Discrete nonparametric algorithms for outlier detection with genomic data

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Abstract
In high-throughput studies involving genetic data such as from gene expression microarrays, differential expression analysis between two or more experimental conditions has been a very common analytical task. Much of the resulting literature on multiple comparisons has paid relatively little attention to the choice of test statistic. In this article, we focus on the issue of choice of test statistic based on a special pattern of differential expression. The approach here is based on recasting multiple comparisons procedures for assessing outlying expression values. A major complication is that the resulting p-values are discrete; some theoretical properties of sequential testing procedures in this context are explored. We propose the use of q-value estimation procedures in this setting. Data from a gene expression profiling experiment in prostate cancer are used to illustrate the methodology.

1 Introduction
Genomic technologies have permeated scientific experimentation, especially in the area of cancer research (Ludwig and Weinstein, 2005). One of the major tasks using these assays is to find genes that are differentially expressed between two experimental conditions. The simplest example is to find genes that are up- or down-regulated in cancerous tissue relative to healthy tissue. Assessing differential expression in this setting leads to a massive multiple comparisons problem that results from performing thousands of tests univariate for each gene. This has led to an explosion of literature on statistical methods for differential expression in genomic studies; see Ge et al. (2003) for a recent review on the topic.

Many authors have advocated for control of the false discovery rate (FDR) (Benjamini and Hochberg, 1995) relative to the traditional familywise type I error (FWER). Less thought
has been provided on the choice of test statistic to use. The two most commonly used
statistics in the two-sample problem are the t-test and the Wilcoxon rank sum test.

Recently, Tomlins et al. (2005) identified a gene fusion in prostate cancer. They discov-
ered it by making the following observation. For certain genes, only a fraction of samples
in one group were overexpressed relative to those in the other group; the remaining samples
showed no evidence of differential expression. They used a method called Cancer Outlier
Profile Analysis (COPA) to rank such genes using expression data generated by microar-
rays. Using such a score identified one of the genes involved in the fusion event. Tomlins
et al. (2005) proposed a statistic but gave no way of assessing its significance statistically.
Recently, Tibshirani and Hastie (2007) and Wu (2007) have proposed modifications of two-
sample t-tests to address the same problem. Ghosh and Chinnaiyan (2009) have cast these
procedures into a general statistical framework.

In this article we consider discretized versions of the procedures given in Ghosh and
Chinnaiyan (2009). They linked outlier detection to the multiple testing problem. While
many authors have studied and developed methods for controlling the false discovery rate
and related metrics (e.g Efron et al., 2001; Genovese and Wasserman, 2004), they all presume
that the test statistics being used come from a continuous distribution. Consequently, under
the null hypothesis, they are distributed as a Uniform(0,1) random variable. This fact no
longer holds when the test statistics are discrete. One key issue we address here is how to
perform multiple testing with discrete test statistics. We review previous work and show that
sequential testing procedures are instable in a particular technical sense. We then describe
algorithms that effectively smooth the discrete p-values in order to perform false discovery
rate estimation, for which we prove results about asymptotic control of the false discovery
rate.

We also consider the situation of more than two groups. This was studied by Liu and Wu
(2007) using modifications of two-sample testing methodologies. In this article, we address
the problem in two different directions. The first is that we will use multiple testing metrics
for identification of outliers. As in Ghosh and Chinnaian (2009), we cast the relevant statistical problem as one of outlier detection and propose a hypothesis testing framework for outlier detection. Note that while outlier has a very specific statistical interpretation as being aberrant from the usual population, for our purposes, genes with high degrees of ‘outlyingness’ are of great interest. Second, we will again have discrete test statistics, so the multiple testing adjustments proposed by Liu and Wu (2007) do not apply here.

