Closed testing with Globaltest with applications on metabolomics data

Ningning Xu∗1, Aldo Solari†2, and Jelle J. Goeman‡1

1Department of Biomedical Data Sciences, Leiden University Medical Center, The Netherlands
2Department of Economics, Management and Statistics, University of Milano-Bicocca, Italy

Abstract

We derive a shortcut for closed testing with Globaltest, which is powerful for pathway analysis, especially in the presence of many weak features. The shortcut strongly controls the family-wise error rate over all possible feature sets. We present our shortcut in two ways: the single-step shortcut and the iterative shortcut by embedding the single-step shortcut in branch and bound algorithm. The iterative shortcut is asymptotically equivalent to the full closed testing procedure but can be stopped at any point without sacrificing family-wise error rate control. The shortcut improves the scale of the full closed testing from 20 around features before to hundreds. It is post hoc, i.e. allowing feature sets to be chosen after

∗n.xu@lumc.nl
†aldo.solari@unimib.it
‡j.j.goeman@lumc.nl; Corresponding author
seeing the data, without compromising error rate control. The procedure
is illustrated on metabolomics data.

**Keywords:** closed testing; family-wise error rate; high-dimensional inference;
metabolomics; pathway analysis

1 Introduction

In high-dimensional data, it is common that features may be meaningfully taken
together in sets, groups or regions. Instead of calculating many p-values for each
feature in the set, a global p-value might be preferred to test for the associa-
tion between the feature set and a certain outcome of interest. For example,
when analyzing metabolomics data, researchers would like to identify metabolic
pathways that are associated with a certain disease or treatment.

One very popular method for testing sets of features is Globaltest [Goeman
et al., 2004, 2006, 2011]. It is applied in the context of generalized linear models.
It is proved that Globaltest is locally most powerful, i.e. pooling many weak
features together can result in significant association with the outcome even
though the effect of features might be too weak to be individually identifiable.
Moreover, it can be used in high-dimensional data with more features than
observations, often with good power. Globaltest is the default testing method
for pathway enrichment analysis for metabolomics data in MetaboAnalyst [Xia
et al., 2015].

For pathway analysis, it is common to test multiple feature sets so that
multiple testing correction is needed. It has been argued that the family-wise
error rate (FWER) is more appropriate in this context than the false discovery
rate (FDR) [Meijer and Goeman, 2015b], whose interpretation is difficult be-
cause feature sets are not exchangeable. Several methods for FWER control,
especially for multiple Globaltests, have been proposed [Goeman and Mans-
However, all these methods require researchers to specify a limited number of features sets of interest beforehand.

Closed testing [Marcus et al., 1976] is a powerful method to control FWER over all possible feature sets. It is the optimal way to construct multiple testing procedures, as all other multiple testing procedures are either equivalent to closed testing or can be improved using closed testing [Goeman et al., 2019a]. More importantly, closed testing controls FWER for all possible feature sets, allowing researchers to postpone the selection of feature sets of interest until after seeing the data. Applied in this fashion, Goeman et al. [2011, 2019b] used closed testing to obtain the simultaneous confidence bounds for all false discovery proportions, which has been extended as “SEA” [Ebrahimpoor et al., 2019] in genomics and “ARI” in neuroimaging [Rosenblatt et al., 2018]. But, “SEA” and “ARI” both use the Simes test, which requires the assumption on positive dependence of p-values for per-feature hypotheses. It can be conservative when the p-values are strongly dependent.

In this work, we derive a novel FWER controlling procedure based on closed testing with Globaltest for all possible feature sets. The major challenge to perform closed testing, as always, is computational: it requires exponentially many tests. We develop a shortcut to overcome this limitation by reducing the exponential number of Globaltest to a linear one. We first propose the “single-step” shortcut, which is fast but approximate to the full closed testing. It guarantees strong FWER control, but may be conservative. We then embed the “single-step” shortcut within a branch and bound algorithm to gain power. This “iterative” shortcut will approximate the full closed testing procedure closer and closer as we iterate longer, trading computation time for power. Our new shortcut allows exact closed testing with Globaltest to be performed on a regular PC using a data set with the size of typical metabolomics data, with up to
around 500 features.

Although Globaltest is defined for all generalized linear models we focus in this paper on logistic regression only, which is the most popular generalized linear model used with Globaltest. We first revisit Globaltest and its properties in Section 2 and the closed testing procedure in Section 3. We derive the single-step shortcut in Section 4 and the iterative extension in Section 5. In the remaining sections, we explore the application of our method on metabolomics data.

2 Globaltest

Suppose that we have data with \( n \) observations. We adopt a logistic regression model that relates the response variable \( y \) to the linear predictor by the canonical link function \( h \) (e.g. the logit function):

\[
h(\mathbb{E}(y \mid Z, X)) = Z\gamma + X\beta.
\]

Here we partition the design matrix into a matrix \( Z \) and an \( n \times m \) matrix \( X \), possibly in a high-dimensional setting with \( m > n \). Columns of \( Z \) represent the confounders that we need to adjust for, e.g. age and gender, but the number of confounders is assumed to be less than \( n \). \( \gamma \) and \( \beta \) are the unknown regression coefficients. We are interested in testing the association of features in \( X \) with the response \( y \) after adjusting for confounders:

\[
H_0 : \beta = 0,
\]

where \( \beta = (X^\top X)^{-1}X^\top(h(\mathbb{E}(y \mid Z, X)) - Z\gamma) \) for which \((X^\top X)^{-1}\) is the Moore-Penrose inverse of \( X^\top X \) and we replace \( \gamma \) with its ordinary least square estimate.
\(\gamma\) in practice.

