

HYPOTHESIS SETTING AND ORDER STATISTIC FOR ROBUST GENOMIC META-ANALYSIS

BY CHI SONG AND GEORGE C. TSENG

University of Pittsburgh

Meta-analysis techniques have been widely developed and applied in genomic applications, especially for combining multiple transcriptomic studies. In this paper, we propose an order statistic of p -values (r th ordered p -value, rOP) across combined studies as the test statistic. We illustrate different hypothesis settings that detect gene markers differentially expressed (DE) “in all studies”, “in the majority of studies”, or “in one or more studies”, and specify rOP as a suitable method for detecting DE genes “in the majority of studies”. We develop methods to estimate the parameter r in rOP for real applications. Statistical properties such as its asymptotic behavior and a one-sided testing correction for detecting markers of concordant expression changes are explored. Power calculation and simulation show better performance of rOP compared to classical Fisher’s method, Stouffer’s method, minimum p -value method and maximum p -value method under the focused hypothesis setting. Theoretically, rOP is found connected to the naïve vote counting method and can be viewed as a generalized form of vote counting with better statistical properties. The method is applied to three microarray meta-analysis examples including major depressive disorder, brain cancer and diabetes. The results demonstrate rOP as a more generalizable, robust and sensitive statistical framework to detect disease-related markers.

1. Introduction. With the advances in high-throughput experimental technology in the past decade, the production of genomic data has become affordable and thus prevalent in biomedical research. Accumulation of experimental data in the public domain has grown rapidly, particularly of microarray data for gene expression analysis and single nucleotide polymorphism (SNP) genotyping data for genome-wide association studies (GWAS). For example, the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) from the National Center for Biotechnology Information (NCBI) and the Gene Expression Atlas (<http://www.ebi.ac.uk/gxa/>) from the European Bioinformatics Institute (EBI) are the two largest public depository websites for gene expression data and the database of Genotypes and Phenotypes (dbGaP, <http://www.ncbi.nlm.nih.gov/gap/>) has the largest collection of genotype data. Because individual studies usually

AMS 2000 subject classifications: genomics, meta-analysis, order statistic, p -value

contain limited numbers of samples, and the reproducibility of genomic studies is relatively low, the generalizability of their conclusions is often criticized. Therefore, combining multiple studies to improve statistical power and to provide validated conclusions has emerged as a common practice (see recent review papers by [Tseng, Ghosh and Feingold, 2012](#) and [Begum et al., 2012](#)). Such genomic meta-analysis is particularly useful in microarray analysis and GWAS. In this paper, we focus on microarray meta-analysis while the proposed methodology can be applied to the traditional “univariate” meta-analysis or other types of genomic meta-analysis.

Microarray experiments measure transcriptional activities of thousands of genes simultaneously. One commonly seen application of microarray data is to detect differentially expressed (DE) genes in samples labeled with two conditions (e.g. tumor recurrence versus non-recurrence), multiple conditions (e.g. multiple tumor subtypes), survival information or time series. In the literature, microarray meta-analysis usually refers to combining multiple studies of related hypotheses or conditions to better detect DE genes (also called candidate biomarkers). For this problem, two major types of statistical procedures have been used: combining effect sizes or combining p-values. Generally speaking, no single method performs uniformly better than the others in all datasets for various biological objectives, both from a theoretical point of view ([Littell and Folks, 1971, 1973](#)) and from empirical experiences. In combining effect sizes, the fixed effects model and the random effects model are the most popular methods ([Cooper, Hedges and Valentine, 2009](#)). These methods are usually more straightforward and powerful to directly synthesize information of the effect size estimates, compared to p-value combination methods. They are, however, only applicable to samples with two conditions when the effect sizes can be defined and combined. On the other hand, methods combining p-values provide better flexibility for various outcome conditions as long as p-values can be assessed for integration. Fisher’s method is among the earliest p-value methods applied to microarray meta-analysis ([Rhodes et al., 2002](#)). It sums the log-transformed p-values to aggregate statistical significance across studies. Under the null hypothesis, assuming that the studies are independent and the hypothesis testing procedure correctly fits the observed data, Fisher’s statistic follows a chi-squared distribution with degrees of freedom $2K$, where K is the number of studies combined. Other methods such as Stouffer’s method ([Stouffer et al., 1949](#)), minP method ([Tippett, 1931](#)) and maxP method ([Wilkinson, 1951](#)) have also been widely used in microarray meta-analysis. It can be shown that these test statistics have simple analytical forms of null distributions and thus they are easy to apply to the genomic settings. The assumptions and

hypothesis settings behind these methods are, however, very different and have not been carefully considered in most microarray meta-analysis applications so far. In Fisher, Stouffer and minP, the methods detect markers that are differentially expressed in “one or more” studies (see the definition of HS_B in Section 2.1). In other words, an extremely small p-value in one study is usually enough to impact the meta-analysis and cause statistical significance. On the contrary, methods like maxP tend to detect markers that are differentially expressed in “all” studies (called HS_A in Section 2.1) since maxP requires that all combined p-values are small for a marker to be detected. In this paper, we begin in Section 2.1 to elucidate the hypothesis settings and biological implications behind these methods. In many meta-analysis applications, detecting markers differentially expressed in all studies is more appealing. The requirement of DE in “all” studies, however, is too stringent when K is large and in light of the fact that experimental data are peppered with noisy measurements from probe design, sample collection, data generation and analysis. Thus, we describe in Section 2.1 a robust hypothesis setting (called HS_r) that detects biomarkers differentially expressed “in the majority of” studies (e.g. $> 70\%$ of the studies) and we propose a robust order statistic, r th ordered p-value (rOP), for this hypothesis setting.

The remainder of this paper is structured as follows to develop the rOP method. In Section 2.2, the rationale and algorithm of rOP are outlined, and the methods for parameter estimation are described in Section 2.3. Section 2.4 extends rOP with a one-sided test correction to avoid detection of DE genes with discordant fold change directions across studies. Section 3 demonstrates applications of rOP to three examples in brain cancer, major depressive disorder (MDD) and diabetes, and compares the result with other classical meta-analysis methods. We further explore power calculation and asymptotic properties of rOP in Section 4.1, and evaluate rOP in genomic settings by simulation in Section 4.2. We also establish an unexpected but insightful connection of rOP with the classical naïve vote counting method in Section 4.3. Section 5 contains final conclusions and discussions.

2. r th Ordered P-value (rOP).

2.1. *Hypothesis settings and motivation.* We consider the situation when K transcriptomic studies are combined for a meta-analysis where each study contains G genes for information integration. Denote by θ_{gk} the underlying true effect size for gene g and study k ($1 \leq g \leq G$, $1 \leq k \leq K$). For a given gene g , we follow the convention of Birnbaum (1954) and Li and Tseng (2011) to consider two complementary hypothesis settings, depending on

the pursuit of different types of targeted markers:

$$\begin{aligned} \text{HS}_A &: \left\{ H_0 : \bigcap_k \{\theta_{gk} = 0\} \text{ versus } H_a^{(A)} : \bigcap_k \{\theta_{gk} \neq 0\} \right\} \\ \text{HS}_B &: \left\{ H_0 : \bigcap_k \{\theta_{gk} = 0\} \text{ versus } H_a^{(B)} : \bigcup_k \{\theta_{gk} \neq 0\} \right\} \end{aligned}$$

In HS_A , the targeted biomarkers are those differentially expressed in all studies (i.e. the alternative hypothesis is the intersection event that effect sizes of all K studies are non-zero), while HS_B pursues biomarkers differentially expressed in one or more studies (the alternative hypothesis is the union event instead of the intersection in HS_A). Biologically speaking, HS_A is more stringent and more desirable to identify consistent biomarkers across all studies if the studies are homogeneous. HS_B , however, is useful when heterogeneity is expected. For example, if studies analyzing different tissues are combined (e.g. study 1 uses epithelial tissues and study 2 uses blood samples), it is reasonable to identify tissue-specific biomarkers detected by HS_B . We note that HS_B is identical to the classical union-intersection test (UIT) (Roy, 1953) but HS_A is different from intersection-union test (IUT) (Berger, 1982; Berger and Hsu, 1996). In IUT, the statistical hypothesis is in complementary form between null and alternative hypothesis $\left\{ H_0 : \bigcup_k \{\theta_{gk} = 0\} \text{ versus } H_a^{(A)} : \bigcap_k \{\theta_{gk} \neq 0\} \right\}$. Solutions for IUT require more sophisticated mixture or Bayesian modeling to accommodate the composite null hypothesis and are not the focus of this paper (for more details, see Erickson, Kim and Allison, 2009).

