

Overlapping Probabilities of Top Ranking Gene Lists, Hypergeometric Distribution, and Stringency of Gene Selection Criterion

Wen Fury, Franak Batliwalla, Peter K. Gregersen, and Wentian Li

Abstract—When the same set of genes appear in two top ranking gene lists in two different studies, it is often of interest to estimate the probability for this being a chance event. This overlapping probability is well known to follow the hypergeometric distribution. Usually, the lengths of top-ranking gene lists are assumed to be fixed, by using a pre-set criterion on, e.g., p -value for the t -test. We investigate how overlapping probability changes with the gene selection criterion, or simply, with the length of the top-ranking gene lists. It is concluded that overlapping probability is indeed a function of the gene list length, and its statistical significance should be quoted in the context of gene selection criterion.

I. INTRODUCTION

One of the most common tasks in microarray analysis is to identify a list of genes that are differentially expressed under two conditions, such as being affected by a disease vs. normal, before vs. after a medical treatment, and one vs. another disease subtype. The number of genes on the top-ranking list is usually much smaller than the total number of genes on the chip, n . If the same type of microarray chip is used for two different studies (e.g. disease-A vs. control, and disease-B vs. control), two differentially expressed gene lists can be obtained, with n_1 and n_2 genes. Researchers often find the same genes appear in both lists and hypothesize that these common genes are involved the etiology of both diseases.

However, for such a hypothesis to be convincing, one has to first estimate the probability for overlapping genes by chance alone. In other words, if two lists of genes are selected out of n genes randomly, we would like to calculate the probability for m genes in common in the two lists, with the lengths of the two lists being n_1 and n_2 . This overlapping probability is known to follow the hypergeometric distribution¹. The name hypergeometric distribution was first used in [1], and was popularized by its role in Fisher's exact test [2].

In microarray analysis, overlapping probability and hypergeometric distribution mainly appear in testing the enrichment of genes in certain functional category [3], [4], [5], [6], [7], [8], [9], [10]. In this application, the first list is the top-ranking differentially expressed genes, and a gene

selection process is involved. The second list is nevertheless given: n_2 genes are known to be in a pathway, a member of a protein family, described by a gene ontology term, etc. One asks the question on chance probability for m out of n_1 selected genes to be in a given pathway, a protein family, and describable by a gene ontology term. Fixing n_2 or not is the main difference between their application and ours.

When a different gene selection criterion is used, the number of genes in the two top-ranking lists of two studies (n_1 and n_2) will also change. Because the stringency of a gene selection criterion is always adjustable and to some extent arbitrary, we would like to examine whether these changes will affect the overlapping probability. At two extreme situations, very small $n_1 = n_2 \approx 1$ and very large $n_1 = n_2 = n$, it is clear that the number of overlapping genes is $m = 0$ and $m = n$. These m values appear 100% of the times, so the corresponding p -value is equal to 1, i.e., not significant. For intermediate $n_1 \approx n_2$ values, it is not clear what the overlapping probability and significance will be, and it is the topic of this abstract.

II. HYPERGEOMETRIC DISTRIBUTION AND OVERLAPPING P-VALUES

Given integers n, n_1, n_2, m ($\max(n_1, n_2) \leq n$ and $m \leq \min(n_1, n_2)$), the hypergeometric distribution is defined as

$$P(m) = \frac{C(n_1, m)C(n - n_1, n_2 - m)}{C(n, n_2)} = \frac{\binom{n_1}{m} \binom{n - n_1}{n_2 - m}}{\binom{n}{n_2}}$$

where $C(n, m)$ is the number of possibilities of choosing m objects out of n objects: $C(n, m) = n!/[m!(n - m)!]$.

When n_1 genes are randomly chosen from the total of n genes, and another random sampling leads to n_2 genes, the probability that the two lists of genes have m in common is exactly the hypergeometric probability $P(m)$. This can be proven by the following steps: 1) The total number of possible choices for the two lists of genes is $C(n, n_1) \cdot C(n, n_2)$. 2) There are $C(n, n_1)$ possibilities for choosing the first list. 3) Among the n_1 genes in the first list, there are $C(n_1, m)$ possibilities for choosing m genes to be in common with the second list. 4) In the second list, besides the m genes that are in common with the first list, the remaining $n_2 - m$ genes are chosen among the $n - n_1$ "leftover" genes not in the first list, thus $C(n - n_1, n_2 - m)$ possibilities. The $P(m)$ is simply $(\#2 \times \#3 \times \#4) / \#1$. Note that n_1 and n_2 can be switched without changing the $P(m)$ value.