We will not only perform Globaltest for the set of all \(m\) features, but also for its subsets. When considering a subset, we look for the marginal association of the features in the subset with the response, i.e. without correcting such associations for the features not in the subset. We use the index set of the features in \(X\) to denote the feature set. \(F = \{1, \ldots, m\}\) is the full feature set with size \(|F| = m\), where \(|\cdot|\) denotes the cardinality of a set. \(X_R\) is the design matrix corresponding to subset \(R \subseteq F\) with size \(|R| = r\). \(\beta_R = (X_R^\top X_R)^{-1}X_R^\top (h(E(y \mid X_R)) - Z\gamma)\) are the corresponding unknown coefficients. The following logistic model describes the marginal association between the features in subset \(R\) and the response \(y\):

\[
E(y \mid Z, X_R) = h^{-1}(Z\gamma + X_R\beta_R).
\]

Then the null hypothesis is as follows:

\[
H_R : \beta_R = 0,
\]

i.e. features in \(R\) are uncorrelated with the outcome. The Globaltest statistic can then be derived based on the work of Goeman et al. [2004, 2011]:

\[
g_R = y^\top (I - H)X_R X_R^\top (I - H)y, \tag{2}
\]

with identity matrix \(I_{n \times n}\) and the hat matrix \(H = Z(Z^\top Z)^{-1}Z^\top\). We are using the non-standardized variant of Globaltest proposed by Guo and Chen [2016].

It can be easily seen that

\[
g_R = \sum_{i \in R} g_i,
\]

where \(g_i = y^\top (I - H)X_i X_i^\top (I - H)y, \quad i = 1, \ldots, m\) are the individual test
statistics. For the asymptotic null distribution of $g_R$, Goeman et al. [2011] proved that it is equivalent to a weighted sum of independent $\chi^2_1$ variables:

$$g_R = \frac{1}{n} \sum_{i=1}^{n} \lambda^R_i \chi^2_1,$$  \hspace{1cm} (3)

where $\lambda^R = (\lambda^R_1, \cdots, \lambda^R_n)^\top$ are the eigenvalues of the positive semi-definite matrix $\Sigma^{\frac{1}{2}}(I - H)X_RX^\top_R(I - H)\Sigma^{\frac{1}{2}}$ arranged in descending order. Here $\Sigma$ is the diagonal covariance matrix of $y$ under the null hypotheses, which we replace by its estimate.

For a prespecified significance level $\alpha$, the critical value $c_R$, i.e. the $1 - \alpha$ quantile of the null distribution, is a function of $\lambda^R$ mapping from $\mathbb{R}^{n \times 1}$ to $\mathbb{R}^+$:

$$c_R = c(\lambda^R).$$

There are several algorithms developed to calculate $c_R$. For example, Robbins and Pitman [1949] and Imhof [1961] derived methods to calculate the exact critical values. Kuonen [1999] and Pearson [1959] presented approximate but computationally simple methods. We use the algorithm of Robbins and Pitman [1949] in this paper, since it is exact and has fewer convergence problems than Imhof’s algorithm. Once we have calculated the test statistic $g_R$ and the critical value $c_R$, Globaltest rejects $H_R$ with type I error rate controlled at $\alpha$ if $g_R \geq c_R$.

3 Closed testing

Based on null hypotheses defined above, we obtain that $\bigcap_{i \in R} H_{\{i\}} \subseteq H_R$, where $H_{\{i\}} : \beta_{\{i\}} = 0$ denotes the elementary hypothesis. It is clear that $\beta_{\{i\}} = 0, i \in R$ implies $\beta_R = 0$ in terms of the definition of true $\beta$. This results a closed set of hypotheses under intersection and therefore we could use the closed testing
procedure. By definition, a hypothesis is only rejected if all hypotheses which are supersets of it are rejected by a local test, Globaltest in our case.

The only assumption for closed testing is that \( H_T \) is tested by a valid \( \alpha \)-level local test, where \( T = \{ i : H_{\{i\}} \text{ is true, } i \in F \} \) is the index set of all true null elementary hypotheses. To guarantee the validity of Globaltest for model \( T \), we assume a logistic regression to associate \( y \) with features in \( T \). It then follows that the probability that Globaltest does not reject \( H_T \) is at least \( 1 - \alpha \), which implies that all true null hypotheses that are subsets of \( H_T \) are not rejected by closed testing with probability at least \( 1 - \alpha \). Hence, FWER is controlled at level \( \alpha \).

A useful and often overlooked property of closed testing is the model robustness regarding to FWER control. It is possible that misspecification of a model causes unsatisfied type I error rate control from the testing procedure [Hemerik et al., 2019]. The only assumption needed for closed testing is that the local test for hypothesis \( H_T \) is valid. All other models (null and alternative) may be misspecified without jeopardizing FWER control.

For the general case of closed testing with \( m \) elementary hypotheses, the number of operations is of order \( 2^m \). This exponential complexity of closed testing is problematic for large-scale multiple testings. Shortcuts are thus proposed to reduce computation time, see for instance [Brannath and Bretz, 2010], [Gou et al., 2014], [Dobriban, 2018]. Shortcuts can be exact or approximate. Approximate shortcuts control FWER, but sacrifice power relative to the full closed testing procedure. In this paper, we derive an approximate “single-step” shortcut and an exact “iterative” shortcut for closed testing with Globaltest.
4 Single-step shortcut

We introduce the single-step shortcut in this section. Globaltest rejects $H_R$ at level $\alpha$ if and only if

$$g_R \geq c_R.$$  

The closed testing procedure rejects $H_R$ at level $\alpha$ if and only if

$$g_S \geq c_S, \text{ for all } R \subseteq S \subseteq F.$$  

It means that naively we have to calculate test statistics and critical values for a total of $2^{m-r}$ hypotheses.