As discussed in Tseng, Ghosh and Feingold (2012), most existing genomic meta-analysis methods target on HS_B . Popular methods include classical Fisher's method (sum of minus log-transformed p-values; Fisher, 1925), Stouffer's method (sum of inverse-normal-transformed p-values; Stouffer et al., 1949), minP (minimum of combined p-values; Tippett, 1931) and a recently proposed adaptively weighted (AW) Fisher's method (Li and Tseng, 2011). The random effects model targets on a slight variation of HS_A , where the effect sizes in the alternative hypothesis are random effects drawn from a Gaussian distribution centered away from zero (but are not guaranteed to be all non-zero). The maximum p-value method (maxP) is probably the only method available to specifically target on HS_A so far. By taking the maximum of p-values from combined studies as the test statistic, the method requires that all p-values to be small for a gene to be detected. Assuming independence across studies and that the inferences to generate p-values in single studies are correctly specified, p-values (p_k as the

p-value of study k) are i.i.d. uniformly distributed in $[0, 1]$. Fisher’s statistic ($S^{Fisher} = -2 \sum \log p_k$) follows a chi-squared distribution with degree of freedom $2K$ (i.e. $S^{Fisher} \sim \chi^2(2K)$) under null hypothesis H_0 ; Stouffer’s statistic ($S^{Stouffer} = \sum \Phi^{-1}(1-p_k)$, where $\Phi^{-1}(\cdot)$ is the quantile function of a standard normal distribution) follows a normal distribution with variance K (i.e. $S^{Stouffer} \sim N(0, K)$); minP statistic ($S^{minP} = \min\{p_k\}$) follows a Beta distribution with parameters 1 and K (i.e. $S^{minP} \sim Beta(1, K)$); and maxP statistic ($S^{maxP} = \max\{p_k\}$) follows a Beta distribution with parameters K and 1 (i.e. $S^{maxP} \sim Beta(K, 1)$).

The HS_A hypothesis setting and maxP method are obviously too stringent in light of the generally noisy nature of microarray experiments. When K is large, HS_A is not robust and inevitably detects very few genes. Instead of requiring differential expression in all studies, biologists are more interested in, for example, “biomarkers that are differentially expressed in more than 70% of the combined studies.” Denote by $\Theta_h = \left\{ \sum_{k=1}^K I(\theta_{gk} \neq 0) = h \right\}$ the situation that exactly h out of K studies are differentially expressed. The new robust hypothesis setting becomes:

$$HS_r : \left\{ H_0 : \bigcap_k \{\theta_{gk} = 0\} \text{ versus } H_a^{(r)} : \bigcup_{h=r}^K \Theta_h \right\}$$

where $r = \lceil p \cdot K \rceil$, $\lceil x \rceil$ is the smallest integer no less than x and p ($0 < p \leq 1$) is the minimal percentage of studies required to call differential expression (e.g. $p = 70\%$). We note that HS_A and HS_B are both special cases of the extended HS_r class (i.e. $HS_A = HS_K$ and $HS_B = HS_1$), but we will focus on large r (i.e. $p > 50\%$) in this paper and view HS_r as a relaxed and robust form of HS_A .

In the literature, maxP has been used for HS_A and minP has been used for HS_B . An intuitive extension of these two methods for HS_r is to use the r th ordered p-value (rOP). Before introducing the algorithm and properties of rOP, we illustrate the motivation of it by the following example. Suppose we consider four genes in five studies, gene A has marginally significant p-values ($p = 0.1$) in all five studies; gene B has a strong p-value in study 1 ($p = 1e - 20$) but $p = 0.9$ in the other four studies; gene C is similar to Gene A but has much weaker statistical significance ($p = 0.25$ in all five studies); gene D differs from gene C in that studies 1-4 have small p-values ($p = 0.15$) but study 5 has a large p-value ($p = 0.9$). Table 1 shows the resulting p-values from five meta-analysis methods that are derived from classical parametric inference in Section 1. Comparing Fisher and minP in HS_B , minP is sensitive to a study that has a very small p-value (see gene B) while Fisher, as an evidence aggregation method, is more sensitive

when all or most studies are marginally statistically significant (e.g. gene A). Stouffer behaves similarly to Fisher except that it is less sensitive to the extremely small p-value in gene B. When we turn our attention to HS_A , gene C and gene D cannot be detected by all three of Fisher, Stouffer and minP methods. Gene C can be detected by both maxP and rOP as expected ($p = 0.001$ and 0.015 , respectively). For gene D, it cannot be identified by maxP method ($p = 0.59$) but can be detected by rOP at $r = 4$ ($p = 0.002$). Gene D gives a good motivating example that maxP may be too stringent when many studies are combined and rOP provides additional robustness when one or a small portion of studies are not statistically significant. In genomic meta-analysis, genes similar to gene D are common due to the noisy nature of high-throughput genomic experiments or when a low quality study is accidentally included in the meta-analysis. Although the types of desired markers (under HS_A , HS_B or HS_r) depend on the biological goal of a specific application, gene A, C and D are normally desirable marker candidates that researchers wish to detect in most situations while gene B is not (unless study-specific markers are expected as mentioned in Section 1). This toy example motivates the development of a robust order statistic of rOP below.

TABLE 1
Four hypothetical genes to compare different meta-analysis methods and to illustrate the motivation of rOP (*: p-values smaller than 0.05)

	gene A	gene B	gene C	gene D
Study 1	0.1	1E-20	0.25	0.15
Study 2	0.1	0.9	0.25	0.15
Study 3	0.1	0.9	0.25	0.15
Study 4	0.1	0.9	0.25	0.15
Study 5	0.1	0.9	0.25	0.9
Fisher (HS_B)	0.01*	1E-15*	0.18	0.12
Stouffer (HS_B)	0.002*	0.03*	0.07	0.10
minP (HS_B)	0.41	5E-20*	0.76	0.56
maxP (HS_A)	1E-5*	0.59	0.001*	0.59
rOP ($r = 4$) (HS_r)	5E-4*	0.92	0.015*	0.002*

2.2. *The rOP method.* Below is the algorithm for rOP when the parameter r is fixed. For a given gene g , without loss of generality, we ignore the subscript g and denote by $S_r = p_{(r)}$ where $p_{(r)}$ is the r th order statistic of p-values $\{p_1, p_2, \dots, p_K\}$. Under the null hypothesis H_0 , S_r follows a beta distribution with shape parameters r and $K - r + 1$, assuming that the model to generate p-values under the null hypothesis is correctly specified and all studies are independent. To implement rOP, one may apply this paramet-

ric null distribution to calculate the p-values for all genes and perform a Benjamini-Hochberg (BH) correction (Benjamini and Hochberg, 1995) to control the false discovery rate (FDR) under general dependence structure. The Benjamini-Hochberg procedure can control the FDR at the nominal level or less when the multiple comparisons are independent or positively dependent. Although the Benjamini-Yekutieli (BY) procedure can be applied to a more general dependence structure of the comparisons, it is often too conservative and unnecessary (Benjamini and Yekutieli, 2001), especially in gene expression analysis where the comparisons are more likely to be positively dependent and the effect sizes are usually small to moderate (also see Section 4.2 for simulation results). As a result, we will not consider the BY procedure in this paper. The parametric BH approach has the advantage of fast computation but in many situations the parametric beta null distribution may be violated because the assumptions to obtain p-values from each single study are not met and the null distributions of p-values are not uniformly distributed. When such violations of assumptions are suspected, we alternatively recommend a conventional permutation analysis (PA) instead. Class labels of the samples in each study are randomly permuted and the entire DE and meta-analysis procedures are followed. The permutation is repeated for B times ($B = 500$ in this paper) to simulate the null distribution and assess the p-values and q-values. The permutation analysis is used for all meta-analysis methods (including rOP, Fisher, Stouffer, minP and maxP) in this paper unless otherwise stated.