The paper is structured as follows. In section 2, we describe the data setup and formulate the statistical model for outlier detection. We also show how to formulate outlier detection as a hypothesis testing problem, which leads to a natural link with multiple testing procedures. We then review previous methods for multiple testing with discrete statistics and discuss a notion of instability for sequential testing procedures. We then propose a false discovery rate estimation procedure in Section 4, along with its extension to multiple groups and a result proving that it asymptotically controls the false discovery rate. In Section 5, the proposed methods are applied to data from a prostate cancer gene expression study. We conclude with some discussion in Section 6.

2 Preliminaries

2.1 Data and outlier detection/multiple testing paradigm

The data consist of \((Y_{gij})\), where \(Y_{gij}\) is the gene expression measurement on the \(g\)th gene for the \(i\)th subject in group \(g = 1, \ldots, G, j = 0, \ldots, (J − 1), i = 1, \ldots, nj\). The indexing for \(j\) is such that we have \(J\) groups. We will refer to the group with \(j = 0\) as the baseline group.

For a given gene \(g\), we wish to identify the samples that have outlying values with respect to the gene. We assume that we will use the samples with \(j = 0\) (i.e., the baseline group) as the baseline distribution with which to assess ‘outlyingness.’

We can phrase this as the following hypothesis testing problem: test the hypothesis \(H^i_0\) : the \(i\)th individual does not have outlying expression values of gene \(g\) relative to the baseline population against \(H^i_1\) : the \(i\)th disease individual does have outlying expression values of
gene \( g \) relative to the baseline population. For each gene we can perform such a test. We can summarize the results of \( n_1 \) such tests in Table 1:

<table>
<thead>
<tr>
<th>Decide Outlier</th>
<th>Decide Non-outlier</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Non-Outlier</td>
<td>( W )</td>
<td>( V )</td>
</tr>
<tr>
<td>True Outlier</td>
<td>( T )</td>
<td>( A )</td>
</tr>
<tr>
<td>( R )</td>
<td>( Q )</td>
<td>( n )</td>
</tr>
</tbody>
</table>

**Note:** The rows represent each sample being a true outlier or a true non-outlier. In the columns, Decide Outlier means that we reject \( H_0 \); Decide Non-outlier means that we fail to reject \( H_1 \). This is the results for a single gene; dependence on gene is suppressed in the notation.

In Table 1 everything is unobserved except for \( n \) and \((R, Q)\). This is because the rows of the table represent the true status of the samples and are unknown to the data analyst. Table 1 shows a correspondence between outlier detections with testing multiple hypotheses. Based on the table, we can construct appropriate error measures to control. There are two kinds of errors that can be made, corresponding to the off-diagonal elements in Table 1. One error is to declare a sample to be an outlier when it is not an outlier in truth. The second is to not declare a sample as an outlier when in fact it is an outlier. Most error rate measures control the first type of error. Two popular error measures to control are the familywise type I error (FWER) (Shaffer, 1995) and the false discovery rate (FDR) (Benjamini and Hochberg, 1995). In words, the FWER is the probability of making at least one false declaration of a sample being an outlier, while the FDR is the average number of false outliers among the samples declared to be outliers. Using the notation of Table 1, FWER equals \( Pr(V \geq 1) \), while the FDR is \( E[V/Q|Q > 0]Pr(Q > 0) \).

We will use multiple testing ideas to develop outlier detection procedures. Everything is done for a fixed gene \( g \). We first estimate \( F_{0g} \) using the empirical cumulative distribution function of \( Y_{gi} \) in the baseline population, \( i = 1, \ldots, n_0 \). This yields an estimator \( \hat{F}_{0g}(y) = \)
\[ \sum_{i=1}^{n_0} I(Y_{gi} \leq y)/n_0. \] Then we transform the gene expression measurements for the other samples by \( \tilde{p}_{gi}^j = 1 - \hat{F}_{0g}(Y_{gji}), \ j = 1, \ldots, K. \) We then use the \( \tilde{p}_{gi}^j \) to test \( H_0^i \) versus \( H_1^i; \) in particular smaller values indicate evidence against \( H_0^i \) for the \( g \)th gene in group \( j. \) Note that if \( F_{0g} \) were known and continuous, then \( 1 - F_{0g}(Y_{gi}) \) would have a uniform(0,1) distribution under \( H_0^i \) and a distribution of a stochastically smaller random variable under \( H_1^i. \)