4.1 Main idea

For a fixed set $R$ of interest, we will derive a shortcut to decide whether $H_R$ can be rejected by closed testing. Before deriving the shortcut, let us define the level of the hypothesis $H_R$ as $\ell_R$ with

$$\ell_R = \sum_{i=1}^{n} \lambda_{i}^{R} = \sum_{i \in R} w_i,$$

where $w_i = \mathbf{X}_i^\top (\mathbf{I} - \mathbf{H}) \Sigma (\mathbf{I} - \mathbf{H}) \mathbf{X}_i$ denotes the weight of the elementary hypothesis $H_{\{i\}}$.

The main idea of the shortcut is as follows. We will first find the minimum test statistic $g_{\min}(\ell)$ and the maximal critical value $c_{\max}(\ell)$, both as a function of the level $\ell$ such that for all $S$ with $R \subseteq S \subseteq F$ we have

$$g_S \geq g_{\min}(\ell_S)$$  (i)
and
\[ c_S \leq c_{\text{max}}(\ell_S). \]  \hspace{1cm} \text{(ii)}

We then derive the following shortcut.

**Lemma 1.** The closed testing procedure rejects \( H_R \) at level \( \alpha \), if

\[ g_{\text{min}}(\ell) \geq c_{\text{max}}(\ell), \forall \ell \in [\ell_R, \ell_F]. \]

**Proof.** For any set \( S \) with \( R \subseteq S \subseteq F \), we have, obviously, that \( g_S \geq g_{\text{min}}(\ell_S) \geq c_{\text{max}}(\ell_S) \geq c_S \) on the basis of (i) and (ii).

For illustration, we use a recurring toy example with \( n = 100 \) observations and \( m = 5 \) features \( x_1, \cdots, x_5 \), fitted by logistic regression. The elementary hypotheses are \( H_{\{i\}} : \beta_i^{\{i\}} = 0, i = 1, \cdots, 5 \). Suppose that we want to test \( H_R \) with \( R = \{3\} \) and \( F = \{1, 2, 3, 4, 5\} \). Figure 1 presents the minimum test statistic versus the maximal critical value for all levels between \( \ell_R \) and \( \ell_F \), from which we conclude that \( H_{\{3\}} \) is rejected by closed testing because \( g_{\text{min}}(\ell) \geq c_{\text{max}}(\ell) \) holds for all \( \ell \in [\ell_R, \ell_F] \). Instead of comparing the exponentially many test statistics and critical values, we only need to compare the minimum test statistic curve and the maximal critical value curve. If \( g_{\text{min}} \) curve is totally above \( c_{\text{max}} \) curve, we conclude that \( H_R \) is rejected by closed testing. We will show how to calculate \( g_{\text{min}}(\ell) \) and \( c_{\text{max}}(\ell) \) that satisfy (i) and (ii) respectively in the following.
Figure 1: Single-step shortcut for testing $H_{(3)}$. The solid line represents $g_{\min}(\ell)$ and the dashed line represents $c_{\max}(\ell)$. Circles denote the exact test statistics and triangles are the exact critical values for all possible $H_S$ with $R \subseteq S \subseteq F$.

4.2 The minimum test statistic

We first consider how to calculate the minimum test statistics $g_{\min}(\ell)$. The supersets of $R$ are unions of $R$ with every possible subsets of the complement of $R$, i.e. $S = R \cup I$ with $I \subseteq V$, $V = F \setminus R$.

Remember that $g_i$ and $w_i$ are the individual test statistics and weights, respectively. Let $q_i = \frac{g_i}{w_i}$ denote the ratios of individual test statistics to the corresponding weights. Let $\{\pi_1, \cdots, \pi_v\}$ be a permutation of $V$ with $|V| = v$, which sorts $\{q_i, i \in V\}$ in ascending order, i.e. $q_{\pi_1} \leq \cdots \leq q_{\pi_v}$. We can then find out which feature can result in the minimum increase on the test statistic per increase on the level. For example, for any $H_S$ whose level is between $\ell_R$ and $\ell_{R\cup\{\pi_1\}}$, it is clear that $\frac{g_S - g_R}{\ell_S - \ell_R} \geq q_{\pi_1}$, i.e. $g_S - g_R \geq (\ell_S - \ell_R)q_{\pi_1}$. More generally, for any $H_S$ with level in the range of $\ell_{R\cup\{\pi_1, \cdots, \pi_{k-1}\}}$ to $\ell_{R\cup\{\pi_1, \cdots, \pi_k\}}$.
we have that
\[ g_S - g_{R \cup \{\pi_1, \ldots, \pi_{k-1}\}} \geq (\ell_S - \ell_{R \cup \{\pi_1, \ldots, \pi_{k-1}\}})q_{\pi_k} \tag{4} \]
for all \( k = 1, \cdots, v \). We let \( \{\pi_1, \cdots, \pi_{k-1}\} = \emptyset \) for \( k = 1 \).

We define the minimum test statistic \( g_{\min}(\ell) \) for \( \ell \in [\ell_R, \ell_F] \) as:
\[ g_{\min}(\ell) = g_{R_\ell} + (\ell - \ell_{R_\ell})q_{\pi_{k_\ell}}, \tag{5} \]
where \( R_\ell = R \cup \{\pi_1, \cdots, \pi_{k_\ell-1}\} \) with \( k_\ell = \min\{j : \ell_R + \sum_{i=1}^{j} w_{\pi_i} \geq \ell\} \). Note
that \( R_\ell = R \) for \( \ell - \ell_R < w_{\pi_1} \). Equation (5) also indicates that \( g_{\min}(\ell_S) = g_S \) for \( S = R_\ell \). The two extreme cases are \( g_{\min}(\ell_R) = g_R \) and \( g_{\min}(\ell_F) = g_F \). Clearly, \( g_{\min}(\ell) \) is a monotone increasing function of \( \ell \), as illustrated in Figure 1.

