metaMA: an R package implementing meta-analysis approaches for microarrays

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8 July 2009

Introduction

Context :

- Research of differentially expressed genes between two conditions (e.g. normal/tumor)
- Several studies available with the same biological question but their direct comparison is impossible
- Small sample size in individual microarray studies, many genes

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Meta-analysis : combining data or results from different studies

- Increase of sensitivity
- Better accuracy

metaMA

Two main approaches in metaMA :

- effect size combination, which extends the methodology implemented in the Bioconductor package GeneMeta (effect sizes : indices measuring the magnitude of an effect)
- p-value combination

Effect sizes and p-values to be combined are derived from t-statistics or moderated t-statistics $\Rightarrow$ several options for each combination.

Effect Size combination

g : gene
s : study
i and j : conditions

Let $Y_{sigr} \sim \mathcal{N}(\mu_{sig}, \sigma^2_{sg})$ and $Y_{sjgr} \sim \mathcal{N}(\mu_{sjg}, \sigma^2_{sg})$

Standard Effect Size (ES) :

$$\delta_{sg} = (\mu_{sig} - \mu_{sjg})/\sigma_{sg}$$

Simple relationship between Student t statistic and standardized mean difference d :

$$d = t/\sqrt{\tilde{n}}$$

with $\tilde{n} = n_i n_j/(n_i + n_j)$

Effect size combination

Hierarchical model (Choi et al., 2003)

$$d_{sg} = \theta_{sg} + e_{sg}, \quad e_{sg} \sim \mathcal{N}(0, w_{sg}^2)$$

$$\theta_{sg} = \mu_g + v_{sg}, \quad v_{sg} \sim \mathcal{N}(0, \tau_g^2)$$

with d_{sg} effect size for study s and gene g,
τ_g^2 between-study variance
w_{sg}^2 within-study variances (assumed to be known, actually estimated before the procedure)

Effect size combination

- Method of moments to estimate τ_g^2 the between-study variance.
- Z-score to test for differential expression :

$$z_g = \frac{\widehat{\mu_g}(\tau_g^2)}{\sqrt{Var(\widehat{\mu_g}(\tau_g^2))}}$$

- z is assumed to follow a normal distribution
- p-values are corrected for multiple testing by the Benjamini Hochberg procedure

Effect size combination

Bioconductor package GeneMeta : gene-by-gene approach
many parameters $\Rightarrow$ lack of sensitivity

Extension in metaMA : definition of shrinkage effect sizes to take advantage of information from other genes
$\Rightarrow$ increase of sensitivity not only in individual studies but also in meta-analysis.

Effect Size combination

In addition to gene-by-gene effect sizes, two moderated effect size calculations are implemented :

- from limma (Smyth, 2004) moderated t-tests :

$$d_{Limma} = t_{Limma}/\sqrt{\tilde{n}}$$

(direct extension from the relationship between the standard t-test and the standard effect size since the same variance is assumed for both conditions)

- from SMVar (Jaffrézic et al., 2007)
Different variances in each condition k.

Effect Size combination

- SMVar (Jaffrézic et al., 2007)
  Different variances in each condition k.

$$ln(\sigma^2_{gk}) = \mu_k + \delta_{gk}, \quad \delta_{gk} \sim \mathcal{N}(0, \phi^2_k)$$

  t_{SMVar} follows a Welch statistic $\Rightarrow$ Need of another definition of effect size.

Details about effect size calculation from moderated t-tests as well as their bias or estimated variance are given in :

(Marot et al., 2009) **Moderated effect size and p-value combinations for microarray meta-analyses.** Submitted to Bioinformatics.

p-value combination

Inverse normal method (Hedges and Olkin, 1985) to combine p-values :

$$S_g = \sum_{s=1}^{N_s} w_s \Phi^{-1}(1 - \tilde{p}_g(s))$$

$$w_s = \sqrt{\frac{n(s)}{\sum_{i=1}^{N_s} n(i)}}$$

(weights according to the number of replicates in each analysis)
Under the null hypothesis,

$$S_g \sim \mathcal{N}(0, 1)$$

Use of metaMA

Main functions :

- EScombination(esets,classes,moderated="limma","BHth=0.05)
- pvalcombination(esets,classes,moderated="limma","BHth=0.05)

Value :

- indices of differentially expressed genes in each individual study and in the meta-analysis
- test statistics for meta-analysis differential expression for all genes
- Loss, IDD, IDR, etc.

Possibility to perform a meta-analysis from personal p-values or effect sizes with directpvalcombi or directEScombi

Simulations

- Simulations of 3 or 5 experiments with various numbers of replicates
- Each gene is normally distributed with parameters calculated from three real datasets (Singh et al., 2002) (La Tulippe et al., 2002) (Stuart et al., 2004)
- Within-study variances from the real datasets : different per gene, per condition and study.
- Between-study variance simulated as the observed between-study variance averaged over the two conditions

Simulations

Focus on limma based meta-analysis approaches.

Comparison of global limma analyses with p-value and effect size combinations

- $Joint_{L1}$ limma analysis gathering all the data 'naively'
- $Joint_{L2}$ limma analysis including a study effect in the linear model

Results

Criteria of comparison :

Sensitivity : $E(\frac{TP}{TP+FN})$

Discoveries (Disc.) : Number of genes which were not declared differentially expressed in individual studies and are significant in meta-analysis.

Revisions (Revis.) : Number of genes which are not significant anymore in meta-analysis while they were in individual studies.

Results

TABLE: Comparison of global limma analyses - the first one ($Joint_{L1}$) only gathering the expression data, the second one ($Joint_{L2}$) including a study effect in the linear model - with p-value and effect size combinations.