For a fixed gene, we can use multiple testing ideas to identify outliers. Three popular approaches are Bonferroni’s method, Sidák’s procedure and the Benjamini-Hochberg method. Bonferroni’s procedure declares observation \( i \) an outlier if \( \tilde{p}_{gi}^j \leq \alpha/n_j. \) Sidák’s procedure declares it to be an outlier if \( \tilde{p}_{gi}^j \leq 1 - (1 - \alpha)^{1/n_j}. \) Finally the Benjamini-Hochberg procedure proceeds as follows:

1. Set an error rate \( \alpha. \)
2. Sort the \( \tilde{p}_{gi}^j \) in increasing order, \( \tilde{p}_{g(1)}^j \leq \tilde{p}_{g(2)}^j \leq \cdots \leq \tilde{p}_{g(n_j)}^j. \)
3. Take as outliers the samples corresponding to \( \tilde{p}_{g(i)}^j \), where \( k_{gj} = \max\{1 \leq i \leq n_j : \tilde{p}_{g(i)}^j \leq i\alpha/n_j\}. \) If no such \( k \) exits, conclude that there are no outliers.

Conditional on \( \hat{F}_{0g} \), all the tests for outliers are independent. This implies that the Bonferroni and Sidák procedure will control the FWER, while the Benjamini-Hochberg procedure will control the FDR exactly for the multiple outlier hypothesis tests for a fixed gene. Observe that the multiplicity being adjusted for in this step of the algorithm is the number of samples in group \( j. \)

Suppose we now wish to make gene-specific inferences regarding outlierliess. If we use the Bonferroni procedure, we compute for \( j = 1, \ldots, (J - 1). \)

\[ T_{jg}^B = \sum_{i=1}^{n_j} I\{\tilde{p}_{gi}^j \leq \alpha/n_j\}. \] (1)

For the Sidák procedure, it is

\[ T_{jg}^S = \sum_{i=1}^{n_j} I\{\tilde{p}_{gi}^j \leq 1 - (1 - \alpha)^{n_j}\}, \] (2)
while for the Benjamini-Hochberg procedure the score would be

\[ T^{BH}_{jg} = \hat{k}_{jg}, \]  

(3)
defined in the previous paragraph.

To derive the null distribution of (1), (2) and (3), we adopt a conditional approach in which we permute the labels of the \( j \)th group versus the baseline group conditional on the observed row and sum totals corresponding to Table 1. This is analogous to a Fisher's exact test-type in which a hypergeometric distribution is used to calculate a p-value. To be precise, for gene \( g \) in group \( j \) (\( g = 1, \ldots, G \); \( j = 1, \ldots, (J - 1) \)), we apply a one-sided Fisher's exact test to the contingency table

\[
\begin{bmatrix}
T^M_{jg} & n_j - T^M_{jg} \\
0 & n_0 - n_{jg}
\end{bmatrix},
\]

where the superscript \( M \) refers to \( B, S \) or \( BH \). This leads to p-values \( p^M_{jg}, g = 1, \ldots, G, j = 1, \ldots, (J - 1) \).