In terms of Equation (5), we have the following result:

**Lemma 2.** Equation (4) holds for all \( S \) with \( R \subseteq S \subseteq F \).

**Proof.** \( g_S \geq g_{\min}(\ell_S) \) obviously holds by Equation (4). \( \square \)

### 4.3 The maximal critical value

We know that the critical value of Globaltest is a function of the eigenvalue vector, so the subsequent step is to find such an eigenvalue vector whose corresponding critical value is maximal.

Let us first introduce the definition of majorization [Horn and Johnson, 2012]:

**Definition** Let vector \( \lambda = (\lambda_1, \cdots, \lambda_n) \) with \( \lambda_1 \geq \cdots \geq \lambda_n \) and \( \delta = (\delta_1, \cdots, \delta_n) \) with \( \delta_1 \geq \cdots \geq \delta_n \) be given. Then vector \( \lambda \) is said to majorize vector \( \delta \), i.e. \( \lambda \succ \delta \), if \( \sum_{i=1}^{s} \lambda_i \geq \sum_{i=1}^{s} \delta_i \) for all \( s = 1, \cdots, n \) with equality for \( s = n \).
Based on the *inclusion principle* for eigenvalues of hermitian and positive semi-definite matrix [Horn and Johnson, 2012], we know that $\lambda_i^R \leq \lambda_i^S \leq \lambda_i^F$, $i = 1, \cdots, n$ for $R \subseteq S \subseteq F$, where $\lambda_i^R$, $\lambda_i^S$ and $\lambda_i^F$ are the $i$-th largest eigenvalues of matrices as defined in Equation (3) in Section 2. Thus, $\lambda_S$ are bounded by the upper bound $\lambda_F$ and the lower bound $\lambda_R$. We then define a “majorizing” vector at level $\ell$ as

$$\hat{\lambda}(\ell) = (\lambda_1^F, \cdots, \lambda_i^F, \eta, \lambda_{i+2}^R, \cdots, \lambda_n^R).$$

(6)

It simply takes the first few biggest values of the upper bound $\lambda_F$ as head and the last few smallest values of the lower bound $\lambda_R$ as tail, and connecting them by an $\eta$ such that $\hat{\lambda}(\ell)$ is in descending order and its sum is $\ell$. Obviously, $\hat{\lambda}(\ell)$ is still bounded by $\ell_R$ and $\ell_F$, but it majorizes $\lambda_S$ for $\ell = \ell_S$.

We will use the following theorem:

**Theorem 3.** [Bock et al., 1987] Suppose that $\lambda \succ \delta$, then there exists an $\alpha_0$ such that for $\alpha \leq \alpha_0$, we have

$$c(\lambda) \geq c(\delta).$$

A proof of Theorem 3 is in Bock et al. [1987], we only changed notations.

Theorem 3 can be also rephrased, equivalently, that there exists a $\ell_0$ such that

$$P\{\sum_{i=1}^{n} \lambda_i \chi_1^2 \leq \ell_0\} = P\{\sum_{i=1}^{n} \delta_i \chi_1^2 \leq \ell_0\} = \alpha_0,$$

then for $\ell \geq \ell_0$, $P\{\sum_{i=1}^{n} \lambda_i \chi_1^2 \leq \ell\} \leq P\{\sum_{i=1}^{n} \delta_i \chi_1^2 \leq \ell\}$.

The maximal critical value is then defined as:

$$c_{\text{max}}(\ell) = c(\hat{\lambda}(\ell)).$$

(7)

Based on Theorem 3, the following lemma can then be obtained:
Lemma 4. Equation (ii) holds for all $S$ with $R \subseteq S \subseteq F$, for $\alpha \leq \alpha_0$.

Proof. For any $S$ with $R \subseteq S \subseteq F$, $\hat{\lambda}(\ell_S) \succ \lambda_S$ so that $c_{max}(\ell_S) \geq c_S$ can be obtained directly from Theorem 3. \qed

In above lemma we may see that the validity of $c_{max}$ depends on $\alpha_0$, which therefore has to be sufficiently large for Lemma 3 to be useful. [Diaconis and Perlman 1990] compared the tail probabilities of $\sum_{i=1}^{n} \lambda_i \chi^2_1$ and $\sum_{i=1}^{n} \delta_i \chi^2_1$ with $\lambda \succ \delta$. They conjectured that their corresponding cumulative distribution functions (cdf) cross exactly once, implying that $\alpha_0$ would be far from 0 or 1. However, their conjecture was disproved by [Yu 2017] who showed that the two cdf cross an odd number of times (but sometimes more than once). However, cdf of $\sum_{i=1}^{n} \lambda_i \chi^2_1$ will be always below that of $\sum_{i=1}^{n} \delta_i \chi^2_1$ after the last crossing point, as Theorem 3 claims. The value of $t_0$ is exactly the last crossing point and $\alpha_0$ is the corresponding tail probability. Usually, practitioners would like to take significance level $\alpha = 0.05$. This requires $\alpha_0 \geq 0.05$. We test this in the real data applications, where we find that $\alpha_0$ is typically safely in the range of $0.25 - 0.3$. We know that, because of the robustness property of closed testing mentioned in Section 3, the assumption that $\alpha_0 \geq 0.05$ is only needed for the single hypothesis $H_T$, not for all true null hypotheses.

