAY"           "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"           "STRESSPATHWAY"         "MEF2DPATHWAY"
[43] "STAT3PATHWAY"          "HSP27PATHWAY"          "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"
```

DIRAC and EVA have been shown mathematically similar. The main advantages of the EVA is efficiency in calculation as well as easier interpretation. Figure 3 a graphical example of such comparison. One can see that the p-values generated by DIRAC and EVA have high correlation, i.e. 0.88. Note that EVA is much faster than DIRAC. For example, in this case, we ran the computations on a Lenovo Thinkpad with Core(TM) i7-3720QM Intel CPU @2.6 GHz. For a thousand permutation, the DIRAC analysis took 207.47 seconds while the latter only took 0.3 seconds. Note that for multiple hypothesis adjustment, a thousand permutations may not be satisfactory and we require hundreds of thousand or a million permutation which may not be feasible.

Note that it is possible that some of the genes in a pathway are not represented in the expression data or are too short (e.g. less than 5 genes). Both `GSReg.GeneSets.EVA` and `GSReg.GeneSets.DIRAC` may ignore such pathways through parameter `minGeneNum`. Please see the manual for more details. If we the user wants to compare the results of DIRAC and

EVA, they can run the following code for plot DIRAC diagram of significantly perturbed pathways:

```
> DIRACAn =GSReg.GeneSets.DIRAC(exprsdata,diracpathways,phenotypes,Nperm=1000)
```

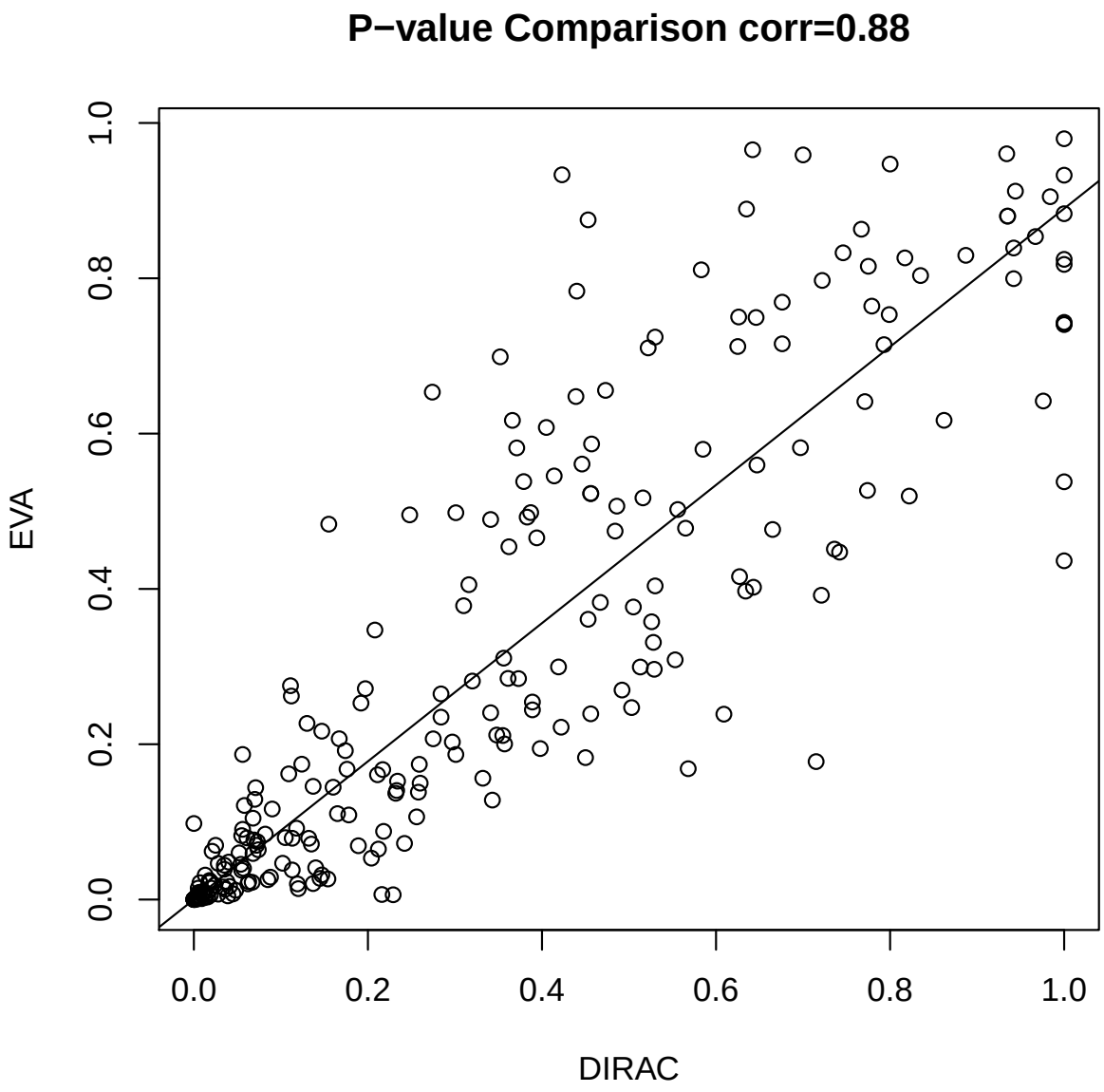
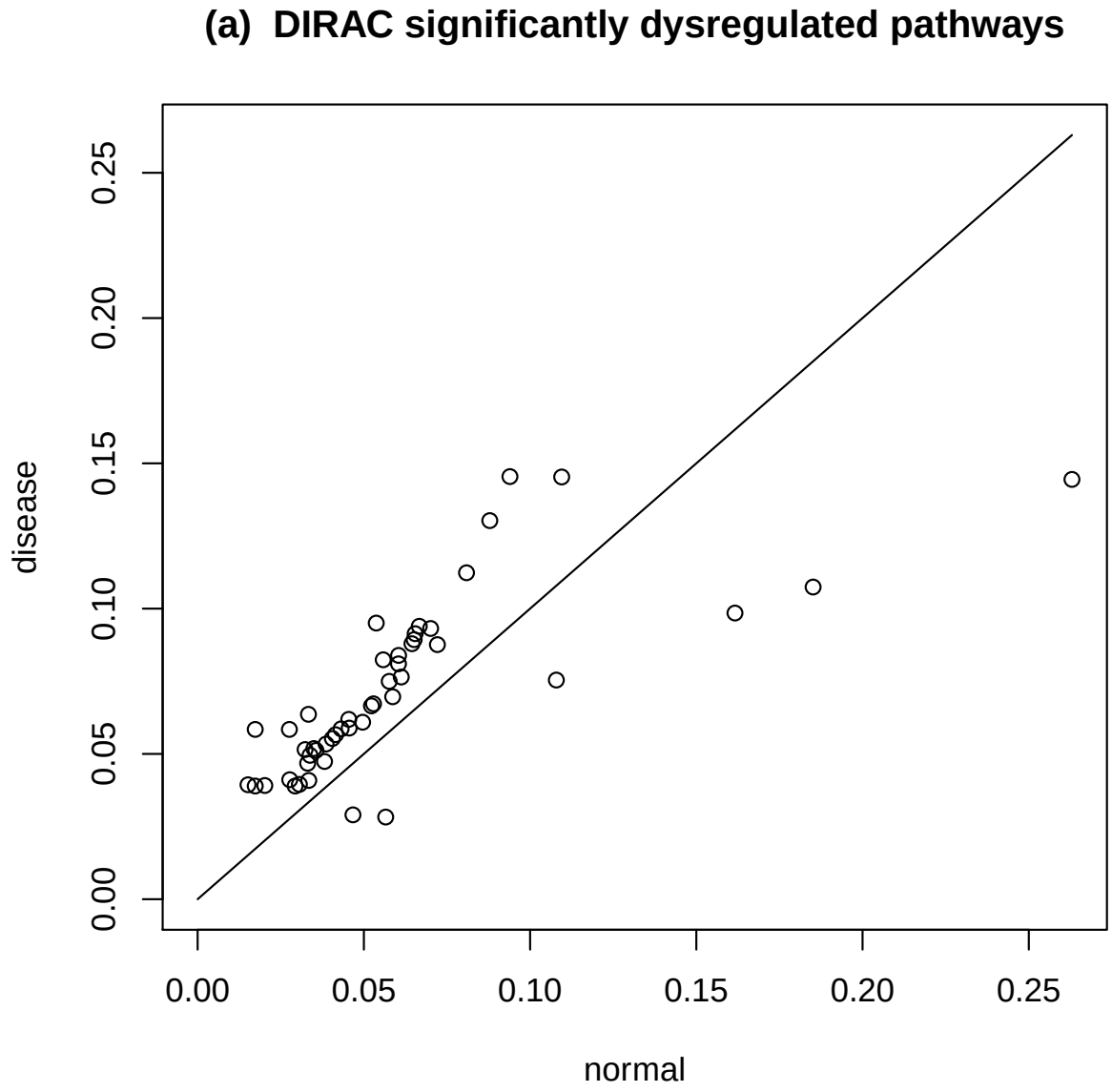


Figure 3: Comparing p-values EVA versus DIRAC. The correlation is 0.88.