We note that both minP and maxP are special cases of rOP, but in this paper we mainly consider properties of rOP as a robust form of maxP (specifically, $K/2 \leq r \leq K$).

2.3. Selection of r in an application. The best selection of r should depend on the biological interests. Ideally, r is a tuning parameter that is selected by the biologists based on the biological questions asked and the experimental designs of the studies. However, in many cases, biologists may not have a strong prior knowledge for the selection of r and data-driven methods for estimating r may provide additional guidance in applications. The purpose of selecting $r < K$ is to tolerate potentially outlying studies and noises in the data. The noises may come from experimental limitations (e.g. failure in probe design, erroneous gene annotation or bias from experimental protocol) or heterogeneous patient cohorts in different studies. Another extreme case may come from inappropriate inclusion of a low-quality study into the genomic meta-analysis. Below we introduce two complementary guidelines to help select r for rOP. The first method comes from the

adjusted number of detected DE genes and the second is based on pathway association (a.k.a. gene set analysis), incorporating external biological knowledge.

2.3.1. Evaluation based on the number of detected DE genes. In the first method, we use a heuristic criterion to find the best r such that the number of detected DE genes is the largest. The dashed line in Figure 1(a) shows the number of detected DE genes using different r in rOP in a brain cancer application. The result shows a general decreasing trend in the number of detected DE genes when r increases. However, when we randomly permute the p-values across genes within each study, the detected number of DE genes also shows a bias towards small r 's (dotted line). It shows that a large number of DE genes can be detected by a small r (e.g. $r=1$ or 2) simply by chance. To eliminate this artifact, we apply a de-trending method by subtracting the dotted permuted baseline from the dashed line. The resulting adjusted number of DE genes (solid line) is then used to seek the maximum that correspond to the suggested r . This de-trend adjustment is similar to what was used in GAP statistic (Tibshirani, Walther and Hastie, 2001) when estimating the number of clusters in cluster analysis. In such a scenario, the curve of number of clusters (on x-axis) versus sum of squared within-cluster dispersions is used to estimate the number of clusters. The curve always has a decreasing trend even in random datasets and the goal is usually to find an ‘‘elbow-like’’ turning point. GAP statistic permutes the data to generate a baseline curve and subtract it from the observed curve. The problem becomes to find the maximum point in the de-trended curve, a setting very similar to ours.

Below we describe the algorithm for the first criterion. Using the original K studies, the number of DE genes detected by rOP using different r ($1 \leq r \leq K$) is first calculated as N_r (under certain false discovery rate threshold, e.g. FDR = 5%; see dashed line in Figure 1(a)). We then randomly permute p-values in each study independently and re-calculate the number of DE genes as $N_r^{(b)}$ in the b th permutation. The permutation is repeated for B times ($B = 100$ in this paper) and the adjusted number of detected DE genes is defined as $N'_r = N_r - \sum_{b=1}^B N_r^{(b)} / B$ (see solid line in Figure 1(a)). In other words, the adjusted number of DE genes is de-trended so that it is purely contributed by the consistent DE information among studies. The parameter r is selected so that N'_r is maximized (or we manually select r as large as possible when N'_r reaches among the largest).

REMARK 1. Note that N'_r could sometimes be negative. This happens mostly when the signal in a single study is strong and r is small. However,

since we usually apply rOP for relatively large K and r , the negative value is usually not an issue. We also note that, unlike GAP statistic, the criterion to choose r with the maximal adjusted number of detected DE genes is heuristic and has no theoretical guarantee. In simulations and real applications to be shown later, this method performs well and provides results consistent with the second criterion described below.

2.3.2. Evaluation based on biological association. Pathway analysis (a.k.a. gene set analysis) is a statistical tool to infer the correlation of differential expression evidence in the data with pathway knowledge (usually sets of genes with known common biological function or interactions) from established databases. In this approach, we hypothesize that the best selection of r will produce a DE analysis result that generates the strongest statistical association with “important” (i.e. disease-related) pathways. Such pathways can be provided by biologists or obtained from pathway databases. However, it is well-recognized that our understanding of biological and disease-related pathways are relatively poor and subject to change every few years. This is especially true for many complex diseases, such as cancers, psychiatric disorders and diabetes. In this case, it is more practical to use computational methods to generate “pseudo” disease-related pathways that are further reviewed by biologists before being utilized to estimate r . Below, we develop a computational procedure for selecting disease-related pathways. We perform pathway analysis using a large pathway database (e.g. GO, KEGG or BioCarta) and select pathways that are top-ranked by aggregated committee decision of different r from rOP. The detailed algorithm is as follows:

STEP I. Identification of disease-related pathways: (committee decision by $[K/2] + 1 \leq r \leq K$)

1. Apply rOP method to combine studies and generate p-values for each gene. Run through different r , $[K/2] + 1 \leq r \leq K$.
2. For a given pathway m , apply Kolmogorov-Smirnov test to compare the p-values of genes in the pathway and those outside the pathway. The pathway enrichment p-values are generated as $p_{r,m}$. Its rank among all pathways for a given r is calculated as $R_{r,m} = \text{rank}_m(p_{r,m})$. Small ranks suggest strong pathway enrichment for pathway m .
3. The sums of ranks of different r are calculated as $S_m = \sum_{r=[K/2]+1}^K R_{r,m}$. The top $U = 100$ pathways with the smallest S_m scores are selected and denoted as M . We treat M as the “pseudo” disease-related pathway set.

STEP II. Sequential testing of improved pathway enrichment significance:

1. We perform sequential hypothesis testing that starts from $r' = K$ since conceptually we would like to pick r as large as possible. We first perform a Wilcoxon signed rank test to test for difference of pathway enrichment significance for $r' = K$ and $r' = K - 1$. In other words, we perform a two-sample test on the paired vectors of $(p_{K,m}; m \in M)$ and $(p_{K-1,m}; m \in M)$ and record the p-value as $\tilde{p}_{K,K-1}$.
2. If the test is rejected (using the conventional type I error of 0.05), it indicates that reducing from $r = K$ to $r = K - 1$ can generate DE gene list that produce more significant pathway enrichment in M . We will continue to reduce r' by one (i.e. $r' = K - 1$) and repeat the test between $(p_{r',m}; m \in M)$ and $(p_{r'-1,m}; m \in M)$. Similarly, the resulting p-values are recorded as $\tilde{p}_{r',r'-1}$. The procedure is repeated until the test from r' is not rejected. The final r' is selected for rOP.

REMARK 2. Note that for simplicity and since this evaluation should be examined together with the first criterion in Section 2.3.1, we will not perform p-value correction for multiple comparison or sequentially dependent hypothesis testings here. Practically, we suggest to select r based on the diagnostic plots of the two criteria simultaneously. Examples of the selection will be shown in Section 3.

REMARK 3. We have tested different U in real applications. As can be expected, the selection of U did not affect the result much. In Supplement Figure 7, we show that the ranks for rOP with different selection of r as well as other methods become stable enough when $U = 100$ for all our applications.

2.4. *One-sided test modification to avoid discordant effect sizes.* Methods combining effect sizes (e.g. random or fixed effects models) are suitable to combine studies with binary outcome, in which case the effect sizes are well-defined as the standardized mean differences or odds ratios. Methods combining p-values, however, have advantages in combining studies with non-binary outcomes (e.g. multi-class, continuous or censored data), in which case F-test, simple linear regression or Cox proportional hazard model can be used to generate p-values for integration. On the other hand, p-value combination methods usually combine two-sided p-values in binary outcome data. A gene may be found statistically significant with up-regulation in one study and down-regulation in another study. Such a confusing discordance, although sometimes a reflection of the biological truth, is often undesirable in most applications. Therefore, we make a one-sided test modification to the rOP method similar to the modification that Owen (2009) and Pearson

(1934) applied on Fisher’s method. The modified rOP statistic is defined as the minimum of the two rOP statistics combining the one-sided tests of both tails. Details of this test statistic can be found in the Supplement Text.