For a fixed \( j \), we now have \( G \) p-values for which we need to account for multiple comparisons; the multiplicity is now the number of genes. This is the more familiar domain for differential expression analyses and multiple testing adjustments. There are two notable differences. First, we used the estimated distribution in the group \( j = 0 \) to calculate the “p-values” so that a known theoretical distribution is not being used. This is different from much of the previous literature on multiple testing. A second major difference is that the p-values obtained are highly discrete. For example, if \( n_j = 10 \) for all \( j \), then the p-value distribution will be discrete and only take one of ten possible values. Examples of discrete p-value distributions can be found in the data analysis done in Section 5. The majority of the recent statistical literature on multiple testing has not dealt with this situation, with some notable exceptions, which we describe next.
3 Multiple testing procedures with discrete data

3.1 Sequential testing procedures

In this section, we assume that \( J = 2 \). It is possible to come up with a more powerful testing procedure by exploiting the discrete nature of the test statistics. This was also done by Tarone (1990) and Gilbert (2005) in related settings. To do this, first note that the minimum achievable significance level for a score is

\[
\alpha_g^* = \left( \frac{n_1}{T_g^M} \right) / \left( \frac{n}{T_g^M} \right).
\]

Note that the significance level is one-sided, as outlyingness is based on extreme expression measurements in one direction.

We now review the modified procedures proposed by Tarone (1990) and Gilbert (2005). The modified Bonferroni procedure would work in the following way. For each \( g = 1, \ldots, G \), let \( n(g) \) be the number of tests for which \( \alpha_g^* < \alpha/g \), and let \( N \) be the smallest value of \( g \) such that \( n(g) \leq g \). Let \( R_N = \{ 1 \leq i \leq G : \alpha_i^* < \alpha/N \} \). For \( i \in R_N \), one rejects the \( i \)th null hypothesis if \( p_{i}^M < \alpha/N \). By the arguments in Tarone (1990), this procedure will control the FWER. The modified Sidák procedure would work in a similar way, except that we would reject the \( i \)th null hypothesis if \( p_{i}^M < 1 - (1 - \alpha)^{1/N} \). Both procedures control the FWER and also the FDR. The modified Benjamini-Hochberg procedure (Gilbert, 2005) would proceed in the following way:

- For \( i \in R_N \), sort the p-values in increasing order \( p_{(1)}^M \leq p_{(2)}^M \leq \cdots p_{(R)}^M \), where \( R \) is the cardinality of \( R_N \).

- Reject the null hypotheses corresponding to \( p_{(1)}^M, \ldots, p_{(D)}^M \), where

\[
D = \max\{ 1 \leq i \leq R : p_{(i)}^M \leq i\alpha/R \}.
\]

If no such \( D \) exists, no hypotheses are rejected.

Assuming the same conditions as in Benjamini and Hochberg (1995), the modified procedure will also control the false discovery rate at level \( \alpha \).
Before describing our proposed multiple testing procedure, we discuss one issue that has not been explored very much in the multiple testing literature, which we term stability.

3.2 Stability

While we have proposed several multiple testing procedures for gene-specific inference, one issue we have not considered so far is their variability. It is desirable to have testing procedures that have small variability. One comment about the Benjamini-Hochberg procedure is that it is claimed to be more powerful than a single-step procedure like the Bonferroni or Sidák procedure because the former controls the FDR, while the latter controls FWER. However, there is a certain instability in the Benjamini-Hochberg procedure that does not exist for the Sidák or Bonferroni procedure. Assume that $R$ is known. For ease of presentation, we assume that $J = 2$ and that $R = n_1$. The multiple testing procedure can be expressed as a function $f : [0, 1]^R \rightarrow Z$, where $Z$ is the set \{0, 1, 2, \ldots, R\}. The function $f$ inputs the $n_1$-dimensional vector of p-values and outputs the number of hypotheses rejected. We have the following result:

**Proposition:** Let $\epsilon > 0$ be an arbitrary constant (a) For the Bonferroni and Sidák procedures, for all $p$ and all $\epsilon > 0$,

$$\max_\epsilon |f(p) - f(p^\epsilon)| = 1,$$

where $p^\epsilon = p$ with the $i$th component equaling $p_i + \epsilon$.