In the toy example, given the upper bound $\lambda_F$ and the lower bound $\lambda_R$ with $R = \{3\}$, Figure 1 shows the maximal critical values $c_{max}(\ell)$ (broken line) and its exact critical values (triangle point-up) for all $H_S$. It is clear that $c_{max}(\ell)$ is above all exact critical values and it is an increasing function of levels. We further note that calculating $\hat{\lambda}(\ell)$ for all possible levels only requires eigenvalue calculation of $\lambda_R$ and $\lambda_F$ once. This significantly reduce the calculation time for large matrices (i.e. large $n$).
4.4 Sure or unsure outcomes

With everything set in place, we check whether $H_R$ can be rejected by the single-step shortcut via (1) checking if the minimum test statistics are greater than the maximal critical values for all levels between $\ell_R$ and $\ell_F$, i.e. $g_{\min}(\ell) \geq c_{\max}(\ell)$ for $\ell \in [\ell_R, \ell_F]$; (2) if necessary, checking if the exact test statistics are greater than the exact critical values for $S = \tilde{R}_\ell$, i.e. $g_{\tilde{R}_\ell} \geq c_{\tilde{R}_\ell}$ for all $\tilde{R}_\ell$.

If $g_{\min}(\ell) \geq c_{\max}(\ell)$ for all $\ell \in [\ell_R, \ell_F]$, $H_R$ is surely rejected by closed testing at level $\alpha$. For example, $H_{\{3\}}$ in Figure 1 can be rejected by closed testing at level 5% as the $g_{\min}$ curve is totally above the $c_{\max}$ curve. Otherwise, we further check $g_{\tilde{R}_\ell}$ versus $c_{\tilde{R}_\ell}$ for all $\tilde{R}_\ell$. If there exists a $\tilde{R}_\ell$ such that $g_{\tilde{R}_\ell} < c_{\tilde{R}_\ell}$, we are then sure that $H_R$ cannot be rejected by closed testing. For example when testing $H_{\{2\}}$ in Figure 2, we find that Globaltest does not reject $H_{\{24\}}$ and $H_{\{245\}}$ so that $H_{\{2\}}$ cannot be rejected by closed testing. On the other hand, if $g_{\tilde{R}_\ell} \geq c_{\tilde{R}_\ell}$ holds for all $\tilde{R}_\ell$, we are inconclusive about the rejection of $H_R$, which is the case in Figure 3, where $H_{\{1\}}$ is unsure to be rejected or not by closed testing.

Clearly, the shortcut drastically reduces the calculation intensity of the full closed testing procedure. The shortcut allows the application of closed testing with Globaltest in large-scale multiple testing problems. Moreover, it strongly controls FWER by Lemma 1. It is approximate to the full closed testing procedure in the sense that it gives as most the same rejections as the full closed testing procedure, but possibly fewer.
Figure 2: Single-step shortcut for testing $H_{(2)}$. Filled circles and triangles represent the exact test statistics and critical values for $S = \bar{R}_\ell$. 
4.5 Algorithm to test whether $g_{\text{min}}(\ell)$ is above $c_{\text{max}}(\ell)$

In this section we introduce an algorithm to test whether $g_{\text{min}}(\ell)$ curve and $c_{\text{max}}(\ell)$ curve cross. Obviously, if $g_R < c_R$ or $g_F < c_F$, $H_R$ cannot be rejected by closed testing. If $g_R \geq c_F$, we directly conclude that $H_R$ can be rejected by closed testing. When neither of the above two cases is true, we need to check whether $g_{\text{min}}(\ell)$ curve and $c_{\text{max}}(\ell)$ curve cross. The outline of the algorithm is given in Algorithm 1.

We draw the algorithm as a step function as illustrated in Figure 4 for $H_{(3)}$ and $H_{(1)}$ in the toy example. In fact, we only need to calculate $c_{\text{max}}$ for the corresponding points (filled square) rather than for all levels. $\epsilon$ is a threshold to limit the number of calculations of $c_{\text{max}}$ when the two curves are truly crossed. Smaller $\epsilon$ results in more times of calculations of $c_{\text{max}}$, and also makes the single-step shortcut more powerful. We set $\epsilon = 10^{-4}$ in the toy example.
Algorithm 1 Test whether $g_{\min}(\ell)$ and $c_{\max}(\ell)$ cross.

Require: $g_{\min}(\ell)$, $c_{\max}(\ell)$

\[
g_R \leftarrow g_{\min}(\ell_R) \\
c_1 \leftarrow c_{\max}(\ell_F) \\
\ell_0 \leftarrow \ell_F
\]

repeat
\[
\ell_1 \leftarrow \ell_0 \\
\ell_0 \leftarrow \{ \ell : g_{\min}(\ell) = c_1 \} \\
c_1 \leftarrow c_{\max}(\ell_0)
\]
until $c_1 \leq g_R$ or $|\ell_1 - \ell_0| \leq \epsilon$

\[\triangleright \epsilon \text{ is a threshold.}\]

if $c_1 \leq g_R$ then
\[\text{return } g_{\min}(\ell) \text{ is above } c_{\max}(\ell).\]
else $|\ell_1 - \ell_0| \leq \epsilon$
\[\text{return } g_{\min}(\ell) \text{ and } c_{\max}(\ell) \text{ cross.}\]

Figure 4: Illustrations of Algorithm 1. We calculate $c_{\max}$ only for filled square points.
5 Iterative shortcut

Clearly, the above single-step shortcut avoids the exponentially many calculations of Globaltest, but it is approximate as we might get unsure outcomes. Next, we investigate how we can make it exact. If an unsure outcome is obtained from the single-step shortcut, we turn to the branch and bound algorithm to approach a certain outcome, i.e. surely rejected or not rejected. Branch and bound algorithm was first proposed by Land and Doig [1960] for discrete programming.