```
> significantPathwaysDIRAC = names(DIRACAn$mu1)[which(DIRACAn$pvalues<0.05)];
> mu1 = DIRACAn$mu1[significantPathwaysDIRAC];
> mu2 = DIRACAn$mu2[significantPathwaysDIRAC];
> #The dysregulated pathways
> names(mu1)

 [1] "DEATHPATHWAY"          "NEUTROPHILPATHWAY"     "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"         "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"       "TGFBPATHWAY"           "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"           "PYK2PATHWAY"           "MYOSINPATHWAY"
[16] "BETAOXIDATIONPATHWAY" "IL7PATHWAY"            "FMLPPATHWAY"
[19] "VITCBPATHWAY"          "CD40PATHWAY"           "CDC25PATHWAY"
[22] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
[28] "ALKPATHWAY"            "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"           "RELAPATHWAY"           "METPATHWAY"
[34] "TNFR2PATHWAY"          "AT1RPATHWAY"           "FREEPATHWAY"
[37] "ARAPPATHWAY"           "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"           "STRESSPATHWAY"         "MEF2DPATHWAY"
[43] "STAT3PATHWAY"          "HSP27PATHWAY"          "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"

> plot(x=mu1,y=mu2,
      xlim=c(0,max(mu1,mu2)),ylim=c(0,max(mu1,mu2)),xlab="normal",ylab="disease",
      main="(a)  DIRAC significantly dysregulated pathways")
> lines(x=c(0,max(mu1,mu2)),y=c(0,max(mu1,mu2)))
```



Now, if we do the analysis using EVA, we have:

```
> significantPathwaysGSV = names(which(pvalustat<0.05));
 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"    "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"           "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"   "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"           "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"           "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"           "CDC42RACPATHWAY"
```

```
[22] "MYOSINPATHWAY"         "BETAOXIDATIONPATHWAY" "IL7PATHWAY"
[25] "FMLPPATHWAY"           "FASPATHWAY"           "VITCBPATHWAY"
[28] "CD40PATHWAY"           "IGF1PATHWAY"          "CDC25PATHWAY"
[31] "MTORPATHWAY"           "RNAPATHWAY"           "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"          "HIVNEFPATHWAY"
[37] "ALKPATHWAY"            "PEPIPATHWAY"          "MSPPATHWAY"
[40] "EDG1PATHWAY"           "GSK3PATHWAY"          "RELAPATHWAY"
[43] "METPATHWAY"            "TNFR2PATHWAY"         "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"        "GLYCOLYSISPATHWAY"    "TIDPATHWAY"
[49] "EPOPATHWAY"            "WNTPATHWAY"           "ARAPPATHWAY"
[52] "MRPPATHWAY"            "P53HYPOXIAPATHWAY"    "PITX2PATHWAY"
[55] "IL18PATHWAY"           "STRESSPATHWAY"        "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY"   "STAT3PATHWAY"         "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"      "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"
```

