GSEA
(work in progress)

Robert Gentleman

BIOCONDUCTOR

FRED HUTCHINSON CANCER RESEARCH CENTER
A LIFE OF SCIENCE

Outline

- Description of the experimental setting
- A brief description of differential gene selection
- gene sets and how to use them
- Related ideas
- Example: ALL data set from the Ritz Lab
- Concluding Remarks

Experiments/Data

- There are n samples
- for each sample we measure mRNA expression levels on G genes
- we consider the case where there are two phenotypes (e.g. BCR/ABL vs NEG)
- A t-test can be computed, for each gene comparing the two samples (other test statistics can be handled easily)

Differential Expression

- Usual approach is to try and find the set of differentially expressed genes [those with extreme values of the univariate statistic, **x**]
- Often adjusting in some way for multiple comparisons
- This can be criticized on many grounds
  - it introduces an artificial distinction - differentially expressed
  - it focuses attention on only a few genes that change by a large amount

Differential Expression

- *p*-value correction methods don't really do what we want
- *p-values* are not signed, so the effects may be in different directions
- to see if too many genes of a particular type have been selected a Hypergeometric calculation is made, but it relies on the articial distinction between expressed and not expressed
- we (and others) propose a different approach: find sets of genes whose expression changes in concert, possibly not by a large amount

Holistic Approach

- we will attempt to find gene sets, or sets, of genes where there are potentially small but coordinated changes in gene expression
- for example, if all genes are expressed at slightly higher (or all at slightly lower) values for one phenotype versus the other

Related Work

- PGC-1 alpha-responsive genes involed in oxidative phosphorylation are coordinately downregulated in human diabetes. Mootha et al, Nature Genetics, 2003
- mTOR inhibition reverses Akt-dependent prostate intraepithelial neoplasia through regulation of apoptotic and HIF-1 dependent pathways, Majumder et al, Nature Medicine, 2004
- Discovering statistically significant pathways in expression profiling studies. Tian et al, PNAS, 2005,

Gene Set Enrichment

- proposed by Mootha et al (2003)
- very similar (and was one of the motivations for this work) but is more complex and computationally expensive
- they discuss gene sets, S, which are the same as gene sets
- they sidestep multiple testing issues by testing a single hypothesis (the maximal observed per set statistic)
- I will sidestep multiple testing issues by simply reporting unadjusted *p-values*

Gene Set Enrichment

- For each gene set S, a Kolmogorov-Smirnov running sum is computed
- The assayed genes are ordered according to some criterion (say a two sample *t*-test; or signal-to-noise ratio SNR).
- Beginning with the top ranking gene the running sum increases when a gene in set S is encountered and decreases otherwise
- The enrichment score (ES) for a set S is defined to be the largest value of the running sum.

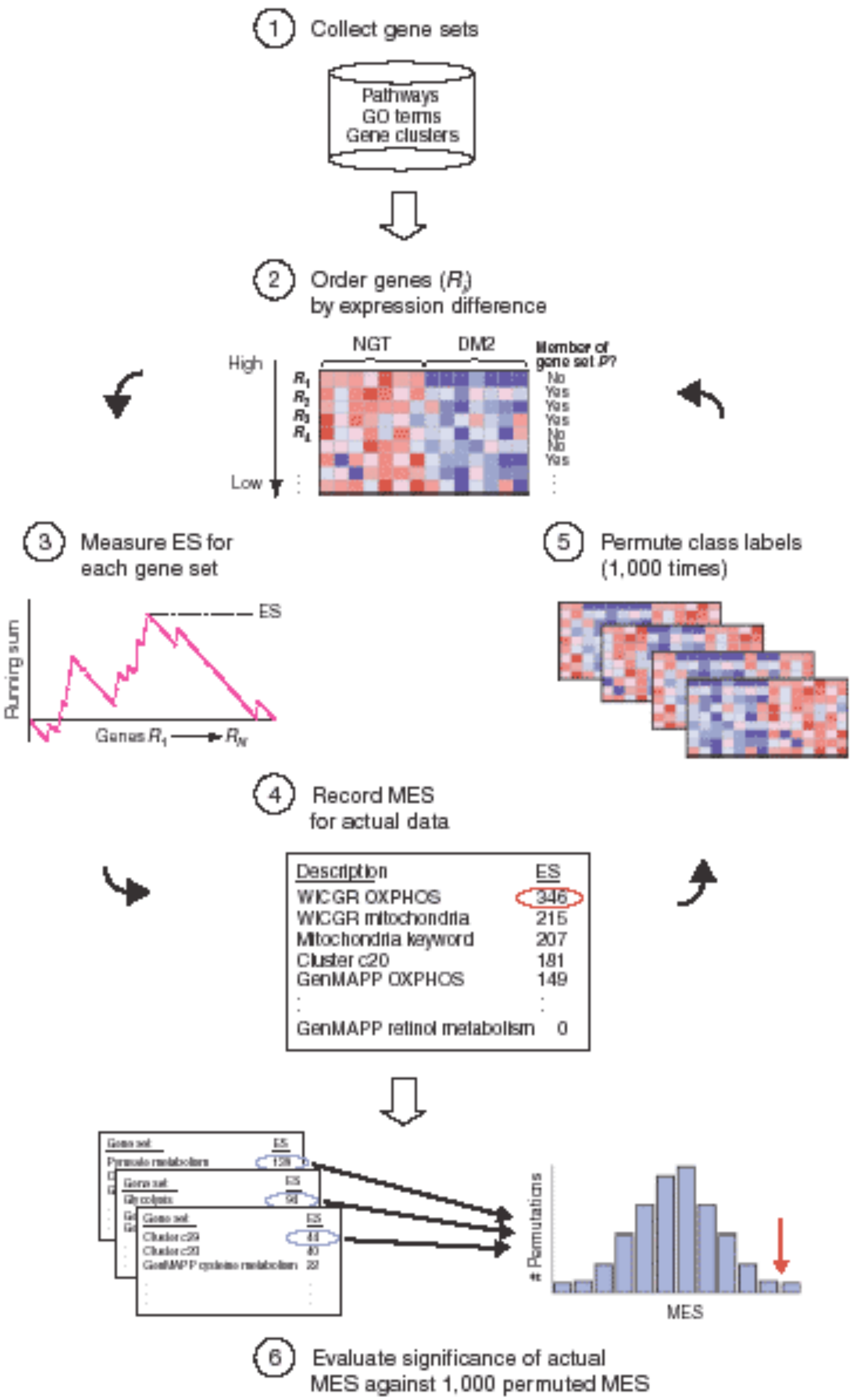
Gene Set Enrichment

- The maximal ES (MES), over all sets S under consideration is recorded.
- For each of B permutations of the class label, ES and MES values are computed.
- The observed MES is then compared to the B values of MES that have been computed, via permutation.
- This is a single *p*-value for all tests and hence needs no correction (on the other hand you are testing only one thing).