We embed the single-step shortcut in the branch and bound algorithm to make a more powerful iterative shortcut. If we iterate the single-step shortcut long enough so that no unsure outcomes left, the full closed testing solution will be obtained. Moreover, we allow to prespecify the number of iterations, i.e. how many times we iterate the single-step shortcut, to save computation time but without sacrificing FWER control. We can make the iterative shortcut more powerful by increasing the number of iterations, i.e. trading time for power.

Branch and bound algorithm is based on the principle that the total space of feasible solutions can be partitioned into smaller sub-spaces of solutions. These smaller spaces can then be evaluated systematically until the best solution is found. It therefore consists of a branching rule that defines how to generate sub-spaces and a bounding rule that defines how to compute a bound. The single-step shortcut defined above will take the role of the bound in the algorithm. In our case, we are interested in the existence of an $H_S$ that is not rejected by Globaltest within a certain subspace. Once we find such an $H_S$, we can stop iterating the single-step shortcut with conclusion that $H_R$ cannot be rejected by closed testing.

We explain our iterative shortcut with branch and bound algorithm in more detail in Algorithm 2.

In our method, we choose $u \in F \setminus R$ in such a way that $g_u$ is largest among
Algorithm 2 Iterative shortcut with branch and bound

Require: \( H_R \): unsure outcome from single-step shortcut in space \( S(F, R) = \{H_S : R \subseteq S \subseteq F\} \)

1: Partition \( S(F, R) \) into two disjoint sub-spaces: \( S^1(F \setminus \{u\}, R) \) and \( S^2(F, R \cup \{u\}), u \in F \setminus R \)

2: Do single-step shortcut in both sub-spaces

3: if There exists a sub-space such that \( H_R \) is not reject then
4: \( \text{return Not reject } H_R \)
5: else if \( H_R \) are rejected in both then
6: \( \text{return Rejected } H_R \)
7: else
8: Refine the sub-spaces with unsure outcomes
9: Repeat the single-step shortcut
10: Until \( H_R \) is rejected in all sub-spaces \( \text{return Rejected } H_R \)
11: or The number of iterations exceeds the preset value
12: \( \text{return Unsure} \)

\( \{g_i, i \in F \setminus R\} \). This enables our iterative shortcut to get conclusive outcomes with the fewest iterations. The number of iterations is exactly the number of branches generated by the branch and bound algorithm. The more the iterations are, the more the iterative shortcut rejects. For a prespecified number of iterations \( \tau \), the iterative shortcut generates \( d_\tau \) sub-spaces without any successors that satisfy (1) \( H_R \in S_i, i = 1, \cdots, d_\tau \), (2) \( S_i \cap S_j = \emptyset, i \neq j \), (3) \( \bigcup_{i=1}^{d_\tau} S_i = S = \{H_S : R \subseteq S \subseteq F\} \). The iterative shortcut rejects \( H_R \) in the following way:

**Lemma 5.** Closed testing rejects \( H_R \) only if \( H_R \) is rejected by the single-step shortcut in each sub-space \( S_i, i = 1, \cdots, d_\tau \) satisfying the above conditions.

**Proof.** Based on Lemma[1] if \( H_R \) is rejected by the single-step shortcut in sub-space \( S_i, i = 1, \cdots, d_\tau \), we have \( g_S \geq g_{\min}(\ell_S) \geq c_{\max}(\ell_S) \geq c_S \) with \( H_S \in S_i, i = 1, \cdots, d_\tau \). Because \( \bigcup_{i=1}^{d_\tau} S_i = S \), \( g_S \geq c_S \) holds for \( H_S \in S \). Thus, closed testing rejects \( H_R \). \( \square \)

It is obvious that \( H_R \) cannot be rejected by closed testing if there exists a sub-space where \( H_R \) is not rejected by shingle-step shortcut. We are still
inconclusive about the rejection of $H_R$ if there exists a sub-space where shingle-step shortcut still has unsure outcomes. In this case, increasing number of iterations will gain power.

Let $\mathcal{X}$ be the rejection set of closed testing, $\mathcal{X}_\tau$ be the rejection set of the iterative shortcut with $\tau$ iterations prespecified. Specifically, $\mathcal{X}_0$ is the set of rejections by the single-step shortcut and $\mathcal{X}_\infty = \lim_{\tau \to \infty} \mathcal{X}_\tau$ is the asymptotic rejection set of iterative shortcut. Obviously, $\mathcal{X} = \mathcal{X}_\infty$ as the subspaces created by infinite branching are exactly all possible $H_S$ with $R \subseteq S \subseteq F$. Now, we introduce the following lemma to investigate the convergence property of the iterative shortcut:

**Lemma 6.** $\mathcal{X}_0 \subseteq \mathcal{X}_\tau \subseteq \mathcal{X}_\infty = \mathcal{X}$.

**Proof.** It is clear that the iterative shortcut rejects as least as many feature sets as the single-step shortcut, whose unsure outcomes are tested repeatedly within branch and bound algorithm. Hence, iterative shortcut rejects more if we iterate more. For hypothesis $H_R$, the iterative shortcut with infinite branches exactly generates $2^{m-r}$ subspaces, each of them only includes one hypothesis to be tested by local test. It is therefore the full closed testing.