```
> eta1 = sapply(VarAnKendallV,function(x) x$E1)[significantPathwaysGSV];
```

```
       DEATHPATHWAY   TCAPOPTOSISPATHWAY    NEUTROPHILPATHWAY         PGC1APATHWAY
          0.09441352           0.08600289           0.35559678           0.04477053
        TERCPATHWAY        RARRXRPATHWAY       SKP2E2FPATHWAY  KERATINOCYTEPATHWAY
          0.07316017           0.06914038           0.03367003           0.07404055
    CHEMICALPATHWAY    METHIONINEPATHWAY          TGFBPATHWAY           PS1PATHWAY
          0.08521303           0.09913420           0.05028305           0.08080808
  PROTEASOMEPATHWAY          CDK5PATHWAY          MAPKPATHWAY          NTHIPATHWAY
          0.09617180           0.07975863           0.08504987           0.08091115
        PDGFPATHWAY   BIOPEPTIDESPATHWAY          SPPAPATHWAY          PYK2PATHWAY
          0.08698709           0.06767807           0.09690598           0.04711514
    CDC42RACPATHWAY        MYOSINPATHWAY BETAOXIDATIONPATHWAY           IL7PATHWAY
          0.08948195           0.09005280           0.02683983           0.10609668
        FMLPPATHWAY           FASPATHWAY         VITCBPATHWAY          CD40PATHWAY
          0.05736961           0.08676830           0.07888408           0.05294705
        IGF1PATHWAY         CDC25PATHWAY          MTORPATHWAY           RNAPATHWAY
          0.09115972           0.06265031           0.04188827           0.03009689
        FBW7PATHWAY    LYMPHOCYTEPATHWAY          LAIRPATHWAY        HIVNEFPATHWAY
          0.03174603           0.22390572           0.15222872           0.09020600
         ALKPATHWAY          PEPIPATHWAY          MSPPATHWAY          EDG1PATHWAY
          0.09130361           0.05194805           0.12150072           0.08801738
        GSK3PATHWAY          RELAPATHWAY          METPATHWAY         TNFR2PATHWAY
          0.11946928           0.06302309           0.10561315           0.05051566
        AT1RPATHWAY       ATRBRCAPATHWAY    GLYCOLYSISPATHWAY           TIDPATHWAY
          0.05943314           0.07166907           0.02308802           0.07331013
         EPOPATHWAY           WNTPATHWAY          ARAPPATHWAY           MRPPATHWAY
          0.07319696           0.10526414           0.05988456           0.04877345
  P53HYPOXIAPATHWAY         PITX2PATHWAY          IL18PATHWAY        STRESSPATHWAY
          0.07708666           0.09375387           0.13015873           0.05505364
       MEF2DPATHWAY  MITOCHONDRIAPATHWAY         STAT3PATHWAY       EPONFKBPATHWAY
          0.04399740           0.12572150           0.05179344           0.07910272
     NKCELLSPATHWAY      MONOCYTEPATHWAY       CARM_ERPATHWAY          HCMVPATHWAY
          0.07718643           0.25171192           0.06462137           0.07781385
```