From Mootha *et al*

ES=enrichment score
for each gene
= scaled K-S dist

A set called OXPHOS got the largest ES score, with *p=0.029* on *1,000* permutations.



1 Collect gene sets

2 Order genes (R_i) by expression difference

3 Measure ES for each gene set

4 Record MES for actual data

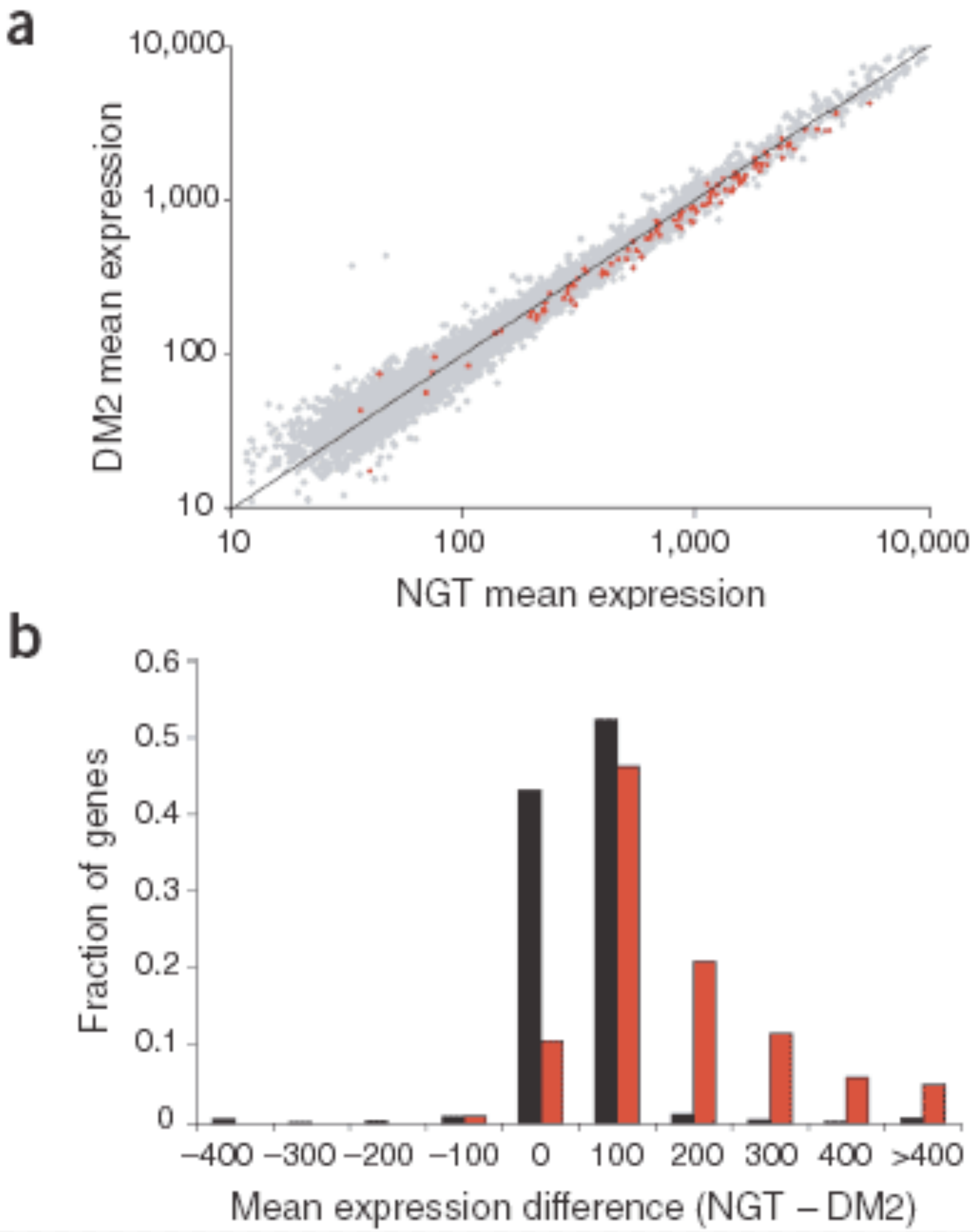
Description	ES
WICGR OXPHOS	346
WICGR mitochondria	215
Mitochondria keyword	207
Cluster c20	181
GenMAPP OXPHOS	149
⋮	⋮
GenMAPP retinol metabolism	0