(b) For the Benjamini-Hochberg procedure, there exists $p$ such that for all $\epsilon > 0$,

$$|f(p) - f(p^\epsilon)| = R.$$

**Proof:** For (a), perturbing the $i$th p-value only affects its rejection decision and not that of the other hypotheses. We prove (b) by construction. Define a configuration of p-values whose $j$th component is $p_j \equiv \alpha$. Then the Benjamini-Hochberg procedure will reject all hypotheses, since the maximal index $k$ for which $p(k) \leq k\alpha/R$ is in fact $R$. However, if we
now consider \( p^i \) with any \( \epsilon > 0 \), then the Benjamini-Hochberg procedure will fail to reject any null hypotheses. This because \( p^i \) will consist of \((R - 1)\) p-values equalling \( \alpha \) and the largest p-value equalling \( \alpha + \epsilon \).

**Remark 1.** The result of the proposition suggests that there is an inherent stability of sequential multiple testing procedures. In particular, part (b) of the proposition implies the possibility of a slight perturbation in the p-values can lead from rejecting all null hypotheses to rejecting no hypotheses. By contrast, part (a) shows that this will never happen for a single-step testing procedure. This instability will potentially manifest itself in the Benjamini-Hochberg procedure having a greater variance in terms of the number of hypotheses rejected relative to a single-step procedure.

**Remark 2.** The result of the proposition applies to any multiple testing error metric. In particular, this result applies to FWER and generalized FWER procedures as well.

**Remark 3.** The proposition makes no assumption about the dependence structure among the p-values. Observe that \( f \) itself has a distribution. If we wished to make distributional statements about \( f \) (e.g., its mean and variance), then this would require making assumptions on the joint distribution of the p-values.

**Remark 4.** The proposition is valid for any sample size. An implication of the proposition is that the Benjamini-Hochberg procedure is not ‘continuous’ in a certain sense; it is possible for a small and local perturbation in the p-values to lead to a drastically different result in terms of the number of hypotheses rejected. By contrast, this never happens for the single-step (Bonferroni and Sidák methods) because the local perturbation only affects the decision to reject one p-value. It turns out that the problem does not happen to be with the error rate being controlled (FDR versus FWER) but rather the use of sequential multiple testing methods versus single-step procedures.

**Remark 5.** If p-values were continuous, then the configuration described in the proof of part (b) of the proposition will have probability zero because ties have zero probability. However, with discrete p-values, the probability of ties becomes substantial and this instability is a
very plausible phenomenon.

In particular, the stability argument also applies to the method of outlier detection described in the previous section. In our experience, for the discrete test statistics, we have found that the Benjamini-Hochberg procedure described in Section 2.1 tends to yield much higher variability results than the Bonferroni and Sidák methods, so here and in the sequel, we will use the Bonferroni method for outlier detection.

Other criticisms of the approach of Gilbert were brought up by Pounds and Cheng (2006). We will adapt their approach for false discovery rate estimation to our problem. Unlike Pounds and Cheng (2006), our null distribution is estimated from the data, while they will either use a parametric model or permutation testing to derive their null distribution.

4 Proposed Outlier Detection Methodology

4.1 Two groups

We first consider the case when \( J = 2 \), i.e. there are two groups. Then we can apply the algorithm of Pounds and Cheng [16] for multiple testing. The algorithm proceeds as follows.

1. Let \( \tilde{p}_1, \ldots, \tilde{p}_d \) denote the \( d \) unique p-values, and let the corresponding number of p-values equal to \( \tilde{p}_i \) (\( i = 1, \ldots, d \)) be denoted as \( m_i \).

2. Define the estimator of the expected proportion of tests with a p-value less than \( \alpha \) as

\[
\hat{F}(\alpha) = G^{-1} \sum_{j=1}^{d} I(\tilde{p}_j \leq \alpha)m_j,
\]

where \( G = \sum_{j=1}^{d} m_j \).