```
> eta2 = sapply(VarAnKendallV,function(x) x$E2)[significantPathwaysGSV];
```

```
      DEATHPATHWAY   TCAPOPTOSISPATHWAY    NEUTROPHILPATHWAY         PGC1APATHWAY
         0.13103827          0.04531025          0.23546691          0.05588351
         TERCPATHWAY       RARRXRPATHWAY       SKP2E2FPATHWAY  KERATINOCYTEPATHWAY
         0.12770563          0.09090909          0.05856181          0.09013983
    CHEMICALPATHWAY    METHIONINEPATHWAY          TGFBPATHWAY          PS1PATHWAY
         0.11832612          0.15454545          0.07165057          0.12332852
  PROTEASOMEPATHWAY          CDK5PATHWAY          MAPKPATHWAY          NTHIPATHWAY
         0.13083213          0.10251869          0.10114801          0.09532055
       PDGFPATHWAY   BIOPEPTIDESPATHWAY          SPPAPATHWAY          PYK2PATHWAY
         0.11066711          0.08559859          0.12604711          0.06023958
    CDC42RACPATHWAY       MYOSINPATHWAY BETAOXIDATIONPATHWAY          IL7PATHWAY
         0.11283954          0.11997526          0.06147186          0.13455988
       FMLPPATHWAY          FASPATHWAY         VITCBPATHWAY          CD40PATHWAY
         0.06835017          0.10725265          0.04252044          0.08041958
       IGF1PATHWAY        CDC25PATHWAY          MTORPATHWAY          RNAPATHWAY
         0.12087036          0.04413179          0.05994640          0.08719852
       FBW7PATHWAY    LYMPHOCYTEPATHWAY          LAIRPATHWAY        HIVNEFPATHWAY
         0.06307978          0.16065416          0.11574140          0.11884884
        ALKPATHWAY          PEPIPATHWAY          MSPPATHWAY          EDG1PATHWAY
         0.11181840          0.13593074          0.17344877          0.11010728
       GSK3PATHWAY          RELAPATHWAY          METPATHWAY         TNFR2PATHWAY
         0.16320909          0.08203463          0.12858234          0.07308378
       AT1RPATHWAY       ATRBRCAPATHWAY    GLYCOLYSISPATHWAY          TIDPATHWAY
         0.08115533          0.08771185          0.05112348          0.09458733
        EPOPATHWAY          WNTPATHWAY          ARAPPATHWAY          MRPPATHWAY
         0.09569080          0.12762130          0.07615440          0.08571429
  P53HYPOXIAPATHWAY        PITX2PATHWAY          IL18PATHWAY       STRESSPATHWAY
         0.09759247          0.11618223          0.18989899          0.07523998
       MEF2DPATHWAY  MITOCHONDRIAPATHWAY        STAT3PATHWAY      EPONFKBPATHWAY
         0.05508870          0.16778499          0.09647495          0.13372688
     NKCELLSPATHWAY      MONOCYTEPATHWAY       CARM_ERPATHWAY         HCMVPATHWAY
         0.09574739          0.17662338          0.08397641          0.08899711
```