5 Permute class labels (1,000 times)

6 Evaluate significance of actual MES against 1,000 permuted MES

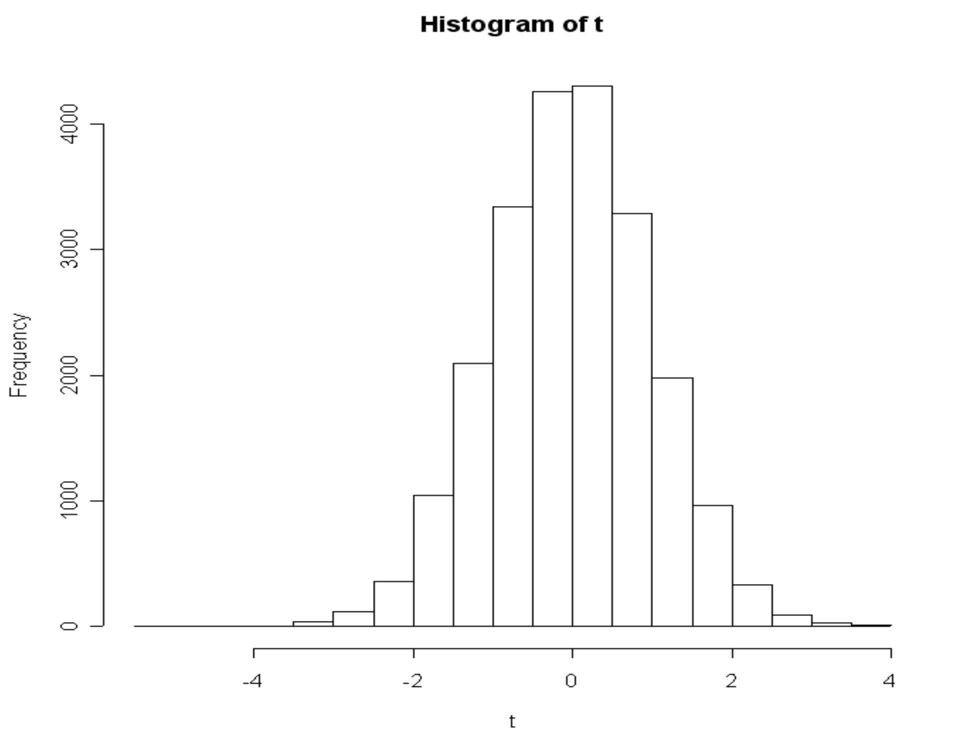
OXPHOS

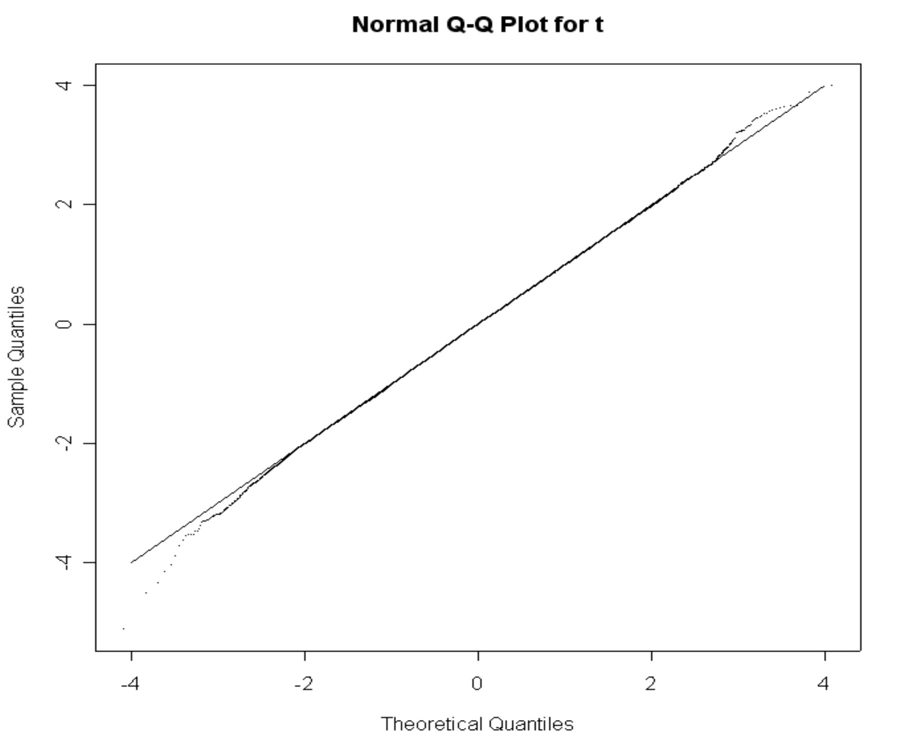
(A small difference for many genes)