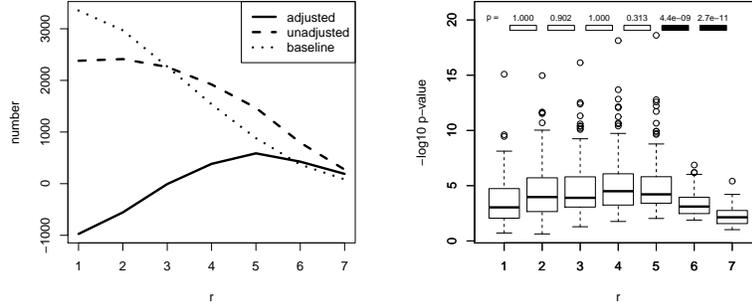
3. Applications. We applied rOP as well as other meta-analysis methods to three microarray meta-analysis applications with different strength of DE signal and different degrees of heterogeneity. Supplement Table 1A-1C lists the detailed information on seven brain cancer studies, nine major depressive disorder (MDD) studies, and 16 diabetes studies for meta-analysis. Data were preprocessed and normalized by standard procedures in each array platform. Affymetrix datasets were processed by RMA method and Illumina datasets were processed by manufacturer’s software with quantile normalization for probe analysis. Probes were matched to the same gene symbols. When multiple probes (or probe sets) matched to one gene symbol, the probe that contained the largest variability (i.e. inter-quartile range) was used to represent the gene. After gene matching and filtering, 5,836, 7,577 and 6,645 genes remained in brain cancer, MDD and diabetes datasets, respectively. The brain cancer studies were collected from the GEO database. The MDD studies were obtained from Dr. Etienne Sibille’s lab. A random intercept model adjusted for potential confounders was applied to each MDD study to obtain p-values (Wang et al., 2012a). Preprocessed data of 16 diabetes studies described by Park et al. (2009) were obtained from the authors. For studies with multiple groups, we followed the procedure of Park et al. by taking the minimum p-value of all the pairwise comparisons and adjusted for multiple tests. All the pathways used in this paper were downloaded from Molecular Signatures Database (MSigDB, Subramanian et al., 2005). Pathway collections c2, c3 and c5 were used for r selection purpose.

3.1. *Application of rOP.* In all three applications, we demonstrate the estimation of r for rOP using the two evaluation criteria in Section 2.3. In the first dataset, two important subtypes of brain tumors - anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM) - were compared in seven microarray studies. To estimate an adequate r for rOP application, we calculated the unadjusted number, the baseline number from permutation and the adjusted number of detected DE genes using $1 \leq r \leq 7$ under FDR=5% (Figure 1(a)). The result showed a peak at $r = 5$. For the second estimation method by pathway analysis, boxplots of $-\log_{10}(p)$ (p-values calculated from association of DE gene list with top pathways) versus r were plotted (Figure 1(b)). The Wilcoxon signed rank tests showed that the result from $r = 6$ is significantly more associated with pathways than that from $r = 7$

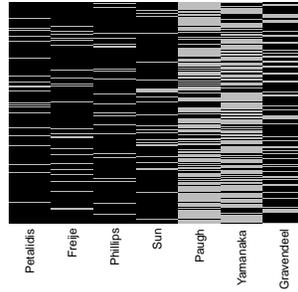
($p = 2.7e - 11$) and similarly for $r = 5$ versus $r = 6$ ($p = 4.4e - 9$). Combining the results from Figures 1(a) and 1(b), we decided to choose $r = 5$ for this application. Figure 1(c) shows the heatmap of studies effective in rOP (when $r = 5$) for each detected DE gene (a total of 1,469 DE genes on the rows and seven studies on the columns). For example, if p-values for the seven studies are (0.13, 0.11, 0.03, 0.001, 0.4, 0.7, 0.15), the test statistic for rOP is $S^{rOP} = 0.15$ and the five effective studies that contribute to rOP are indicated as (1, 1, 1, 1, 0, 0, 1). In the heatmap, effective studies were indicated by black color and non-effective studies were in light gray. As shown in Figure 1(c), Paugh and Yamanaka were non-effective studies in almost all detected DE genes, suggesting that the two studies did not contribute to the meta-analysis and may potentially be problematic studies. This finding agrees with a recent MetaQC assessment result using the same seven studies (Kang et al., 2012). In our application, AA and GBM patients were compared in all seven studies. We expected to detect biomarkers that have consistent fold change direction across studies and the one-sided corrected rOP method was more preferable. Supplement Figure 1 showed plots similar to Figure 1 for one-sided corrected rOP. The result similarly concluded that $r = 5$ was the most suitable choice for this application.

For the second application, nine microarray studies used different areas of post-mortem brain tissues from MDD patients and control samples (Supplement Table 1B). MDD is a complex genetic disease with largely unknown disease mechanism and gene regulatory networks. The post-mortem brain tissues usually result in weak signals, compared to blood or tumor tissues, which makes meta-analysis an appealing approach. In Supplement Figure 2(a), the maximizer of adjusted DE gene detection was at $r = 6$ ($r = 7$ or 8 are also good choices). For Supplement Figure 2(b), the statistical significance improved “from $r = 9$ to $r = 8$ ” ($p = 5.6e - 14$), “from $r = 8$ to $r = 7$ ” ($p = 8.7e - 7$) and “from $r = 7$ to $r = 6$ ” ($p = 0.045$). We also obtained 98 pathways that were potentially related to MDD from Dr. Etienne Sibille. As shown in Supplement Figure 2(c), the statistical significance improved “from $r = 8$ to $r = 7$ ” using the 98 expert selected pathways. Combining the results, we decided to choose $r = 7$ (since $r = 6$ only provided marginal improvement in both criteria and we preferred r as large as possible) for the rOP method in this application. Supplement Figure 2(d) showed the heatmap of effective studies in rOP. No obvious problematic study was observed. The one-sided rOP was also applied (results not shown), good selection of r appeared to be between 5 and 7.

In the last application, 16 diabetes microarray studies were combined. These 16 studies were very heterogeneous in terms of the organisms, tis-



(a) Adjusted and unadjusted number of detected DE genes using different logical association evaluation. P-values for Wilcoxon signed rank tests are shown on the top. Black filled rectangles represent a p-value smaller than 0.05.



(c) Heatmap to show effective studies of rOP in each gene. Effective studies are shown in black and non-effective ones are in light gray.

FIG 1. Results of brain cancer dataset applying rOP.

sues and experimental design (Supplement Table 1C). Supplement Figure 7 showed diagnostic plots to estimate r . Although the number of studies and heterogeneity across datasets were relatively larger than the previous two examples, we could still observe similar trends in Supplement Figure 7. Specifically, for Supplement Figure 3(a), it was shown that $r = 7 \sim 12$ detected higher adjusted number of DE genes. For pathway analysis, results from $r = 12$ was more associated with the top pathways. As a result, we decided to use $r = 12$ in this application. It was noticeable that the r selec-

tion in this diabetes example was relatively vague, compared to the previous examples. Supplement Figure 3(c) showed the heatmap of effective studies in rOP. Two to four studies (s01, s05, s08 and s14) appeared to be candidates of problematic studies, but the evidence was not as clear as the brain cancer example in Figure 1(c). It should be noted that the results of Supplement Figure 3 used the beta null distribution inference and Benjamini-Hochberg correction. Permutation analysis generated a relatively unstable result (Supplement Figure 4), although it suggested a similar selection of r . This was possibly due to the unusual *ad hoc* DE analysis from minimum p-values of all possible pairs of comparisons (procedures that were used in the original paper [Park et al., 2009](#)).

Next, we explored the robustness of rOP by mixing a randomly chosen MDD study into seven brain cancer studies as an outlier. The results in Supplement Figure 5 showed that $r = 5$ or 6 may be a good choice (Supplement Figures 5(a) and 5(b)). We used $r = 6$ in rOP for this application. Supplement Figure 5(c) interestingly showed that the mixed MDD study, together with Paugh and Yamanaka studies, were potentially problematic studies in the rOP meta-analysis. This result verified our intuition that rOP is robust to outlying studies and the p-values of the outlying studies minimally contribute to the rOP statistic.

3.2. Comparison of rOP with other meta-analysis methods. We performed rOP using r determined from Section 3.1 in four applications (brain cancer, MDD, diabetes, and brain cancer + 1 random MDD) and compared to Fisher’s method, Stouffer’s method, minP, maxP and vote counting. The vote counting method will be discussed in greater detail in Section 4.3. Two quantitative measures were used to compare the methods. The first measure compared the number of detected DE genes from each method as a surrogate of sensitivity (although the true list of DE genes is unknown and sensitivity cannot be calculated). The second approach was by pathway analysis, very similar to the method we introduced to select parameter r . However, in order to avoid bias in top pathway selection, single study analysis results were used as the committee to select disease-related pathways. KEGG, BioCarta, Reactome and GO pathways were used in the pathway analysis. Wilcoxon signed rank test was then used to test if two methods detected DE genes with differential association with disease-related pathways.