We illustrate the iterative shortcut with the toy example in Section 4.1 in Figure 5 for hypothesis $H_{\{1\}}$. It is shown that $H_{\{1\}}$ is rejected by closed testing in both sub-spaces: $S(F \setminus \{3\}, R)$ and $S(F, R \cup \{3\})$. By Lemma 6, $H_{\{1\}}$ is rejected by the iterative shortcut with FWER controlled at level 0.05.
There is clearly a trade-off between computing time and coming close to closed testing when applying branch and bound algorithm. Iterative shortcut with more branches will get closer to the full closed testing procedure. Applying branch and bound algorithm still requires exponentially many calculations but the computation time dramatically reduced in practice.

6 Applications on metabolomics data

6.1 Assumption on $\alpha_0$

From Section 4.3 we know that $\alpha_0$ is the tail probability of the null distribution of Globaltest statistic at point $t_0$, after which the cdf corresponding to the true
eigenvalue vector is greater than that corresponding to the majorizing vector. Namely, only for $\alpha \leq \alpha_0$ we have a valid maximal critical value and accordingly a valid shortcut procedure. $\alpha = 0.05$ is the most commonly used significance level by researchers. We would like to show that $0.05 < \alpha_0$ holds so that the shortcut procedure with significance level $\alpha = 0.05$ can be safely used in practice. This is illustrated by an example data set in MetaboAnalyst [Xia et al., 2015], published by Eisner et al. [2011]. It includes 63 metabolites for 77 urine samples, 47 of them are experiencing muscle wasting and 30 are in the control group. It is normalized by the logarithm transformation with base 2 and then fitted by logistic regression.

We randomly choose hypotheses of interest $H_{R_1}$, $H_{R_{10}}$, $H_{R_{30}}$ and $H_{R_{50}}$ with different sizes. $R_{10}$ is, for example, the set of 10 metabolites. For each of the four hypotheses, we randomly select 500 around supersets at diverse levels. We then calculate $\alpha_0 = P\{\sum_{i=1}^{n} \lambda_i \chi^2_i \leq t_0\} = P\{\sum_{i=1}^{n} \hat{\lambda}_i \chi^2_i \leq t_0\}$ per superset, where $\hat{\lambda}_i$ is the $i$-th element of the majorizing vector $\hat{\lambda}$ at level of the hypothesis corresponding to that superset. In Figure 6, we make a scatter plot with levels on x-axis and $\alpha_0$ on y-axis, the horizontal broken line denotes $\alpha = 0.05$. It can be seen that $\alpha_0$ is much larger than 0.05 so that our shortcut procedure basically works for significance level $\alpha = 0.05$. We only show $\alpha_0$ for this data but same behavior can be found for other data sets used below.

6.2 Real data analysis

To investigate the properties of closed testing with Globaltest (CTGT), we apply it to four real metabolomics data sets, whose role on regulatory pathways of human pathophysiology, ranging from aging to disease, has been highlighted. We compare CTGT with other FWER-controlling methods, such as SEA [Ebrahim-
Figure 6: Assumption on $\alpha_0$ that $\alpha_0 > 0.05$.

The data sets are referred to as Eisner (47 subjects with cachexic muscle loss, 30 in control group, [Eisner et al., 2011]), Bordbar (6 subjects with lipopolysaccharide stimulated, 6 in control group, [Bordbar et al., 2012]), Taware (53 subjects with head and neck carcinoma, 39 in control group, [Taware et al., 2018]) and Al-Mutawa (only the tumour group, 25 subject with hypoxic preconditioning and 19 in control group, [Al-Mutawa et al., 2018]). The detailed information of the four data sets are listed in Table 1. For each data, pathways are generated by ‘rWikiPathways’ [Slenter et al., 2017], an R package to interface with the WikiPathways database. Some of the data sets have metabolites with the same CheBI ID. In this case, we take all such metabolites as a small pathway.
Eisner can be acquired via MetaboAnalyst and the others can be downloaded from ‘MetaboLights’ \cite{Haug2012}, a database for metabolomics experiments and derived information. We use the logarithm transformation with base 2 to normalize the data containing only positive values, such as Eisner and Bordbar, and use the generalized logarithm \cite{Xia2015} to normalize the data containing negative values or zeros, such as Taware and Al-Mutawa. A logistic regression model is used to fit these data sets.

| Method          | Eisner | Bordbar | Taware | Al-Mutawa |
|-----------------|--------|---------|--------|-----------|
| sample size     | 77     | 12      | 92     | 44        |
| metabolites     | 63     | 50      | 48     | 261       |
| Pathways & Metabolites | 113 | 63      | 50     | 421       |
| mean pathway size | 1.92  | 1.73    | 1.08   | 10.83     |
| minimum pathway size | 1     | 1       | 1      | 1         |
| maximal pathway size | 10    | 14      | 4      | 103       |

Table 1: Four metabolomics data sets.

We apply our single-step shortcut and iterative shortcut to these four data sets, and compare them with the above-mentioned methods. CTGT$_0$ represents the single-step shortcut. CTGT$_i$, $i = 500, 20000$ represents iterative shortcut with $i$ iterations. Table 2 shows the number of rejected pathways per method per data set. Numbers in the parenthesis for CTGT methods are the number of unsure outcomes remained.

CTGT methods with more iterations are more powerful, as we can see from Table 2 that CTGT$_0$ rejects less pathways than CTGT$_{500}$, which rejects less than CTGT$_{20000}$. CTGT methods with zero unsure rejections is the full closed testing procedure. For example, CTGT$_{20000}$ is in fact the full closed testing procedure for all these data sets. Noting that not all unsure outcomes become surely
rejected when applying the iterative shortcut. For example, for Al-Mutawa, CTGT has 11 unsure outcomes remained, among which 5 are surely rejected by CTGT\textsubscript{500} and one is surely not rejected.