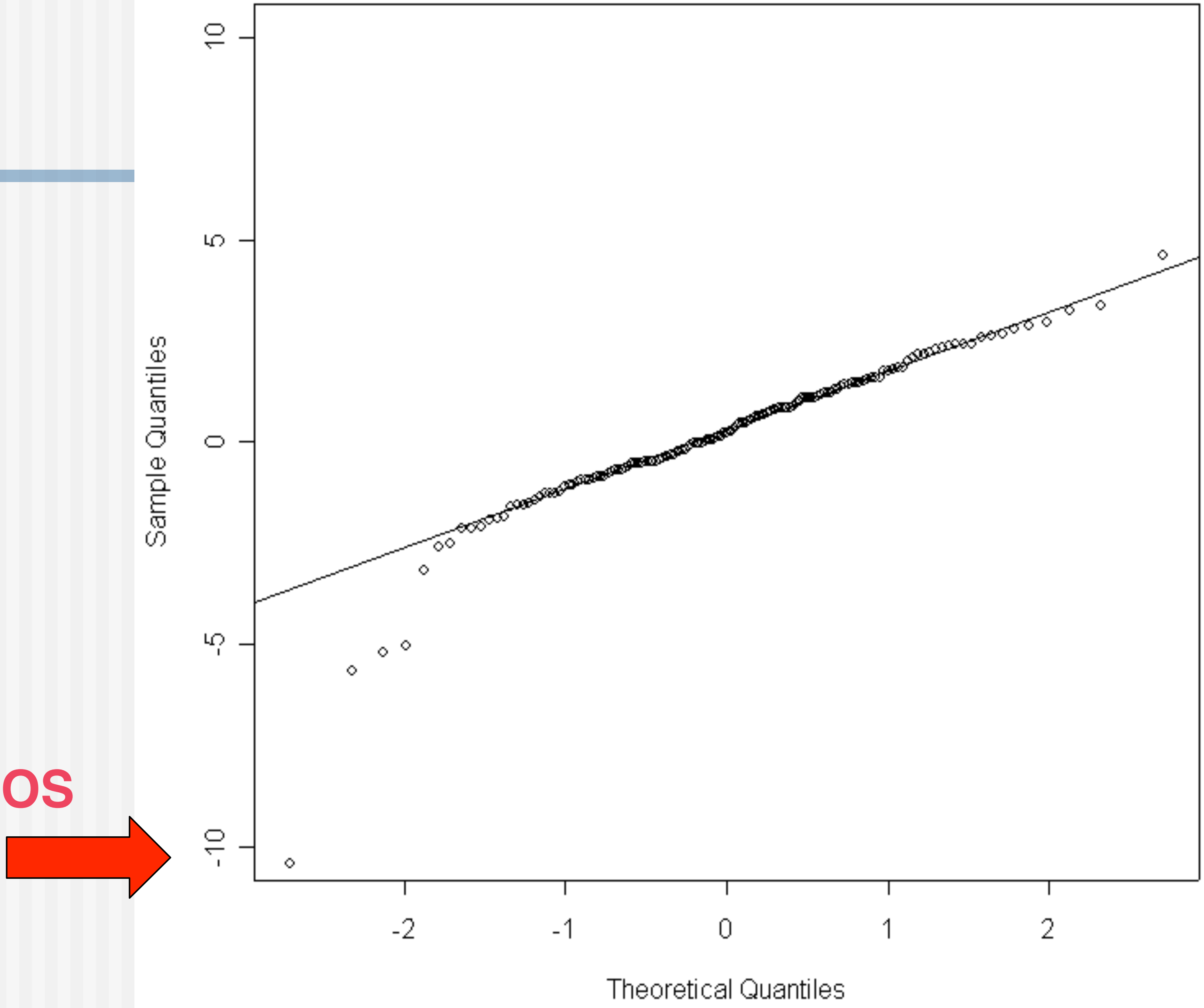
All genes
OXPHOS

Mootha’s ts are approx normal


Histogram of t


Normal Q-Q Plot for t

Normal qq-plot of $\sqrt{n}\ x\ \bar{t}$



OXPHOS

Selection of gene sets

- pathways (KEGG, cMAP, BioCarta)
- molecular function, biological process cellular location (GO)
- predefined sets from the published literature etc
- regions of synteny; chromosome bands
- some care should be exercised to select gene sets that are of interest *a priori*
  - there are more gene sets than genes so you will simply end up back in the multiple comparison problem

Gene Sets

- a set of gene sets is merely a grouping of genes (entities)
- the groups do not need to be exhaustive or disjoint
- we do not need to be completely right, we can have some genes that are not in the gene set, and we can miss some, but not too many
- we are relying on averaging to help adjust for mistakes
- given the state of genomic knowledge this seems reasonable

Software

- There are a number of Bioconductor packages that you can use
- GSEAbase: has basic infrastructure classes etc
- Category: tools for creating incidence matrices and performing tests
- PGSEA, sigPathways, GlobalAncova, are other packages you should consider

Simple Statistical Approach

- the data matrix has G rows (one for each gene) and N columns (one for each sample)
- let’s assume that there are two phenotypes of interest, so we have a two-sample comparison
- we can compute univariate test statistics, **x**, a G-vector
- select some set of gene sets, or gene sets, and let C denote the number of such sets
- you should address the problem that very commonly some genes are represented by a single probe and others by many (same for Hypergeometric testing)

Gene Sets

- define **A**, a C by G matrix, such that **A**[*i,j*]=1 if gene *j* is in gene set *i,* and **A**[*i,j*]=0, otherwise
- the row sums of **A** represent the number of genes in each gene set
- the column sums of **A** represent the number of gene sets a gene is in
- if two rows are identical (for a given set of genes) then the two gene sets are aliased (in the usual statistical sense)
- other patterns can can cause problems and need some study

Gene Sets

- the simplest transformation is to simply sum up the *t*-statistics for all genes in each gene set,

  $$\mathbf{z} = \mathbf{A}\mathbf{x}$$

- we divide the sum by the square root of the number of genes per gene set (this is right if genes are independent - very unrealistic)
- then the resultant statistics, under the null hypothesis, have approximately a N(0,1) distribution
- we could also use other, per gene set, test statistics such as the median, or sign-test

Gene Sets: Reference Distribution

- an alternative is to generate many versions of **x**, the per gene set test statistic, from some reference distribution
- e.g. go back to the original expression data and either permute the sample labels or bootstrap to provide a reference distribution
- you should not (as Tian et al do) permute the gene labels [what is your null hypothesis?]

Comparisons

- you can do either within gene set comparisons
  - for a given gene set is the observed test statistic unusual
- or overall comparisons
  - are any of the observed gene set statistics unusually large with respect to the entire reference distribution
- the former requires some consideration of multiple testing issues
- note that the approach is inherently multivariate, one data set gives G test statistics (one per gene) and these are transformed to yield one per gene set

Bayesian Approach

- following Newton et al, we could compute the posterior probability that a gene is differentially expressed
- then **x**, our G vector is a set of probabilities
- **z** = **Ax**, is then a C vector of the expected number of differentially expressed genes in each gene set