Table 2 showed the number of detected DE genes under FDR=5%. We can immediately observe that Fisher and Stouffer generally detected many more biomarkers because they targeted on HS_B (genes differentially expressed in one or more studies). Although minP also targeted on HS_B , it

sometimes detected extremely small numbers of DE genes in weak-signal data such as the MDD and diabetes examples. This is reasonable because minP has very weak power to detect consistent but weak signals across studies (e.g. p-values=(0.1, 0.1, ..., 0.1)). The stringent maxP method detected few numbers of DE genes in general. Vote counting detected very few genes especially when the effect sizes were moderate (in the MDD and diabetes examples). rOP detected more DE genes than maxP because of its relaxed HS_r hypothesis setting. It identified about 50 ~ 65% fewer DE genes than the Fisher's and Stouffer's methods but guaranteed that the genes detected were differentially expressed in the majority of the studies. We also performed the one-sided corrected rOP for comparison. This method detected similar numbers of DE genes compared to two-sided rOP, and the majority of detected DE genes in two-sided and one-sided rOP were overlapped in the brain cancer example. The result showed that almost all DE genes detected by two-sided rOP had a consistent fold change direction across studies. In MDD, the one-sided rOP detected much fewer genes than the two-sided method. This implied that many genes related to MDD acted differently in different brain regions and in different cohorts.

Figure 2 showed the results of biological association from pathway analysis that were similarly shown in 1(b). The result showed that the DE gene lists generated by Fisher and Stouffer were more associated with biological pathways. The rOP method generally performed better than maxP and minP and had similar biological association performance to Fisher's and Stouffer's methods.

TABLE 2
Number of DE gene detected by different methods under FDR=5%.

	rOP		Fisher	Stouffer	minP	maxP	VC
	Two-sided	One-sided					
Brain Cancer	1469 ($r = 5$)	1625 ($r = 5$) overlap=1139	2918	2449	2380	273	328
MDD	617 ($r = 7$)	86 ($r = 7$) overlap=48	1124	1423	0	310	0
Diabetes	636 ($r = 12$)	Not applicable	1698	1492	1	85	0
Brain + 1 MDD	751 ($r = 6$)	Not applicable	2081	1773	1648	132	64

4. Statistical properties of rOP.

4.1. *Power calculation of rOP and asymptotic properties.* When K studies are combined, suppose r_0 of the K studies have equal non-zero effect sizes and the rest of the $(K - r_0)$ studies have zero effect sizes. That is,

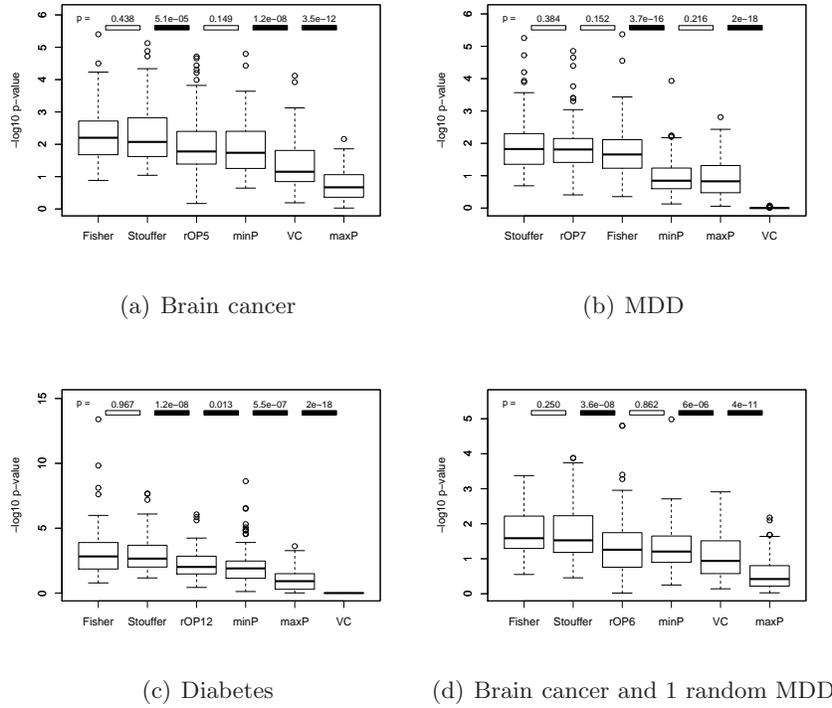


FIG 2. Comparison of different meta-analysis methods using pathway analysis.

$$H_0 : \theta_1 = \dots = \theta_K = 0$$

$$H_a : \theta_1 = \dots = \theta_{r_0} = \theta \neq 0, \theta_{r_0+1} = \dots = \theta_K = 0$$

For a single study, the power function given effect size θ is known as $\Pr(p_i \leq \alpha_0|\theta)$. We will derive the statistical power of rOP under this simplified hypothesis setting when r_0 and r for rOP are given. Under H_0 , the rejection threshold for rOP statistic is $\beta = B_\alpha(r, K-r+1)$ (the α quantile of a beta distribution with shape parameters r and $K-r+1$), where the significance level of the meta-analysis is set at α . The power of rejection threshold β under H_a is $\Pr(p_{(r)} \leq \beta|H_a) = \Pr(\sum_{i=1}^K I(p_i \leq \beta) \geq r|H_a)$. By definition $\Pr(p_i \leq \beta|\theta_i = 0) = \beta$ and we further denote $\beta' = \Pr(p_i \leq \beta|\theta_i = \theta)$. The power calculation of interest is equivalent to finding the probabilities of having at least r successes in K independent Bernoulli trials, among which

r_0 have success probabilities β' , and $K - r_0$ have success probabilities β :

$$\Pr\left(p_{(r)} \leq \beta | H_a\right) = \sum_{i=r}^K \sum_{j=\max(0, i-K+r_0)}^{\min(i, r_0)} \binom{r_0}{j} \beta'^j (1 - \beta')^{r_0-j} \binom{K-r_0}{i-j} \beta^{i-j} (1 - \beta)^{K-r_0-i+j}$$

REMARK 4. We note that the assumption of r_0 equal non-zero effect sizes can be relaxed. When the non-zero effects are not equal, the power calculation can be done in polynomial time using dynamic programming.

Below we demonstrate some asymptotic properties of rOP.

THEOREM 4.1. *Assume r_0 is fixed. When the effect size θ and K are fixed and the sample size of study k $N_k \rightarrow \infty$, $\Pr\left(p_{(r)} \leq \beta | H_a\right) \rightarrow 1$ if $r \leq r_0$. When $r > r_0$, $\Pr\left(p_{(r)} \leq \beta | H_a\right) \rightarrow c(r) < 1$ and $c(r)$ is a decreasing function in r .*

PROOF. When $N_k \rightarrow \infty$, $\beta' \rightarrow 1$. The theorem easily follows from the power calculation formulae. \square

Theorem 4.1 states that asymptotically if the parameter r in rOP is specified less or equal to the true r_0 , the statistical power converges to 1 as intuitively expected. When specifying r greater than r_0 , the statistical power is weakened with increasing r . Particularly, maxP will have weak power. In contrast to Theorem 4.1, for methods designed for HS_B (e.g. Fisher's method, Stouffer's method and minP), the power always converges to 1 if $N_k \rightarrow \infty$ and $r_0 > 0$. Figure 3(a) shows the power curve of rOP for different r when $K = 10$, $r_0 = 6$ and $N_k \rightarrow \infty$.