W. Fury is a Senior Bioinformatics Scientist at Regeneron Pharmaceutical, Inc. Tarrytown, NY 10591, USA. wen.fury@regeneron.com

F. Batliwalla, P.K. Gregersen, and W. Li are Research Scientists with the Robert S Boas Center for Genomics and Human Genetics, Feinstein Institute for Medical Research, North Shore LIJ Health System, Manhasset, NY 11030, USA fb@nshs.edu, peterg@nshs.edu, wli@nslj-genetics.org

¹Despite certain similarity, this problem is not the birthday problem – the probability for two people in a room to have the same birthday.

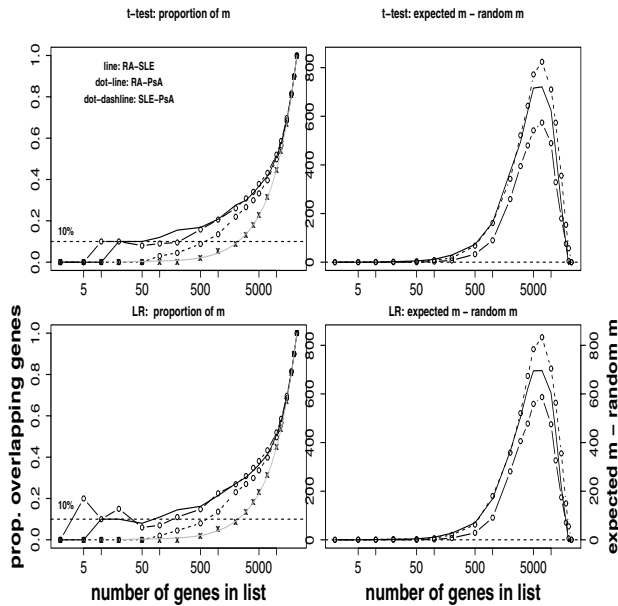


Fig. 1. First column: proportion of overlapping genes between two top ranking gene lists for a pair of studies (m/n_1) as a function of the gene list length ($n_1 (= n_2)$). Top is for gene ranking by t -test and bottom is for gene ranking by logistic regression. The overlapping proportion for two randomly shuffled lists is shown in crosses, and the line $m/n_1 = n_1/n$ is marked. Second column: observed number of overlapping genes (m) subtract the expected number of overlapping genes (n_1^2/n).

It is usually more interesting to calculate the sum of $P(m)$ for m 's equal or larger than the observed value (i.e., the p -value):

$$p\text{-value} = \sum_{k=m}^{\min(n_1, n_2)} p(k) = \sum_{k=0}^{\min(n_1, n_2)} p(k) - \sum_{k=0}^{m-1} p(k)$$

In statistical package *R* (<http://www.r-project.org/>), there are at least two ways to calculate the overlapping p -value. The first is to use the accumulative distribution of hypergeometric distribution, $\text{phyper}(m, n_1, n - n_1, n_2)$: $p\text{-value} = \text{phyper}(\min(n_1, n_2), n_1, n - n_1, n_2) - \text{phyper}(m - 1, n_1, n - n_1, n_2)$ if $m > 0$, and $p\text{-value} = 1$ if $m = 0$. The second method is to use the p -value from the Fisher's exact test on the following 2-by-2 table:

	col_1	col_2	$total$
row_1	m	$n_1 - m$	n_1
row_2	$n_2 - m$	$n - n_1 - n_2 + m$	$n - n_1$
$total$	n_2	$n - n_2$	n

The two approaches lead to the identical result.

III. PROPORTION OF OVERLAPPING GENES IN A COLLECTION OF MICROARRAY DATASET

In hypergeometric distribution, the number of overlapping elements m is an independent variable from the list lengths n_1, n_2 . In order to get a rough idea on how m changes with the list lengths, we use three real microarray datasets. These studies concern three autoimmune diseases: rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and psoriatic arthritis (PsA), described in details in [11], [12],