Bayesian Approach

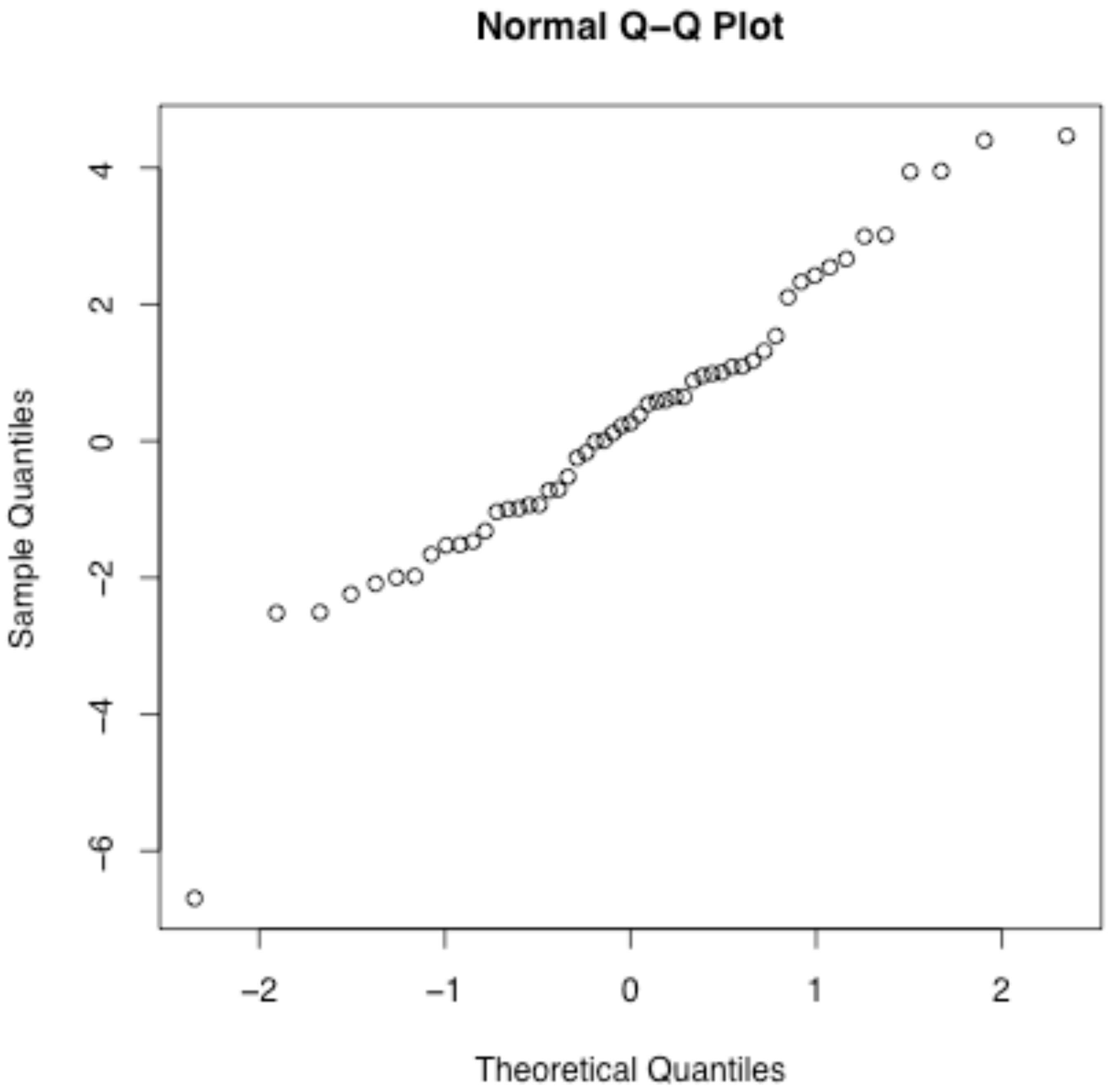
- adjustment for gene set size is needed
- an expected number per gene set can be obtained by using p*=mean of the posterior probabilities and the gene set size
- gene sets that deviate substantially from that expected number are of interest

Example: ALL Data

- samples on patients with ALL were assayed using HGu95Av2 GeneChips
- we were interested in comparing those with BCR/ABL (basically a 9;22 translocation) with those that had no cytogenetic abnormalities (NEG)
- 37 BCR/ABL and 42 NEG

Example: ALL Data

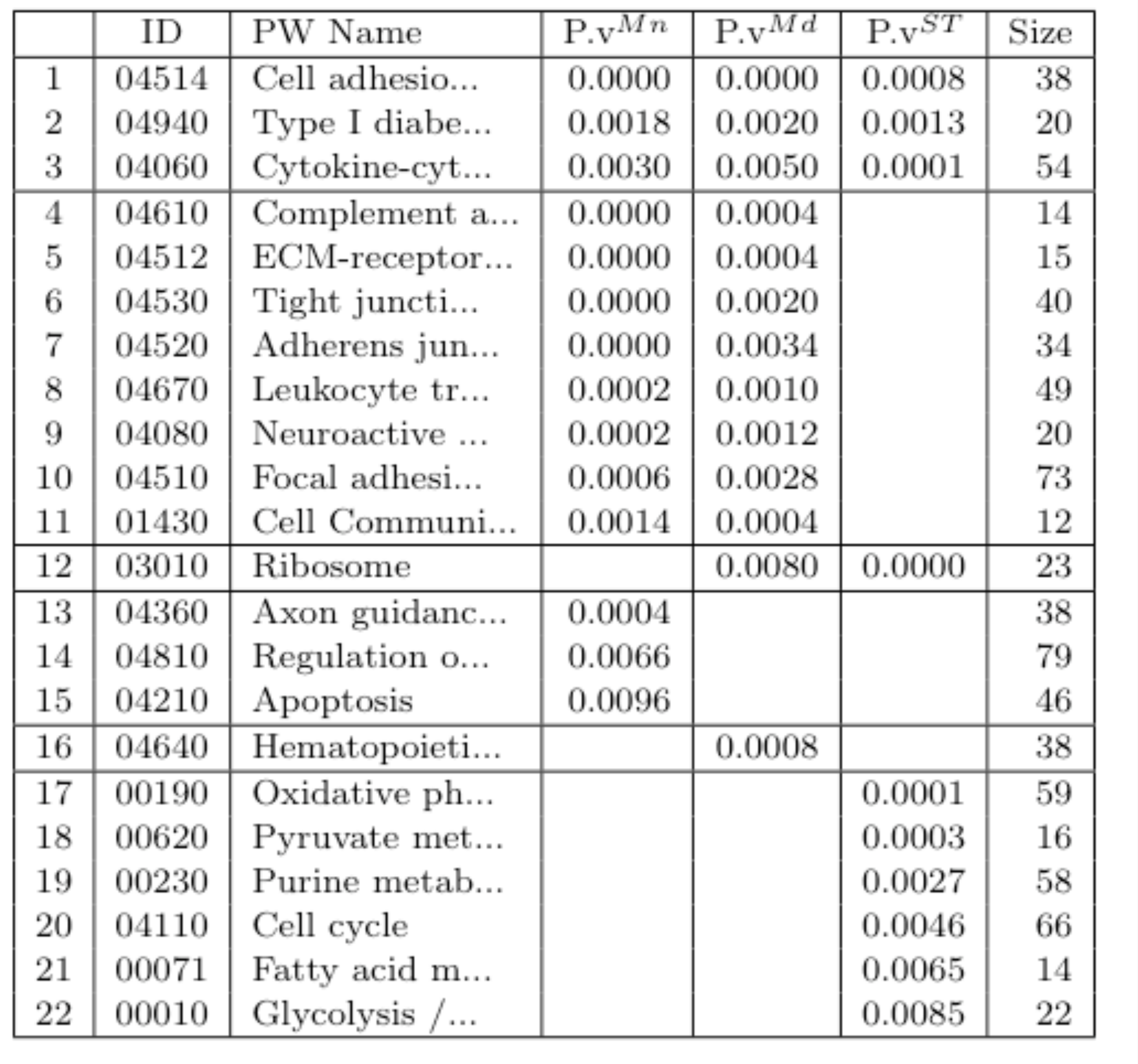
- we then mapped the probes to KEGG pathways
- the mapping to pathways is via EntrezGene ID
  - we have a many-to-one problem and solve it by taking the probe set with the most extreme *t*-statistic
- we chose to only consider pathways with at least 10 genes
- this leaves us with 79 samples, 1036 genes and 70 pathways