```
> #The dysregulated pathways
> names(eta1)
```

```
 [1] "DEATHPATHWAY"         "TCAPOPTOSISPATHWAY"   "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"         "TERCPATHWAY"          "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"       "KERATINOCYTEPATHWAY"  "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"    "TGFBPATHWAY"          "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"    "CDK5PATHWAY"          "MAPKPATHWAY"
[16] "NTHIPATHWAY"          "PDGFPATHWAY"          "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"          "PYK2PATHWAY"          "CDC42RACPATHWAY"
[22] "MYOSINPATHWAY"        "BETAOXIDATIONPATHWAY" "IL7PATHWAY"
[25] "FMLPPATHWAY"          "FASPATHWAY"           "VITCBPATHWAY"
[28] "CD40PATHWAY"          "IGF1PATHWAY"          "CDC25PATHWAY"
[31] "MTORPATHWAY"          "RNAPATHWAY"           "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"    "LAIRPATHWAY"          "HIVNEFPATHWAY"
[37] "ALKPATHWAY"           "PEPIPATHWAY"          "MSPPATHWAY"
[40] "EDG1PATHWAY"          "GSK3PATHWAY"          "RELAPATHWAY"
[43] "METPATHWAY"           "TNFR2PATHWAY"         "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"       "GLYCOLYSISPATHWAY"    "TIDPATHWAY"
```

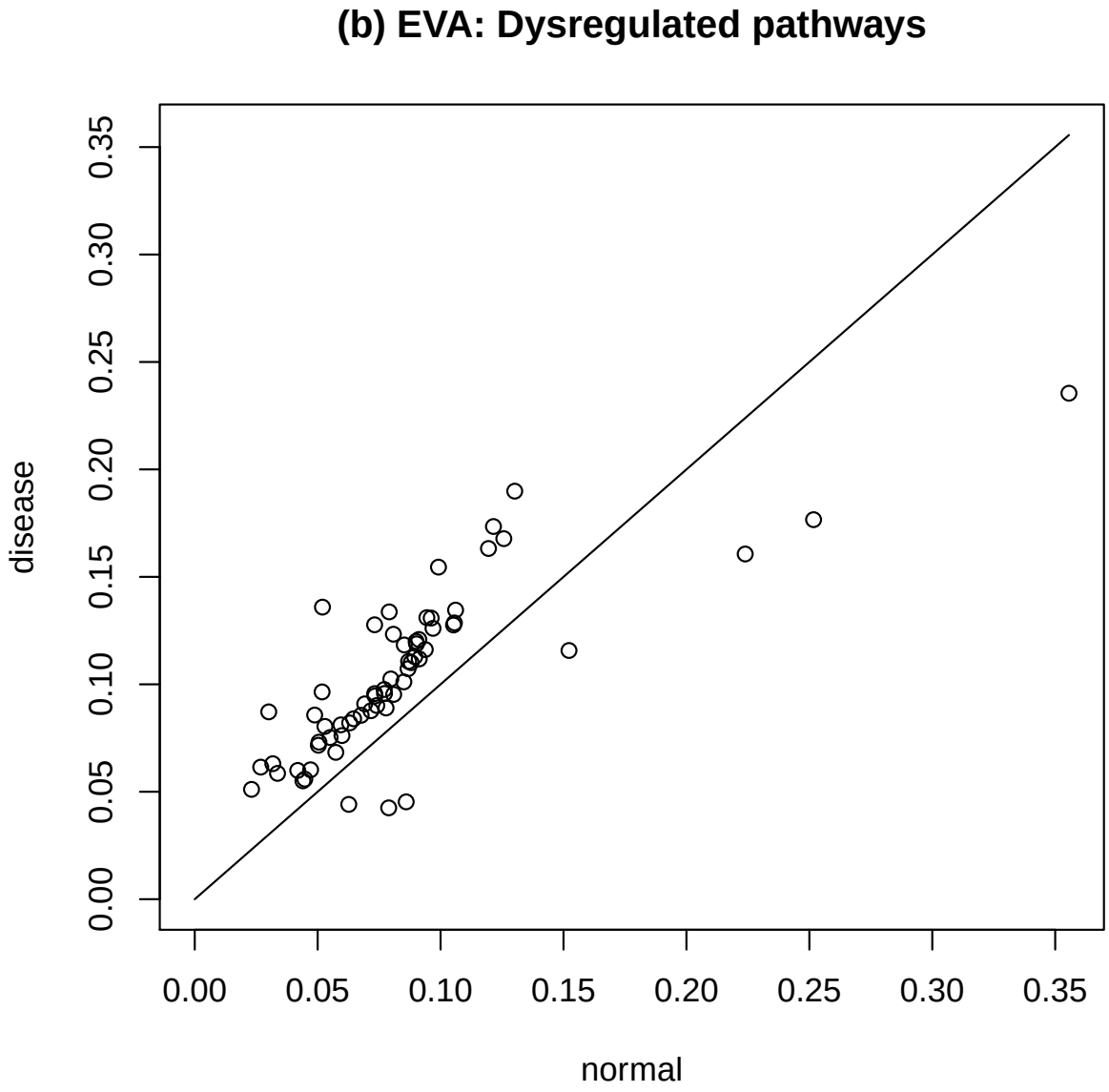
```
[49] "EPOPATHWAY"           "WNTPATHWAY"           "ARAPPATHWAY"
[52] "MRPPATHWAY"           "P53HYPOXIAPATHWAY"    "PITX2PATHWAY"
[55] "IL18PATHWAY"          "STRESSPATHWAY"        "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY" "STAT3PATHWAY"         "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"       "MONOCYTEPATHWAY"      "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"
```

```
> plot(x=eta1,y=eta2,xlim=c(0,max(eta1,eta2)),ylim=c(0,max(eta1,eta2)),xlab="normal",ylab="disease",
      main="(b) EVA: Dysregulated pathways")
```

```
NULL
```

```
> lines(x=c(0,max(eta1,eta2)),y=c(0,max(eta1,eta2)))
```

```
NULL
```



Although there is discrepancy in identified dysregulated pathways (p-value<0.05), the general trend found in [1] holds still true. The trend is that usually the dysregulated pathways have higher variability measure in more dangerous phenotypes. The figures reveal that both DIRAC and EVA have this property. DIRAC found 48 dysregulatd pathways and EVA discovered 64 pathways, 45 pathways showed up in both analysis, and 67 pathways were discovered totally.