3. Define the estimated proportion of true null hypotheses as \( \hat{\pi} = \min(1, 8c) \), where

\[
c = G^{-1} \sum_{i=1}^{m} 2 \min(p_i, 1 - p_i),
\]

and \( \min(a, b) \) denotes the minimum of \( a \) and \( b \).
4. Estimate the false discovery proportion as

\[ \hat{W}(\alpha) = \begin{cases} \hat{\pi}, & \alpha \leq 1/2 \\ \hat{\pi}/2 + \hat{F}(\alpha) - \hat{F}(1/2), & \alpha > 1/2. \end{cases} \]

As described by Pounds and Cheng (2006), what this calculation does is to count every p-value greater than 0.5 as if it were testing a true null hypothesis and count it towards the numerator of the false discovery proportion.

5. Compute \( \tilde{t}_j = \hat{W}(\tilde{p}_j)/\hat{F}(\tilde{p}_j), j = 1, \ldots, d. \)

6. Smooth \( \tilde{t}_j \) using a robust regression algorithm and rank interpolation; details are given in Pounds and Cheng (2006).

7. Construct q-values using the predicted values of \( \tilde{t}_j \) from the previous step.

This algorithm has been coded by Pounds and Cheng in R and is available at the following URL:

www.stjude.org/depts/biostats/.

What is novel in our problem is that we are using the observed data in the baseline group to calculate the p-values. This is identical to what was done in Ghosh and Chinnaiyan (2009). After implementing the algorithm, we can select genes whose q-value is less than a pre-specified threshold. This is referred to as the control paradigm by Pounds and Cheng (2006). They provide extensive simulation evidence to show that the rule of rejecting all genes whose q-value is less than \( \alpha \) will control the false discovery rate at level \( \alpha \).

4.2 Asymptotic Error Rate Control: Heuristics

In Pounds and Cheng (2006), no proof of error control in either a finite-sample or an asymptotic setting is given. If the null distribution is known, it turns out that one can use the arguments in Ferreira (2007) to show that the rule described in the previous paragraph will asymptotically control the false discovery rate. However, the null distribution was assumed to be known, whereas in our problem, it is actually estimated from the data. We give a
sketch of the proof that the proposed procedure asymptotically controls the FDR under certain assumptions.

Let \( \hat{p}_1, \ldots, \hat{p}_G \) denote the \( G \) p-values obtained from the Benjamini-Hochberg procedure described in Section 4.1. Let \( p_1, \ldots, p_G \) denote the p-values of the \( G \) genes if the null distribution were known (i.e., we used \( F_{0g} \) instead of \( \hat{F}_{0g} \)). Of the \( G \) genes, we assume that \( G_0 \) truly have no evidence of outlying expression and \( G_1 \) truly have evidence of outlying expression. Let these two populations have cumulative distribution functions denoted by \( F_0 \) and \( F_1 \). We define the binary random variable \( OE_g \) to denote outlying expression for gene \( g \) (i.e., \( OE_g = 1 \) if there is evidence of outlying expression, 0 otherwise). We make the following assumptions:

A. \( Y_{gij} \) share common support;

B. For \( g = 1, \ldots, G \), \( F_{0g} \) and \( F_{1g} \) are continuously differentiable, and their density functions \( f_{0g} \) and \( f_{1g} \) are also continuous; furthermore, \( f_{0g} \) is bounded on every compact subset of \((0, 1)\) and is positive on \( \{ t : 0 < F_{0g}(t) < 1 \} \).

C. \( n_0 \) and \( n_1 \) go to infinity such that \( n_1/n \to \lambda \in (0, 1) \).

D. Convergence of empirical cdfs:

\[
G_0^{-1} \sum_{i=1}^{G} I(p_i \leq t, OE_i = 0) \to F_0(t)
\]

\[
G_1^{-1} \sum_{i=1}^{G_1} I(p_i \leq t, OE_i = 1) \to F_1(t)
\]

uniformly in \( t \).