Normal Q−Q Plot
Sample Quantiles
4
2
0
−2
−4
−6
Theoretical Quantiles
−2
−1
0
1
2

Which gene sets

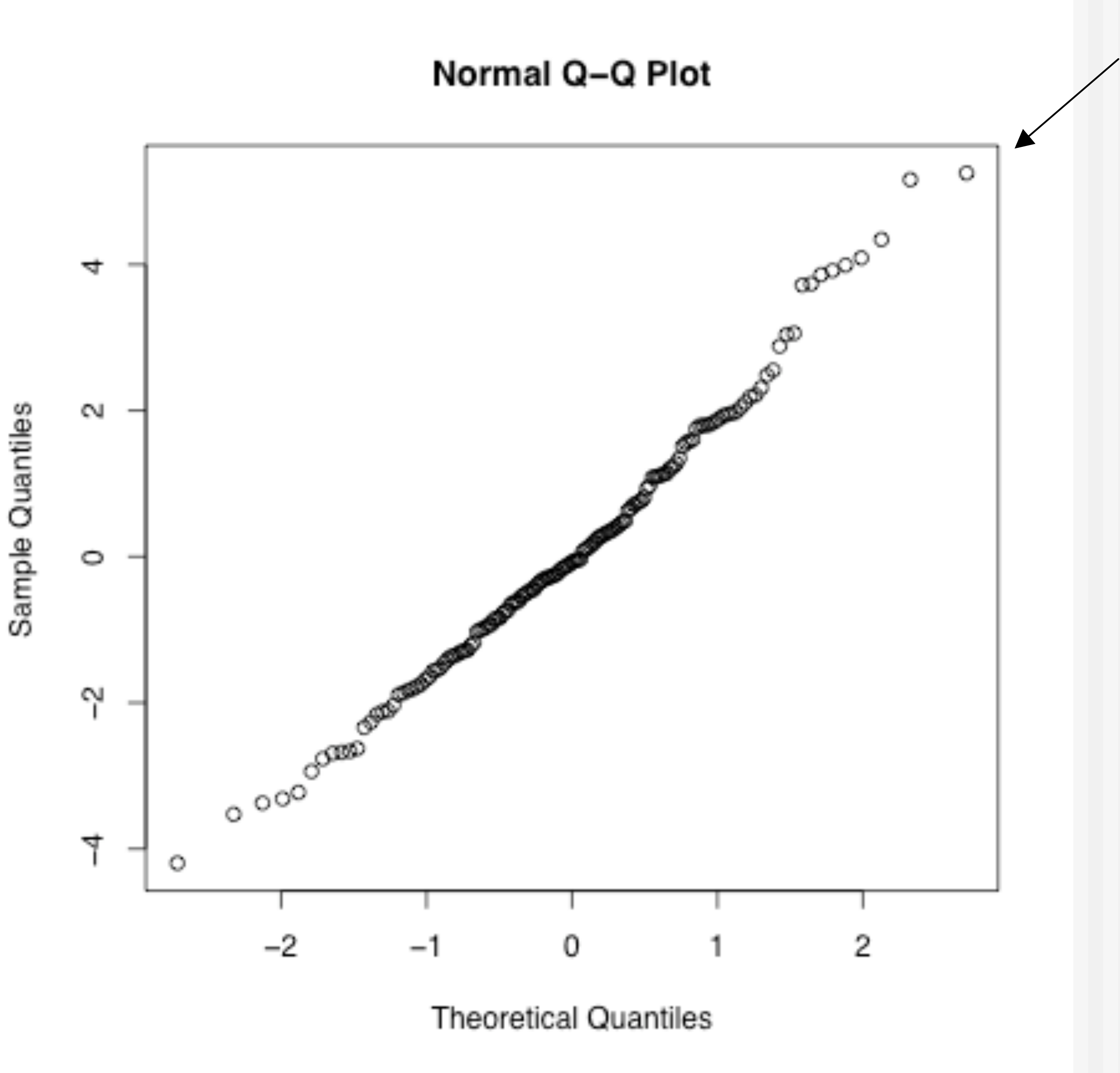
- so the qq-plot looks interesting and identifies at least one gene set that looks interesting
- we identify it, and create a plot that shows the two group means (BCR/ABL and NEG)
- if all points are below or above the 45 degree line that should be interesting

Different Univariate test statistics



	ID	PW Name	P.v^{Mn}	P.v^{Md}	P.v^{ST}	Size
1	04514	Cell adhesio...	0.0000	0.0000	0.0008	38
2	04940	Type I diabe...	0.0018	0.0020	0.0013	20
3	04060	Cytokine-cyt...	0.0030	0.0050	0.0001	54
4	04610	Complement a...	0.0000	0.0004		14
5	04512	ECM-receptor...	0.0000	0.0004		15
6	04530	Tight juncti...	0.0000	0.0020		40
7	04520	Adherens jun...	0.0000	0.0034		34
8	04670	Leukocyte tr...	0.0002	0.0010		49
9	04080	Neuroactive ...	0.0002	0.0012		20
10	04510	Focal adhesi...	0.0006	0.0028		73
11	01430	Cell Communi...	0.0014	0.0004		12
12	03010	Ribosome		0.0080	0.0000	23
13	04360	Axon guidanc...	0.0004			38
14	04810	Regulation o...	0.0066			79
15	04210	Apoptosis	0.0096			46
16	04640	Hematopoieti...		0.0008		38
17	00190	Oxidative ph...			0.0001	59
18	00620	Pyruvate met...			0.0003	16
19	00230	Purine metab...			0.0027	58
20	04110	Cell cycle			0.0046	66
21	00071	Fatty acid m...			0.0065	14
22	00010	Glycolysis /...			0.0085	22