As is shown in Table 2, that CTGT methods decent power property, especially with 20000 iterations. The strength of CTGT is emphasized specifically for data set Bordbar, where there are only 12 samples but 50 metabolites, for which CTGT method already rejected more pathways than the others. This might be caused by the small sample size, which influences the Bonferoni-based procedures more than CTGT methods, which do not need divide $\alpha$ by the number of hypotheses. However, for low-dimensional data set Taware, especially for small-scale multiple testings, the former may outperform the later.

| Method     | Eisner | Bordbar | Taware | Al-Mutawa |
|------------|--------|---------|--------|-----------|
| CTGT\textsubscript{0} | 31(42) | 60(1)  | 6(10)  | 111(11)   |
| CTGT\textsubscript{500} | 50(23) | 61(0)  | 16(0)  | 116(5)    |
| CTGT\textsubscript{20000} | 73(0)  | 61(0)  | 16(0)  | 119(0)    |
| SEA        | 74     | 57      | 21     | 224       |
| SH         | 57     | 43      | 21     | 201       |
| FL         | 45     | 46      | 20     | 186       |
| DAG        | 53     | 43      | 21     | 81        |

Table 2: Number of rejected pathways for Eisner, Bordbar, Taware and Al-Mutawa. Number in the parenthesis represents unsure outcomes remained by CTGT method.

We use Figure 7 and Figure 8 to look into detailed information of rejections per method, where CTGT represents the iterative shortcut with 20000 iterations. Additionally, marginal histograms for x-axis denote the number of shared rejections per method.
It is shown in Figure 7 and Figure 8 that rejected pathways by CTGT are mainly gathered around large pathway size. This implies that CTGT is preferable for testing multiple large-size pathways. SEA, DAG, Focus Level and Structured Holm are, in contrast, better for testing small-size pathways, especially when there are many strong features. Unfortunately, they are sensitive to the total number of hypotheses. For example, for Al-Mutawa, DAG method becomes conservative because of the number of pathways, for which there will be 91 rejected pathways if we reduce to test the first 300 largest pathways. In addition, CTGT identifies metabolites ‘lactose\_lactulose\_118’ (the first rejection of CTGT from the left of Figure 8) and ‘homocutullines’ (the third one) that are significantly associated with the hypoxic preconditioned tumour for Al-Mutawa, but other methods do not.

We would pay more attention to the short bars in the marginal histograms, which imply the ‘short slabs’ of a method who has no rejections. CTGT is potentially powerful for testing large pathways rather than small ones, which can be obviously seen from Figure 8 for AL-Mutawa data set in Figure 8. While DAG, FL, SH or SEA, as shown in Figure 7 and Figure 8 can be easily effected by the number of hypotheses and sample size as they are basically Bonferroni-based methods.
Figure 7: Rejected pathways for Eisner and Bordbar.
Figure 8: Rejected pathways for Taware and Al-Mutawa.
7 Discussion

We have proposed a shortcut for the closed testing with Globaltest (CTGT). Globaltest is a powerful test for pathway analysis, which is specifically powerful when testing the sets with many weak features. It can be used in high-dimensional settings, which is becoming increasingly common especially in the area of biomedical science. Closed testing is a powerful and robust procedure for FWER control. Based on our shortcut, we make the full closed testing feasible for hundreds of features, which was 20 around before.

We derive our shortcut in two ways: the single-step shortcut and the iterative shortcut. They dramatically reduced the computational complexity as a hypothesis can be rejected by closed testing without carrying out the full procedure. The single-step shortcut might have inconclusive outcomes. We then derive the iterative shortcut by applying the branch and bound method to further test these pathways until we found certain outcomes. But we also allow the iterative shortcut stop at any prespecified point with FWER still controlled. Shortcut with more iterations will get closer to the full closed testing procedure but will also be more time-consuming. Once there are no uncertain outcomes left, the full closed testing procedure is obtained.

Furthermore, CTGT methods is post hoc, i.e. it allows researchers to choose a certain feature sets a priori and control FWER for all possible feature sets. One competitive FWER-controlling procedures that is also post hoc is SEA on the basis of Hommel [Meijer et al. 2019], which is fast in computations. It allows flexible null hypothesis testing and provides adjusted p-values and simultaneous confidence bounds for true and false discovery proportions. However, it assumes the positive dependence of p-values and is less powerful than Globaltest when testing large pathways. Our method is more desirable for large pathways. For a given group of feature sets tested by Globaltest, FWER can also be controlled by
DAG, Focus Level, and Structured Holm, whose power property can be largely influenced by the total amount of hypotheses.

We also argue that the shortcut works especially well for small sample size. Small sample size results in less accurate asymptotic null distributions, and accordingly less accurate tail probabilities, especially for extremely small tail probabilities, such as $\frac{\alpha}{m}$ where $m$ is a large number of hypotheses. This causes the Bonferroni-based method less accurate than CTGT for which the significance level is always $\alpha$.

One assumption on the CTGT methods is that it holds only for small significance level $\alpha$, i.e. $\alpha \leq \alpha_0$. We have shown that $\alpha = 0.05$ generally works well with applications on metabolomics data.

To give a better idea of when to use what procedure, we performed all above mentioned methods on real metabolomics data sets to examine the strengths and weaknesses of each method, as there is no one-fit-all method. Noting that our method is not limited to metabolomics data, but also genomics data. Other possibilities that could be analyzed in the future are to build the confidence interval for the number of true discoveries when rejecting any specific feature set or pathway.

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