By assumption D, we can use the result of the main proposition in [4] to show that the Benjamini-Hochberg procedure applied to \( p_1, \ldots, p_G \) controls the FDR at the appropriate
level. By algebraic manipulation,
\[
G_0^{-1} \sum_{i=1}^{G} I(\hat{p}_i \leq t, OE_i = 0) = G_0^{-1} \sum_{i=1}^{G} I(\hat{p}_i \leq t, OE_i = 0) - I(p_i \leq t, OE_i = 0)
\]
\[
+ G_0^{-1} \sum_{i=1}^{G} I(p_i \leq t, OE_i = 0)
\]

Assumptions A)-C) imply that the first summand on the right-hand side of the equality sign converges almost surely to zero uniformly in \(t\), while by assumption D), the second summand converges almost surely to \(F_0\) uniformly in \(t\). An appeal to the arguments in the proof of the main proposition in Ferreira (2007) can then be used to show that the Benjamini-Hochberg procedure applied to \(\hat{p}_1, \ldots, \hat{p}_G\) also asymptotically controls the FDR at the correct level.

### 4.3 More than two groups

We now consider the situation where we have more than two groups, i.e. \(J > 2\). Outlier detection for the multigroup case has been considered recently by Liu and Wu (2007). Their methodology was primarily based on using \(F\)-statistics. As noted by Ghosh and Chinnaiyan (2009), such regression-based statistics only use information on the first two moments, which might lose power in situations where the data are far from normality.

We seek to extend the discrete version of the previous section here. Note that for each gene, we will have a vector of p-values. Again, the p-values are estimated using the distribution of values in the baseline group rather than based on permutation or a theoretical null distribution. Ploner et al. (2006) propose a multivariate density estimation for the local false discovery rate of Efron et al. (2001), while Chi (2008) proposes multivariate extensions of the Benjamini-Hochberg procedure. However, the latter author uses a mixture model for multiple testing that is an extension of that proposed by Genovese and Wasserman (2004). An implication of such a model is that the distribution of p-values is continuous, which is not the case here.

Our approach is the following. First, we convert the multivariate p-values to multivariate q-values applying the Pounds-Cheng algorithm to each component. We now describe two
types of hypotheses we might wish to test:

1. **Subtype hypothesis**: This would be looking for genes that show evidence of outlier expression in at least one group relative to the baseline group.

2. **Uniform effect hypothesis**: This would correspond to genes that consistently show evidence of outlier expression across all groups relative to baseline.

For the first hypothesis, for each gene, we can simply test if the minimum p-value across the \((J - 1)\) dimensions is less than \(\alpha/(J - 1)\), where \(\alpha\) is the FDR we wish to control. The procedure would be to then declare all genes for which this condition holds as satisfying the subtype hypothesis.

For the second hypothesis, we can test if the average of the q-values is less than or equal to \(\alpha\) for each gene. In the case where the tests are independent across groups, it is easy to show that both procedures will control the FDR. While this will not be exactly true because \(\hat{F}_0\) is used to derive p-values, we can argue that FDR control holds conditional on \(\hat{F}_0\).

Similarly, we can rank genes under the first hypothesis by the smallest q-value and by the average q-value in the second situation. It is not as clear how to adapt the approach of Ploner et al. (2006) for these two hypotheses.

## 5 Prostate Cancer Data

The example we use is from a prostate cancer gene expression study, a slightly different version from that considered previously in Ghosh and Chinnaiyan (2009). In this example, there are \(K = 3\) groups, non-cancer, localized prostate cancer and metastatic prostate cancer samples (24, 10, and 6, respectively). While the platform is a two-color gene expression array originally containing 20000 genes, we remove all genes that have any missing measurements, this yields a total of \(G = 8350\) genes.