BCR/ABL vs NEG - gene sets are cytochrome band (only those with more than 10 genes per band)

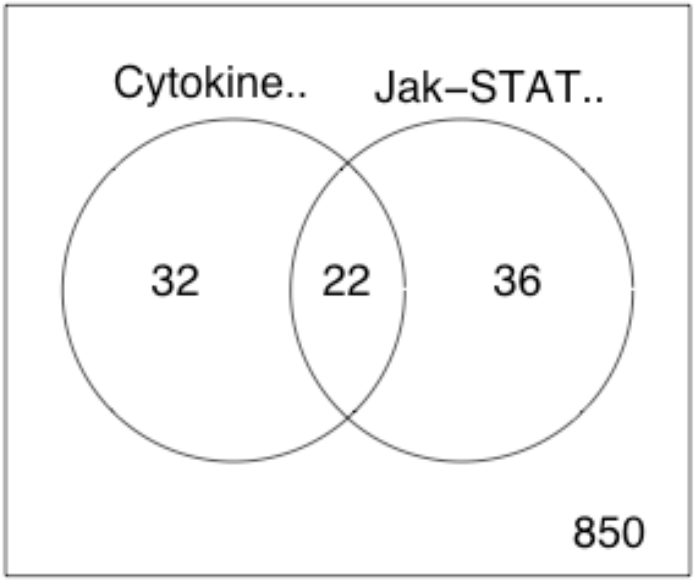


Two largest are 9q34 and 1p36 - both already implicat ed

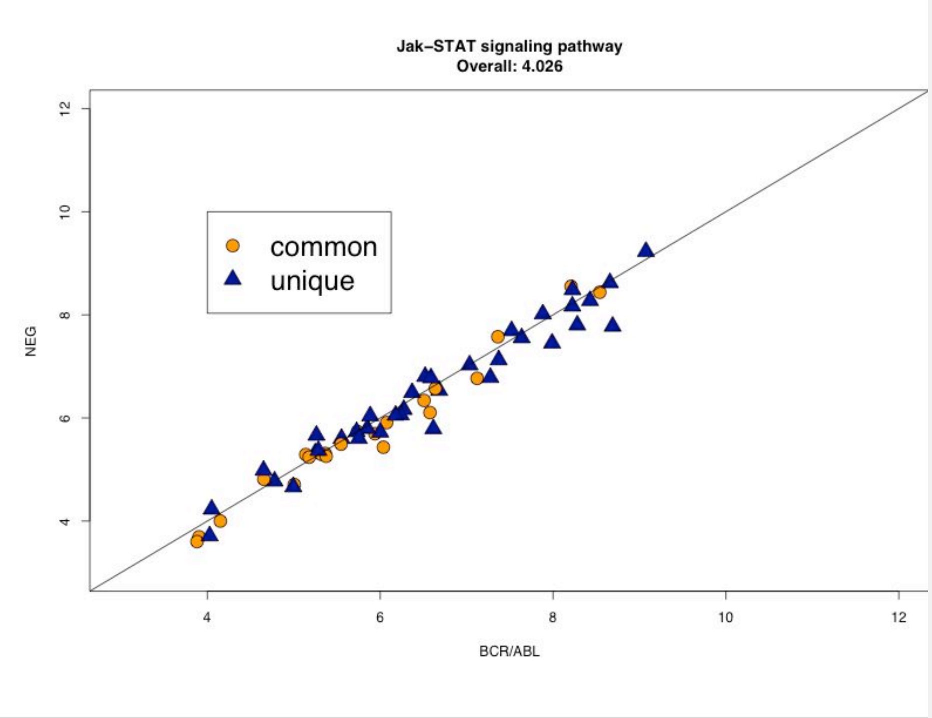
Aliasing

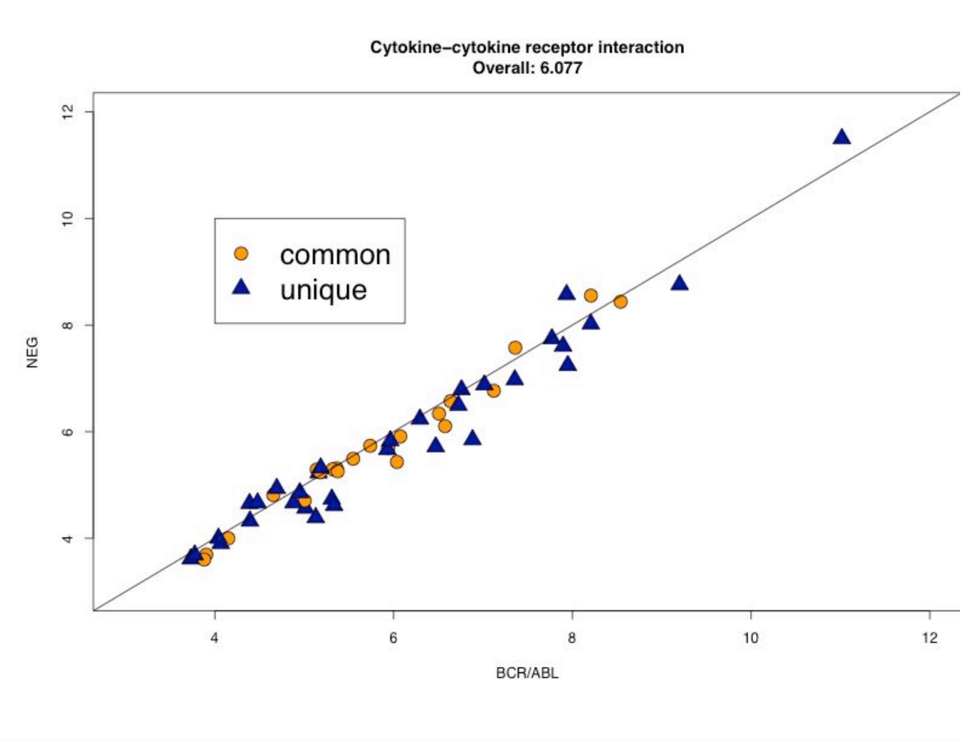
- all others have ignored this - but it does matter
- when we use gene sets, two gene sets can have substantial overlap
- if they are both significant, we might ask why