LEMMA 4.1. *Assume the parameter r used in rOP is fixed. When the effect size θ and K are fixed and the sample sizes $N_k \rightarrow \infty$, $\Pr\left(p_{(r)} \leq \beta | H_a\right) \rightarrow 1$ if $r_0 \geq r$. When $r_0 < r$, $\Pr\left(p_{(r)} \leq \beta | H_a\right) \rightarrow c(r_0) < 1$ and $c(r_0)$ is an increasing function in r_0 .*

Lemma 4.1 takes a different angle from Theorem 4.1. When the parameter r used in rOP is fixed, it asymptotically has perfect power to detect all genes that are differentially expressed in r or more studies. It then does not have strong power to detect genes that are differentially expressed in less than

r studies. Figure 3(b) shows a power curve of rOP for $K = 10$, $r = 6$ and $N_k \rightarrow \infty$ (solid line). We note that the dashed line ($f(r) = 0$ when $0 \leq r_0 < 6$ and $f(r) = 1$ when $6 \leq r_0 \leq 10$) is the ideal power curve for HS_r (i.e. it detects all genes that are differentially expressed in r or more studies but does not detect any gene that are differentially expressed in less than r studies). Methods like Fisher, Stouffer and minP target on HS_B and their power is always 1 asymptotically when $r_0 > 0$. The maxP method has perfect asymptotic power when $r_0 = K = 10$ but has relatively weak power when $r_0 < K$. The rOP method lies between maxP and the methods designed for HS_B . The power of rOP for $r_0 \geq 6$ converges to 1, and for $r_0 \leq 5$, the power is always smaller than 1 as the sample sizes in single studies go to infinity. Although the asymptotic powers of rOP for $r_0 = 4$ and $r_0 = 5$ are not too small, we are less concerned of these genes because they are still very likely to be important biomarkers.

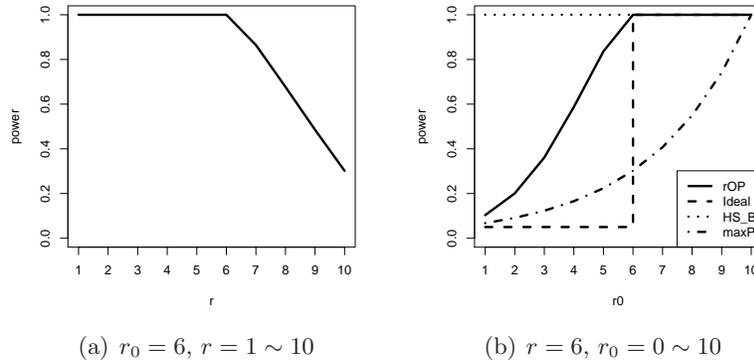


FIG 3. Power of rOP method when $N_k \rightarrow \infty$, $K = 10$.

4.2. *Power comparison in simulated studies.* To evaluate the performance of rOP in the genomic setting, we simulated a dataset using the following procedure.

STEP I. Sample 200 gene clusters, with 20 genes in each and other 6,000 genes that do not belong to any cluster. Denote $C_g \in \{0, 1, 2, \dots, 200\}$ as the cluster membership of gene g , where $C_g = 0$ means that gene g is not in a gene cluster.

STEP II. Sample the covariance matrix Σ_{ck} for genes in cluster c and in study k , where $1 \leq c \leq 200$ and $1 \leq k \leq 10$. First, sample $\Sigma'_{ck} \sim$

$W^{-1}(\Psi, 60)$, where $\Psi = 0.5I_{20 \times 20} + 0.5J_{20 \times 20}$, W^{-1} denotes the inverse Wishart distribution, I is the identity matrix and J is the matrix with all the elements equal 1. Then Σ_{ck} is calculated by standardizing Σ'_{ck} such that the diagonal elements are all 1's.

STEP III. Denote g_{c1}, \dots, g_{c20} as the indices for the 20 genes in cluster c , i.e. $C_{g_{cj}} = c$, where $1 \leq c \leq 200$ and $1 \leq j \leq 20$. Assuming the effect sizes are all zeros, sample gene expression levels of genes in cluster c for sample n as $(X'_{g_{c1}nk}, \dots, X'_{g_{c20}nk})^T \sim MVN(0, \Sigma_{ck})$, where $1 \leq n \leq 100$ and $1 \leq k \leq 10$, and sample expression level for gene g which is not in a cluster (i.e. $C_g = 0$) for sample n as $X'_{gnk} \sim N(0, 1)$, where $1 \leq n \leq 100$ and $1 \leq k \leq 10$.

STEP IV. Sample the true number of studies that gene g is DE, t_g , from a discrete uniform distribution that takes values on $1, 2, \dots, 10$, for $1 \leq g \leq 1,000$; and set $t_g = 0$ for $1,001 \leq g \leq 10,000$.

STEP V. Sample δ_{gk} , which indicates whether gene g is DE in study k , from a discrete uniform distribution that takes values on 0 or 1 and with the constraint that $\sum_k \delta_{gk} = t_g$, where $1 \leq g \leq 1,000$ and $1 \leq k \leq 10$. For $1,001 \leq g \leq 10,000$ and $1 \leq k \leq 10$, set $\delta_{gk} = 0$.

STEP VI. Sample the effect size μ_{gk} uniformly from $[-1, -0.5] \cup [0.5, 1]$. For control samples, set the expression levels as $X_{gnk} = X'_{gnk}$; for case samples, set the expression levels as $Y_{gnk} = X'_{g(n+50)k} + \mu_{gk} \cdot \delta_{gk}$, for $1 \leq g \leq 10,000$, $1 \leq n \leq 50$ and $1 \leq k \leq 10$.

In the simulated dataset, 10 studies with 10,000 genes were simulated. Within each study, there were 50 cases and 50 controls. The first 1,000 genes were DE in 1 to 10 studies with equal probabilities; and the rest 9,000 genes were DE in none of the studies. We denoted t_g as the true number of studies where gene g was DE. To mimic the gene dependencies in real gene expression dataset, within the 10,000 genes, we drew 200 gene clusters with 20 genes in each. We sampled the data such that the genes within the same cluster were correlated. The correlation matrices for different studies and different gene clusters were sampled from an inverse Wishart distribution. Suppose the goal of the meta-analysis was to obtain biomarkers differentially expressed in at least 60% (6 out of 10) studies (i.e. HS_r with $r = 6$). We performed two sample t-tests in each study and combined the p-values using rOP with $r = 6$. $FDR \leq 5\%$ was controlled using the permutation analysis. To compare rOP with other methods in the HS_r setting, we defined two FDR criteria as follows. Note that FDR_1 targets on $H_0 : t_g = 0$ and FDR_2

targets on $H_0 : t_g < r$.

$$\text{FDR}_1 = \frac{\sum_g I(t_g = 0 \text{ and gene } g \text{ is detected})}{\#\{\text{genes detected}\}}$$

$$\text{FDR}_2 = \frac{\sum_g I(t_g < r \text{ and gene } g \text{ is detected})}{\#\{\text{genes detected}\}}$$

Table 3 listed the average FDR_1 and FDR_2 for different methods calculated using 100 simulations. We can see that although FDR_1 was well-controlled, all the methods were anti-conservative in terms of FDR_2 , since the inference of the five methods was based on $H_0 : t_g = 0$ while genes with $1 \leq t_g \leq 5$ existed and were calculated towards FDR_2 . To compare different FDR control methods, we also included the results of the Benjamini-Hochberg and Benjamini-Yekutieli procedures. According to the simulation, the Benjamini-Hochberg procedure controlled FDR similarly to the permutation test. The Benjamini-Yekutieli Procedure, on the other hand, was too conservative that the FDR_1 was controlled at about 1/10 of the nominal FDR level. Figure 4 showed the number of detected DE genes and the statistical power of different methods for genes with t_g from 1 to 10. From Figure 4(a), we noticed that Fisher, Stouffer and minP methods detected many genes with $1 \leq t_g \leq 5$, which violated our targeted HS_r with $r = 6$. MaxP detected very few genes and missed many targeted markers with $6 \leq t_g \leq 9$. Only rOP generated result most compatible with HS_r ($r = 6$). Most genes with $6 \leq t_g \leq 10$ were detected. The high $\text{FDR}_2 = 18.2\%$ mostly came from genes with $4 \leq t_g \leq 5$, genes that were very likely important markers and were minor mistakes. Vote counting detected genes with $t_g \geq 6$ but was less powerful. Relationship of vote counting and rOP will be further discussed in Section 4.3. We also performed rOP ($r = 5$) and rOP ($r = 7$) to compare the robustness of slightly different selections of r . Among the 620.16 DE genes (averaged over 100 simulations) detected by rOP ($r = 6$), 594.15 (95.8%) of them were also detected by rOP ($r = 5$) and 516.28 (83.3%) of them were also detected by rOP ($r = 7$). The result of Figure 4(b) was consistent with the theoretical power calculation as shown in Figure 3(b).