We first begin by collapsing the two types of cancer sample and compare them to the non-cancer samples so that we deal with \(K = 2\) groups. A histogram of the p-values for
the Bonferroni statistic $T^R_g$, $g = 1, \ldots, 8350$ is given in Figure 1. Based on the plot, we see the extremely discrete nature of the p-value distribution. As a comparison, we also applied the q-value approach of Storey and Tibshirani (2003). At a significance threshold of 0.05, the method proposed here calls 136 genes significant, while the q-value approach calls 115 significant. As noted in Pounds and Cheng (2006), the q-value approach will tend to yield a higher estimate for the proportion of true null positives than the proposed method when the p-value distribution is discrete. This will lead to less power in finding differentially expressed genes.

Based on the genes that were found to have a q-value less than or equal to 0.05, we then searched the Census of Cancer Genes database (Futreal et al., 2004) to determine if there are oncogenes that are found by our analysis. Two genes were found: FANCD2 and TSC2. The first gene, FANCD2, is involved in the disorder Fanconi anemia. Fanconi anemia (FAN) is a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. FANCD2 encodes for one of the proteins that makes up a common nuclear protein complex with other proteins encoded by other FAN genes. FANCD2 is known to interact with the BRCA1 and BRCA2 genes (Garcia-Higuera et al., 2001), which are major oncogenes involved in the etiology of breast cancer. Also, in leukemia, FANCD2 is mutated in the germline. The other gene, TSC2 (tuberous sclerosis 2), is a tumor suppressor gene that is a component of membrane microdomains and mediates caveolin-1 localization in post-Golgi transport (Jones et al., 2004). It is mutated in the germline in cancer, primarily in renal cell carcinomas.

The next step is to do the analysis with $K = 3$ groups. We will use the non-cancer samples as the baseline distribution. Note that the uniform effect hypothesis is equivalent to the analysis comparing cancer samples to non-cancer samples, so we instead focus on genes that satisfy the subtype hypothesis. The distribution of p-values for the localized and metastatic prostate cancer (PCA) samples are given in Figure 2. The distribution for
the metastatic PCA samples is more discrete due to the smaller sample size. After having
applied the Pounds-Cheng algorithm to derive multivariate p-values, we select genes based
on the rule that the minimum q-value is less than 0.025. Such an analysis leads to selection of
186 genes. Again repeating the Census of Cancer Genes database, another known oncogene,
TCL6, is found. The function of TCL6 has not yet been defined; however, this protein may
play a role which is similar to or complementary to other T-cell leukemia/lymphoma proteins
during early embrogenesis. The association of this gene with T-cell leukemia chromosome
translocations implicates this gene as a candidate for leukemogenesis.

Notice that while the cancer samples come from subjects with prostate cancer, this anal-
ysis has uncovered oncogenes that have been found in other cancers. An implicit assumption
is that oncogenes are common across all cancers. This is in keeping with a model for tu-
morigenesis as posited by Hanahan and Weinberg (2000). They posited that there existed
oncogenes that regulated or were dysregulated in the following mechanisms: elf-sufficiency in
growth signals; insensitivity to anti-growth signals; evading apoptosis (cell death); limitless
replicative potential; sustained angiogenesis; tissue invasion and metastasis.

6 Discussion

In this article, we have explored the issue of mutliple testing with discrete test statistics in
the context of finding genes that have outlying differential expression in one group relative
to a baseline group. We have given an argument favoring the so-called direct estimation
procedures relative to sequential testing methods. In addition, we have developed nonpara-
metric testing procedures that do not rely on normality. An inspection of previous methods
reveals that these methods will be most powerful when a Gaussian assumption can be made;
their performance is less clear when there is departure from this distributional assumption.

While we have described the discreteness in a problem involving gene expression, tech-
nologies such as next-generation sequencing platforms and single nucleotide polymorphism
arrays offer discrete measurements of molecular activities. Thus, we view the issue of dealing
with discrete data in genomics as one that will not go away anytime soon. Further thought is needed on how to develop multiple comparison procedures for this setting.

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References