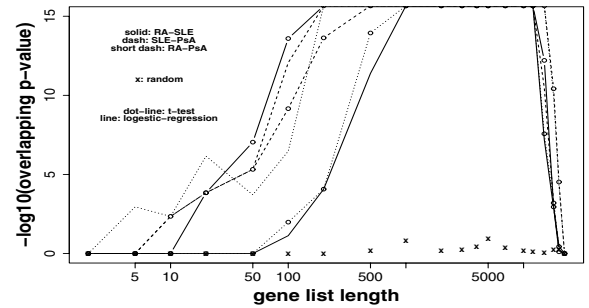


Fig. 2. Overlapping significance as measured by $-\log_{10}(p\text{-value})$ where p -value is obtained by the hypergeometric distribution, as a function of $n_1 (= n_2)$, the number of genes in the top-ranking gene lists. The *R* program reports p -value to be zero whenever it is lower than 2.2×10^{-16} , and we use a ceiling of $15.65758 = -\log_{10}(2.2 \times 10^{-16})$ in the plot. Six lines are shown for three study pairs (RA-SLE, SLE-PsA, RA-PsA) and two tests/models (t -test and logistic regression). Similar overlapping significance for two randomly shuffled lists is also shown (indicated by crosses).

[13]. The number of controls (C) and patients (P) in these three datasets are (C=39, P=46), (C=41, P=81), and (C=19, P=19), respectively. The total number of genes/probe-sets is $n = 22283$, and the expression levels are log transformed. Genes are ranked for their degree of differential expression which can be measured by various tests or models, such as t -test and logistic regression.

For any pair of studies, with a fixed number of top-ranking gene lists $n_1 (= n_2)$, one can count the number of overlapping genes m and the proportion $m/n_1 (= m/n_2)$. Fig.1 (left column) shows this proportion as a function of $n_1 (= n_2)$ for three study-pairs (RA-SLE, SLE-PsA, RA-PsA) as well as for two ranking methods (t -test and logistic regression). Similar overlapping proportion of two random shuffled lists is also indicated in Fig.1 as crosses.

When $n_1 (= n_2)$ is small, m is more likely to be zero, so the proportion is also zero. When $n_1 (= n_2)$ approaches the total number of genes, n , all genes are overlapping genes, and the proportion is 1. Fig. 1 indeed shows these trends at the two extreme points. In order to check behavior in-between, we draw a reference line in Fig.1 (left column) that assume a linear relationship between m/n_1 and n_1/n . Most of the points on Fig.1 are above this line, and the overlapping proportion of two random lists is exactly on this line.

To have an idea of the absolute number of common genes more than expected by random chance, Fig.1 (right column) plots the observed m subtract the expected $m_{exp} = n_1^2/n (= n_2^2/n)$ as a function of $n_1 (= n_2)$. The maximum difference between the observed and expected is reached between $n_1 = 5000$ and $n_1 = 10000$. The difference of observed and expected m 's can be as much as 600–800.

IV. OVERLAPPING SIGNIFICANCE

The overlapping p -value corresponding to the m counts plotted in Fig.1 was calculated by the hypergeometric distribution, and is shown in Fig.2: y -axis is $-\log_{10}(p\text{-value})$, and x -axis is $n_1 (= n_2)$. Six lines are shown for three comparisons (RA-SLE, SLE-PsA, RA-PsA) and two measurements of the differential expression (t -test and logistic regression).

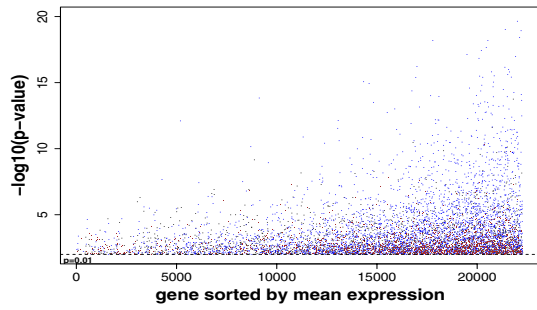


Fig. 3. The test significance ($-\log_{10}(p\text{-value})$) from t -test of $n=22283$ genes sorted by the averaged expression level (log-transformed) across all 245 samples in 3 studies (RA, SLE, PsA). The three t -tests are for RA vs. control (black dots), SLE vs. control (blue dots), and PsA vs. control (brown dots). Only the genes with p -value smaller than 0.01 are displayed.

Zero p -values are converted to 2.2×10^{-16} which is the minimum value reported by R program. Fig.2 shows that besides the two ends ($m = n_1 = n_2 = 0$ and $m = n_1 = n_2 = n$) where the p -value is 1, the overlapping significance quickly increases with the length of top-ranking gene list $n_1 (= n_2)$, and can be extremely significant when a large number of genes are kept in the two lists for comparison.