We also performed the simulation without correlated genes. The results were shown in the Supplement Table 2 and Supplement Figure 6. We noticed that the FDRs were controlled well in both correlated and uncorrelated cases. However, the standard deviations of FDRs with correlated genes were higher than the FDRs with only independent genes, which indicated some instability of the FDR control with correlated genes reported by Qiu et al. (2006).

TABLE 3

Mean FDRs for different methods in HS_r with $r = 6$ by simulation analysis with correlated genes. The standard deviations of the FDRs in using 100 simulations are shown in the parentheses.

	FDR ₁	FDR ₂	# of detected genes
rOP ($r = 6$, PA)	0.0439 (± 0.0106)	0.1818 (± 0.0179)	620.16
rOP ($r = 6$, BH)	0.0472 (± 0.0094)	0.2029 (± 0.0184)	617.53
rOP ($r = 6$, BY)	0.0043 (± 0.0031)	0.1044 (± 0.0139)	539.85
Fisher	0.0441 (± 0.0090)	0.4186 (± 0.0212)	934.91
Stouffer	0.0440 (± 0.0089)	0.3623 (± 0.0217)	858.86
minP	0.0466 (± 0.0103)	0.4567 (± 0.0207)	958.26
maxP	0.0459 (± 0.0199)	0.0729 (± 0.0251)	201.02
Vote Counting	0.0000 (± 0.0000)	0.0003 (± 0.0016)	234.43

4.3. *Connection with vote counting.* Vote counting has been used in many meta-analysis applications due to its simplicity, while it has been criticized as being problematic and statistically inefficient. [Hedges and Olkin \(1980\)](#) showed that the power of vote counting converges to 0 when many studies of moderate effect sizes are combined (see Supplement Theorem 1). We, however, surprisingly found that rOP has a close connection with vote counting, and rOP can be viewed as a generalized vote counting with better statistical properties. There are many variations of vote counting in the literature. One popular approach is to count the number of studies that have p-values smaller than a pre-specified threshold, α . We define this quantity as

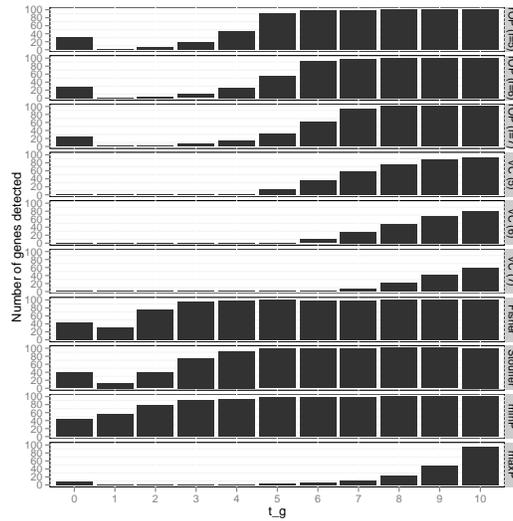
$$(1) \quad r = f(\alpha) = \sum_{k=1}^K I\{p_k < \alpha\},$$

and define its related proportion as $\pi = E(r)/K$. The test hypothesis is

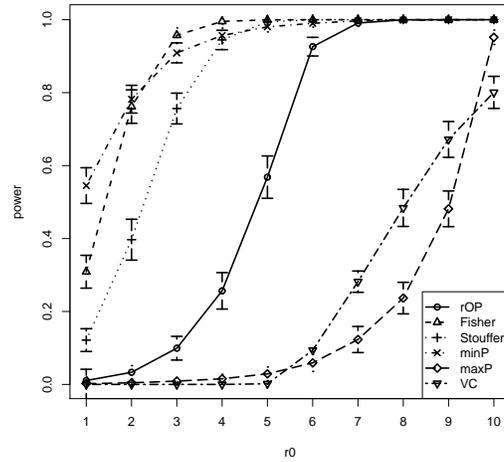
$$\begin{cases} H_0 : \pi = \pi_0, \\ H_a : \pi > \pi_0, \end{cases}$$

where $\pi_0 = 0.5$ is often used in the applications. Under null hypothesis, $r \sim BIN(K, \alpha)$ and $\pi = \alpha$, so the rejection region can be established. In the vote counting procedure, α and π_0 are two preset parameters and the inference is made on the test statistic r .

In the rOP method, we view equation (1) from another direction. We can easily show that if we solve equation (1) to obtain $\alpha = f^{-1}(r)$, the solution will be $\alpha \in [p_{(r)}, p_{(r+1)})$, and one may choose $\alpha = p_{(r)}$ as the solution. In other words, rOP presets r as a given parameter, and the inference is based on the test statistic $\alpha = p_{(r)}$.



(a) Number of genes detected by difference methods. The detected genes are binned according to their t_g 's.



(b) Power of different methods for genes with r_0 non-zero effect sizes.

FIG 4. Simulation results for rOP and other methods with correlated genes.

It is widely criticized that vote counting is powerless because when the effect sizes are moderate and the power of single studies is lower than π_0 ,

as K increases, the percentage of significant studies will converge to the single study power. However, in the rOP method, because the r th quantile is used, tests of the top r studies are combined, which helps the rejection probability of rOP achieve 1 as $K \rightarrow \infty$. It should be noted that the major difference between rOP and vote counting is that the test statistic $\alpha = p_{(r)}$ in rOP increases as K and $r = K \cdot c$ increase, which keeps information of the r smallest p-values. On the contrary, for vote counting, α is often chosen small and fixed when K increases. In Supplement Theorem 1, the power of vote counting converges to 0 as $K \rightarrow \infty$, while the power of rOP converges to 1 asymptotically as proved in Supplement Theorem 2.

5. Conclusion. In this paper, we proposed a general class of order statistics of p-values, called r th ordered p-value (rOP), for genomic meta-analysis. This family of statistics included the traditional maximum p-value (maxP) and minimum p-value (minP) statistics that target on DE genes in “all studies” (HS_A) or “one or more studies” (HS_B). We extended HS_A to a robust form that detected DE genes “in the majority of studies” (HS_r) and developed the rOP method for this purpose. The new robust hypothesis setting has an intuitive interpretation and is more adequate in genomic applications where unexpected noise is common in the data. We developed the algorithm of rOP for microarray meta-analysis and proposed two methods to estimate r in real applications. Under “two-class” comparisons, we proposed a one-sided corrected form of rOP to avoid detection of discordant expression change across studies (i.e. significant up-regulation in some studies but down-regulation in other studies). Finally, we performed power analysis and examined asymptotic properties of rOP to demonstrate appropriateness of rOP for HS_r over existing methods such as Fisher, Stouffer, minP and maxP. We further showed a surprising connection between vote counting and rOP that rOP can be viewed as a generalized vote counting with better statistical property. Applications of rOP to three examples of brain cancer, major depressive disorder (MDD) and diabetes showed better performance of rOP over maxP in terms of detection power (number of detected markers) and biological association by pathway analysis.

There are two major limitations of rOP. Firstly, rOP is for HS_r but the null and alternative hypotheses are not complementary (see Section 2.1). Thus, it has weaker ability to exclude markers that are differentially expressed in “less than r ” studies since the null of HS_r is “differentially expressed in none of the studies”. One solution to improve the anti-conservative inference (which is also our future work) is by Bayesian modeling of p-values with a family of beta distributions (Erickson, Kim and Allison, 2009). Secondly,

selection of r may not always be conclusive from the two methods we proposed; especially the external pathway information may be prone to errors and may not be informative to the data. But since choosing slightly different r usually gives similar results, this is not a severe problem in most applications. We have tested a different approach by adaptively choosing the best gene-specific r that generates the best p-value. The result is, however, not stable and the gene-specific parameter r is hard to interpret in applications.