```
> print(significantPathwaysGSV)
```

```
 [1] "DEATHPATHWAY"          "TCAPOPTOSISPATHWAY"    "NEUTROPHILPATHWAY"
 [4] "PGC1APATHWAY"          "TERCPATHWAY"           "RARRXRPATHWAY"
 [7] "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"   "CHEMICALPATHWAY"
[10] "METHIONINEPATHWAY"     "TGFBPATHWAY"           "PS1PATHWAY"
[13] "PROTEASOMEPATHWAY"     "CDK5PATHWAY"           "MAPKPATHWAY"
[16] "NTHIPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[19] "SPPAPATHWAY"           "PYK2PATHWAY"           "CDC42RACPATHWAY"
[22] "MYOSINPATHWAY"         "BETAOXIDATIONPATHWAY" "IL7PATHWAY"
[25] "FMLPPATHWAY"           "FASPATHWAY"            "VITCBPATHWAY"
[28] "CD40PATHWAY"           "IGF1PATHWAY"           "CDC25PATHWAY"
[31] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
[34] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
[37] "ALKPATHWAY"            "PEPIPATHWAY"           "MSPPATHWAY"
[40] "EDG1PATHWAY"           "GSK3PATHWAY"           "RELAPATHWAY"
[43] "METPATHWAY"            "TNFR2PATHWAY"          "AT1RPATHWAY"
[46] "ATRBRCAPATHWAY"        "GLYCOLYSISPATHWAY"     "TIDPATHWAY"
[49] "EPOPATHWAY"            "WNTPATHWAY"            "ARAPPATHWAY"
[52] "MRPPATHWAY"            "P53HYPOXIAPATHWAY"     "PITX2PATHWAY"
[55] "IL18PATHWAY"           "STRESSPATHWAY"         "MEF2DPATHWAY"
[58] "MITOCHONDRIAPATHWAY"   "STAT3PATHWAY"          "EPONFKBPATHWAY"
[61] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"
[64] "HCMVPATHWAY"

> print(significantPathwaysDIRAC)

 [1] "DEATHPATHWAY"          "NEUTROPHILPATHWAY"     "PGC1APATHWAY"
 [4] "RARRXRPATHWAY"         "SKP2E2FPATHWAY"        "KERATINOCYTEPATHWAY"
 [7] "CHEMICALPATHWAY"       "TGFBPATHWAY"           "PROTEASOMEPATHWAY"
[10] "MAPKPATHWAY"           "PDGFPATHWAY"           "BIOPEPTIDESPATHWAY"
[13] "SPPAPATHWAY"           "PYK2PATHWAY"           "MYOSINPATHWAY"
[16] "BETAOXIDATIONPATHWAY" "IL7PATHWAY"            "FMLPPATHWAY"
[19] "VITCBPATHWAY"          "CD40PATHWAY"           "CDC25PATHWAY"
[22] "MTORPATHWAY"           "RNAPATHWAY"            "FBW7PATHWAY"
[25] "LYMPHOCYTEPATHWAY"     "LAIRPATHWAY"           "HIVNEFPATHWAY"
[28] "ALKPATHWAY"            "P35ALZHEIMERSPATHWAY" "MSPPATHWAY"
[31] "GSK3PATHWAY"           "RELAPATHWAY"           "METPATHWAY"
[34] "TNFR2PATHWAY"          "AT1RPATHWAY"           "FREEPATHWAY"
[37] "ARAPPATHWAY"           "MRPPATHWAY"            "P53HYPOXIAPATHWAY"
[40] "IL18PATHWAY"           "STRESSPATHWAY"         "MEF2DPATHWAY"
[43] "STAT3PATHWAY"          "HSP27PATHWAY"          "EPONFKBPATHWAY"
[46] "NKCELLSPATHWAY"        "MONOCYTEPATHWAY"       "CARM_ERPATHWAY"
```

4 System Information

Session information:

```
> toLatex(sessionInfo())
```

- R version 3.2.0 (2015-04-16), `x86_64-unknown-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=C`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: GSBenchMark 0.101.1, GSReg 1.2.0
- Loaded via a namespace (and not attached): tools 3.2.0

5 Literature Cited

References

[1] James A Eddy, Leroy Hood, Nathan D Price, and Donald Geman. Identifying tightly regulated and variably expressed networks by differential rank conservation (dirac). *PLoS computational biology*, 6(5):e1000792, 2010.

[2] Bahman Afsari. *Modeling cancer phenotypes with order statistics of transcript data*. PhD thesis, Johns Hopkins University, 2013.

[3] Maurice G Kendall. A new measure of rank correlation. *Biometrika*, 1938.