This result confirm our previous suspicion that overlapping significance is a function of the gene list lengths. If the selection of n_1, n_2 is arbitrary, the overlapping significance thus calculated is also arbitrary. It is not surprising that overlapping significance may keep increasing (or, p -value decreasing) with the increase of $n_1 (= n_2)$, because p -value in general depends on the sample size. When a signal is real (true positive), p -value will monotonically decrease with the sample size. On the contrast, if a true signal is absent, the sample size does not affect the conclusion. As can be seen in Fig.2, the overlapping significance for two random lists does not really change with $n_1 (= n_2)$.

One may argue that it is unlikely to consider top 5000 genes as being differentially expressed, because by a typical selection criterion (e.g. p -value of t -test smaller than 0.01, with or without multiple testing correction), the number of genes selected is less than a few hundreds. However, as can be seen in Fig.2, even in the range of 10–500, the overlapping p -value changes dramatically.

This pitfall of gene-list-length dependence of overlapping p -values has not been noticed before perhaps because in other application of hypergeometric distribution for calculating overlapping probability, the length of the second list n_2 is fixed, for example, in the study of overrepresentation of genes in certain pathway. The number of overlapping genes m is then constrained from above by $\min(n_1, n_2)$ even though the length of the first list, n_1 , might increase by relaxing the gene selection criterion.

V. THE EFFECTS OF UNEXPRESSED GENES

There are many genes/probe-sets on the microarray chip that do not register much signal. Since these low-expressed genes are lowly expressed in both control and patient samples, they usually do not appear in the top-ranking differentially expressed gene list. Fig.3 shows $-\log_{10}(p\text{-value})$ of

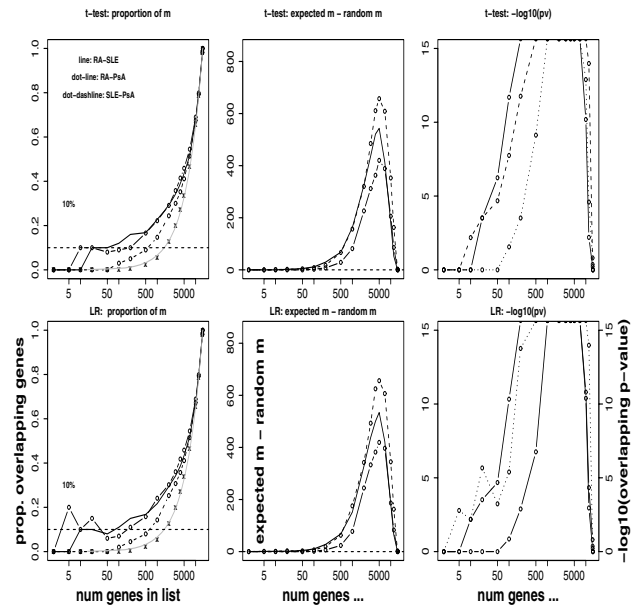


Fig. 4. Several measures of overlapping genes between a pair of studies as a function of the number of genes included in the top-ranking list, for the reduced dataset with 15283 genes. First column: proportion of overlapping genes (m/n_1); second column: number of observed overlapping genes subtracting the number of expected ($m - n_1^2/15283$); third column: $-\log_{10}(p\text{-value})$ by the hypergeometric distribution. First row is for lists ranked by t -test result, and second row is for lists ranked by logistic regression.

each gene of 3 t -tests sorted by average expression (log-transformed) across all 245 samples in 3 datasets (for both cases and controls). Although we cannot use the average expression level to predict the degree of differential expression, there is a general trend for low-expressed genes to rank lower in the differentially expressed list as seen from Fig.3.

We removed 7000 genes with lower overall expression across all samples, leaving $n = 15283$ genes. Figs.1 and 2 are reproduced in Fig.4 for the dataset with a reduced gene pool. As in Figs.1 and 2, the observed number of overlapping genes m is much larger than the expected, though the difference peaks at 400–600, as versus 600–800 in Fig.1. The overlapping significance as measured by $-\log(p\text{-value})$ again quickly moves up with $n_1 (= n_2)$ as shown in the last column of Fig.4.

The qualitative similarity between Figs.1, 2 and Fig.4 indicates that the presence of low-expressed genes does not affect our conclusion.

VI. CONCLUSIONS AND FUTURE WORKS

A. Conclusions

Using the hypergeometric distribution to calculate the overlapping probability between two top-ranking differentially expressed genes in two studies, we have shown that the overlapping significance depends on the stringency of gene selection criterion, or equivalently, the length of the gene lists. This observation presents a problem when an overlapping p -value is reported but the gene selection criterion is not specified. On the other hand, the increase of the overlapping significance with the gene list length can be an indication that the significant overlapping of genes is a true signal.