Although many meta-analysis methods have been proposed and applied to microarray applications, it is still not clear which method enjoys better performance under what condition. The selection of an adequate (or best) method heavily depends on the biological goal (as illustrated by the hypothesis settings in this paper) and the data structure. In this paper, we stated a robust hypothesis setting (HS_r) that is commonly targeted in biological applications (i.e. identify markers statistically significant in the majority of studies) and developed an order statistic method (rOP) as a solution. The three applications covered “cleaner” data (brain cancer) to “noisier” data (complex genetics in MDD and diabetes), and rOP performed well in all three examples. We expect that the robust hypothesis setting and the order statistic methodology will find many more applications in genomic research and traditional univariate meta-analysis in the future.

For multiple comparison control, we propose to either apply the parametric beta null distribution to assess the p-value and perform the Benjamini-Hochberg (BH) procedure for p-value adjustment or conduct a conventional permutation analysis by permuting class labels in each study. The former approach is easy to implement, and the latter approach better preserves the gene correlation structure in the inference. Instead of the BH procedure, we also tested the Benjamini-Yekutieli (BY) procedure which is applicable to general dependence structure but found that it is overly conservative for genomic applications. The problem of FDR control under general high-dimensional dependence structures is beyond the scope of this paper but is critical in applications and deserves future research.

Implementation of rOP is available in the “MetaDE” package in R together with over 12 microarray meta-analysis methods in the package. MetaDE has been integrated with other quality control methods (“MetaQC” package, Kang et al., 2012) and pathway enrichment analysis methods (“MetaPath” package, Shen and Tseng, 2010). The future plan is to integrate the three packages with other genomic meta-analysis tools into a “MetaOmics” software suite (Wang et al., 2012b).

Acknowledgements. The authors would like to thank Etienne Sibille and Peter Park for providing the organized major depressive disorder and diabetes data. We would also like to thank the anonymous associate editor and editor Karen Kafadar for many suggestions and critiques to improve the paper. This study is supported by NIH R21MH094862 and R01DA016750.

References.

- BEGUM, F., GHOSH, D., TSENG, G. C. and FEINGOLD, E. (2012). Comprehensive literature review and statistical considerations for GWAS meta-analysis. *Nucleic acids research* **40** 3777–3784.
- BENJAMINI, Y. and HOCHBERG, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* 289–300.
- BENJAMINI, Y. and YEKUTIELI, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of statistics* 1165–1188.
- BERGER, R. L. (1982). Multiparameter hypothesis testing and acceptance sampling. *Technometrics* 295–300.
- BERGER, R. L. and HSU, J. C. (1996). Bioequivalence trials, intersection-union tests and equivalence confidence sets. *Statistical Science* 283–302.
- BIRNBAUM, A. (1954). Combining independent tests of significance. *Journal of the American Statistical Association* 559–574.
- COOPER, H. M., HEDGES, L. V. and VALENTINE, J. C. (2009). *The handbook of research synthesis and meta-analysis*. Russell Sage Foundation Publications.
- ERICKSON, S., KIM, K. and ALLISON, D. B. (2009). *Meta-analysis and combining information in genetics and genomics* 6 90. Chapman & Hall/CRC.
- FISHER, R. A. (1925). *Statistical methods for research workers*. Edinburgh.
- HEDGES, L. V. and OLKIN, I. (1980). Vote-counting methods in research synthesis. *Psychological Bulletin* **88** 359.
- KANG, D. D., SIBILLE, E., KAMINSKI, N. and TSENG, G. C. (2012). MetaQC: objective quality control and inclusion/exclusion criteria for genomic meta-analysis. *Nucleic Acids Research* **40** e15–e15.
- LI, J. and TSENG, G. C. (2011). An adaptively weighted statistic for detecting differential gene expression when combining multiple transcriptomic studies. *The Annals of Applied Statistics* **5** 994–1019.
- LITTELL, R. C. and FOLKS, J. L. (1971). Asymptotic optimality of Fisher’s method of combining independent tests. *Journal of the American Statistical Association* 802–806.
- LITTELL, R. C. and FOLKS, J. L. (1973). Asymptotic optimality of Fisher’s method of combining independent tests II. *Journal of the American Statistical Association* 193–194.
- OWEN, A. B. (2009). Karl Pearson’s meta-analysis revisited. *The Annals of Statistics* **37** 3867–3892.
- PARK, P. J., KONG, S. W., TEBALDI, T., LAI, W. R., KASIF, S. and KOHANE, I. S. (2009). Integration of heterogeneous expression data sets extends the role of the retinol pathway in diabetes and insulin resistance. *Bioinformatics* **25** 3121.
- PEARSON, K. (1934). ON A NEW METHOD OF DETERMINING “GOODNESS OF FIT.”. *Biometrika* **26** 425.
- QIU, X., YAKOVLEV, A. et al. (2006). Some comments on instability of false discovery rate estimation. *Journal of bioinformatics and computational biology* **4** 1057–1068.

- RHODES, D. R., BARRETTE, T. R., RUBIN, M. A., GHOSH, D. and CHINNAIYAN, A. M. (2002). Meta-Analysis of Microarrays. *Cancer research* **62** 4427.
- ROY, S. (1953). On a heuristic method of test construction and its use in multivariate analysis. *The Annals of Mathematical Statistics* 220–238.
- SHEN, K. and TSENG, G. C. (2010). Meta-analysis for pathway enrichment analysis when combining multiple genomic studies. *Bioinformatics* **26** 1316–1323.
- STOUFFER, S. A., SUCHMAN, E. A., DEVINNEY, L. C., STAR, S. A. and WILLIAMS JR, R. M. (1949). *The American soldier: adjustment during army life*. Princeton Univ. Press.
- SUBRAMANIAN, A., TAMAYO, P., MOOTHA, V. K., MUKHERJEE, S., EBERT, B. L., GILLETTE, M. A., PAULOVICH, A., POMEROY, S. L., GOLUB, T. R., LANDER, E. S. et al. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* **102** 15545–15550.
- TIBSHIRANI, R., WALTHER, G. and HASTIE, T. (2001). Estimating the number of clusters in a data set via the gap statistic. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)* **63** 411–423.
- TIPPETT, L. H. C. (1931). *The Methods of Statistics*. London: Williams Norgate Ltd.
- TSENG, G. C., GHOSH, D. and FEINGOLD, E. (2012). Comprehensive literature review and statistical considerations for microarray meta-analysis. *Nucleic Acids Research* **40** 3785–3799.
- WANG, X., LIN, Y., SONG, C., SIBILLE, E. and TSENG, G. C. (2012a). Detecting disease-associated genes with confounding variable adjustment and the impact on genomic meta-analysis: with application to major depressive disorder. *BMC bioinformatics* **13** 52.
- WANG, X., KANG, D. D., SHEN, K., SONG, C., LU, S., CHANG, L. C., LIAO, S. G., HUO, Z., TANG, S., KAMINSKI, N. et al. (2012b). An R package Suite for Microarray Meta-analysis in Quality Control, Differentially Expressed Gene Analysis and Pathway Enrichment Detection. *Bioinformatics* **28** 2534–2536.
- WILKINSON, B. (1951). A statistical consideration in psychological research. *Psychological Bulletin* **48** 156.

SUPPLEMENTARY MATERIAL

Supplement Text: Supplement Text

(<http://lib.stat.cmu.edu/aoas/???/???>). Details of one-sided test modification to avoid discordant effect sizes.

Supplement Theorems: Supplement Theorems 1 and 2

(<http://lib.stat.cmu.edu/aoas/???/???>). Theorem 1 - Asymptotic property of vote counting as $K \rightarrow \infty$. Theorem 2 - Asymptotic property of rOP as $K \rightarrow \infty$.

Supplement Tables: Supplement Table 1 and 2

(<http://lib.stat.cmu.edu/aoas/???/???>). Table 1 - Detail information of combined datasets. Table 2 - FDRs for simulation analysis without correlated genes.

Supplement Figures: Supplement Figures 1 to 7

(<http://lib.stat.cmu.edu/aoas/???/???>). Figure 1 - Results of brain cancer dataset using one-sided corrected rOP. Figure 2 - Results of MDD dataset. Figure 3 - Results of diabetes dataset. Figure 4 - Permutation results of diabetes dataset. Figure 5 - Results of brain cancer and 1 random MDD dataset. Figure 6 - Simulation results without correlated genes. Figure 7 - Mean rank of different methods for the top U pathways.

E-MAIL: chi.song@yale.edu

E-MAIL: ctseng@pitt.edu