For cytokine-cytokine and Jak-Stat we have



Comparison of Gene Expression





The Analysis

- and when the genes involved, are separated into three groups
  - those in Cytokine-Cytokine only
  - those in Jak-Stat only
  - those common
- then we find that the first and third are significant, but the second (Jak-Stat alone) is not

Some other extensions

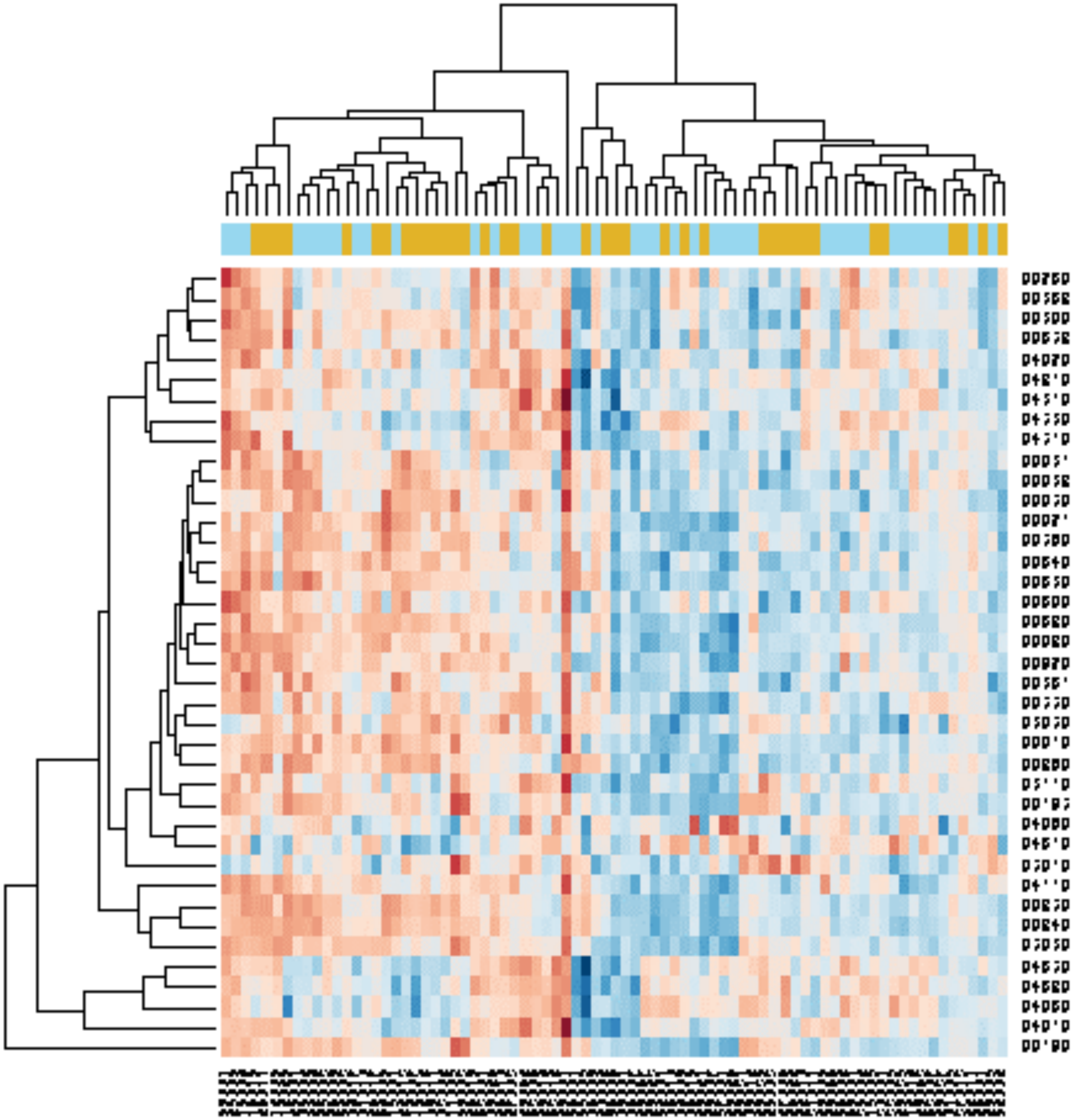
- gene sets might be a better way to do meta-analysis
- one of the fundamental problems with meta-analysis on gene expression data is the gene matching problem
- even technical replicates on the same array do not show similar expression patterns

Extensions

- if instead we compute per gene set effects these are sort of independent of the probes that were used
- matching is easier and potentially more biologically relevant
- the problem of adjustment still exists; how do we make two gene sets with different numbers of expression estimates comparable

Extensions

- you can do per array computations
- residuals are one of the most underused tools for analyzing microarrays
- we first filter genes for variability
- next standardize on a per gene basis - subtract the median divide by MAD
- now X*= AX, is a Cxn array, one entry for each gene set for each sample



Concluding Remarks

- the analysis of gene expression data still requires more research
- we should be looking at mechanisms for coordinated expression
  - transcription factors
  - amplifications
  - deletions
  - change in chromatin structure

Concluding Remarks

- *p*-value corrections are not really the right approach here
- bringing more biology to bear seems to be more likely to bear fruit
- we need some results to indicate how to deal with the coordinated gene expression (lack of independence within a gene set)

Acknowledgements

- Terry Speed (also some slides are his)
- Arden Miller
- Vincent Carey
- Michael Newton
- Kasper Hansen
- Jerry Ritz
- Sabina Chiaretti
- Sandrine Dudoit
- Zhen Jiang
- Seth Falcon