B. Future Works

The overlapping probability calculated here assumes the two top-ranking gene lists are selected from the same pool of n genes. If the two studies are based on different chip platforms, the two initial gene pools are not identical, though there are perhaps certain common genes. We plan to derive the overlapping distribution for this situation.

We also plan to study the probability for genes appearing in three top-ranking gene lists. Although a permutation based approach comparing multiple studies was proposed in [14], there is no analytic formula available.

VII. ACKNOWLEDGMENTS

We would like to thank Prof. Richard Friedberg for suggestions.

REFERENCES

- [1] H.T. Gonin, "The use of factorial moments in the treatment of the hypergeometric distribution and in tests for regression", *Philosophical Mag.*, vol 7, 1936, pp 215-226.
- [2] R.A. Fisher, *Statistical Methods for Research Workers* Oliver and Boyd, Edinburgh; 1934.
- [3] S. Tavazoie, J.D. Hughes, M.J. Campbell, R.J. Cho, G.M. Church, "Systematic determination of genetic network architecture", *Nature Genet.*, vol 22, 1999, pp 281-285.
- [4] S. Drăghici, P. Khatri, R.P. Martins, G.C. Ostermeier, S.A. Krawetz, "Global functional profiling of gene expression", *Genomics*, vol 81, 2003, pp.98-104.
- [5] G. Finocchiaro, F. Mancuso, H. Muller, "Mining published lists of cancer related microarray experiments: identification of a gene expression signature having a critical role in cell-cycle control", *BMC Bioinf.*, vol 6(suppl 4), 2003, S14.
- [6] D.A. Hosack, G. Dennis Jr., B.T. Sherman, H.C. Lane, R.A. Lempicki (2003), "Identifying biological themes within lists of genes with EASE", *Genome Biol.*, vol 4, 2003, R70.
- [7] A. Boorsma, B.C. Foat, D. Vis, F. Klis, H.J. Bussemaker, "T-profiler: scoring the activity of predefined groups of genes using gene expression data", *Nucleic Acids Res.*, vol 33, 2005, pp W592-W595.
- [8] R.K. Curtis, M. Orešič, A. Vidal-Puig, "Pathways to the analysis of microarray data", *Trends Biotech.*, vol 23, 2005, pp 429-435.
- [9] X. Mao, T. Cai, J.G. Olyarchuk, L. Wei, "Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary", *Bioinfo.*, vol 21, 2005, pp 3787-3793.
- [10] L. Tian, S.A. Greenberg, S.W. Kong, J. Altschuler, I.S. Kohane, P.J. Park, "Discovering statistically significant pathways in expression profiling studies", *Proc. Natl. Acad. Sci.*, vol 102, 2005, pp 13544-13549.
- [11] F.M. Batliwalla, E.C. Baechler, X. Xiao, W. Li, S. Balasubramanian, H. Khalili, A. Damle, W.A. Ortmann, A. Perrone, A.B. Kantor, M. Kern, P.S. Gulko, M. Kern, R. Furie, T.W. Behrens, P.K. Gregersen, "Peripheral blood gene expression profiling in rheumatoid arthritis", *Gene and Immunity*, vol 6, 2005, pp 388-397.
- [12] E.C. Baechler, F.M. Batliwalla, G. Karypis, P.M. Gaffney, W.A. Ortmann, K.J. Espe, K.B. Shark, W.J. Grande, K.M. Hughes, V. Kapur, P.K. Gregersen, T.W. Behrens, "Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus", *Proc. Natl. Acad. Sci.*, vol 100, 2003, pp 2610-2615.
- [13] F.M. Batliwalla, W. Li, C.T. Ritchlin, X. Xiao, M. Brenner, T. Laragione, T. Shao, R. Durham, S. Kemshetti, E. Schwarz, R. Coe, M. Kern, E.C. Baechler, T.W. Behrens, P.K. Gregersen, P.K. Gulko, "Microarray analyses of peripheral blood cells identifies unique expression signature in psoriatic arthritis", *Mol. Med.*, 2006, to appear.
- [14] D.R. Rhodes, J. Yu, K. Shanker, N. Deshpande, R. Varambally, D. Ghosh, T. Barrette, A. Pandey, A.M. Chinnaiyan, "Large-scale meta-analysis of cancer microarray data identifies common transcriptional profiles of neoplastic transformation and progression", *Proc. Natl. Acad. Sci.*, vol 101, 2004, pp 9309-9314.