	$Joint_{L1}$	$Joint_{L2}$	pv_{Limma}	ES_{Limma}
DE	54.8(9.3)	853.1(19.1)	1064.3(17.7)	732.0(20.2)
Sens.	3.8(0.7)	57.2(1.2)	71.2(1)	50.4(1.3)
FDR	0.0(0.3)	4.3(0.7)	4.6(0.6)	1.7(0.5)
Disc.	14.1(4.3)	467.2(21.2)	635.1(21.8)	426.4(19.4)
TP Disc.	14.0(4.3)	432.7(18.8)	589.4(19.7)	413.8(18.4)
Revis.	428.8(18.2)	83.8(9.4)	40.4(6.5)	164(13.2)
TP Revis.	43.3(2.5)	8.2(2.7)	4.0(2.1)	16.3(3.6)
AUC	90.0(0.4)	93.9(0.4)	96.6(0.3)	95.9(0.3)

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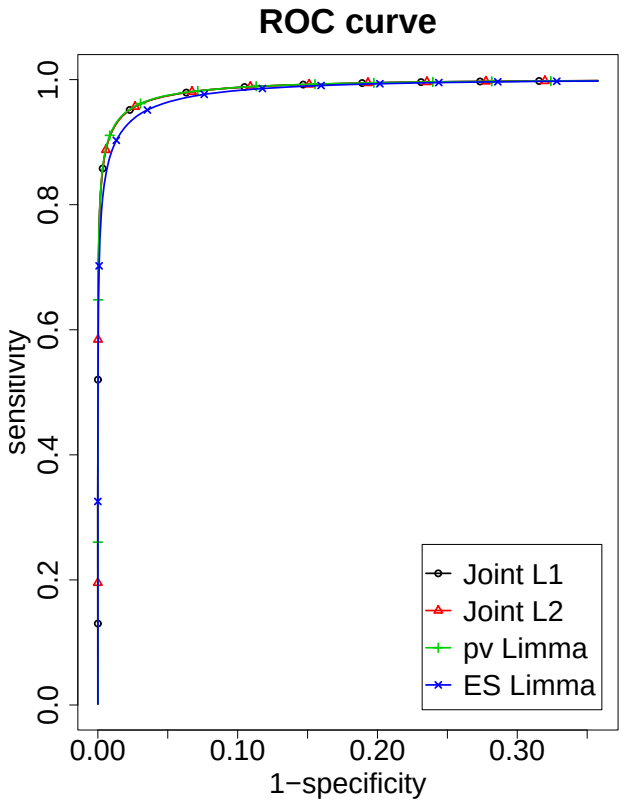
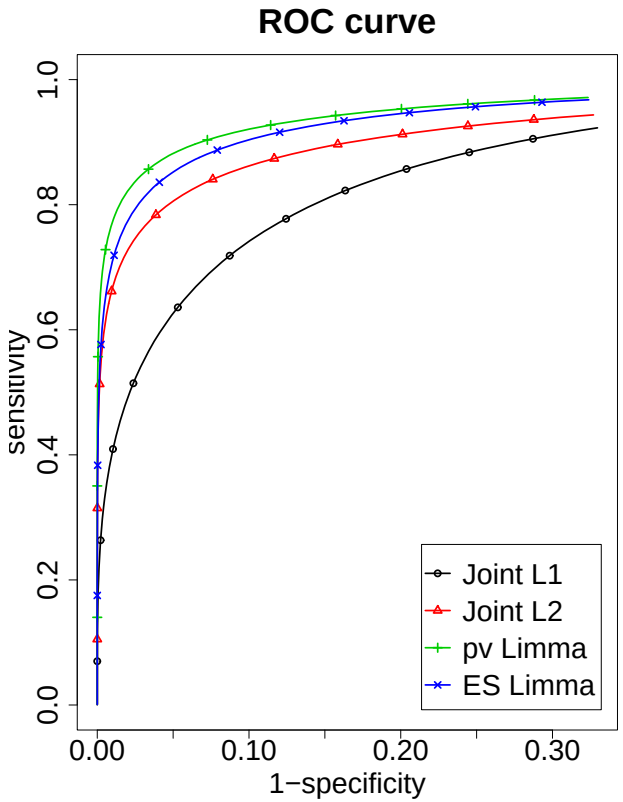
ROC curves





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5 studies with 10 replicates

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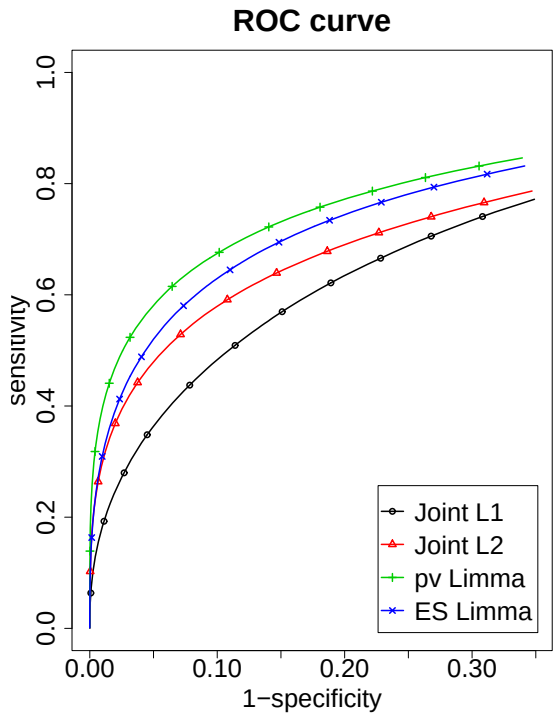
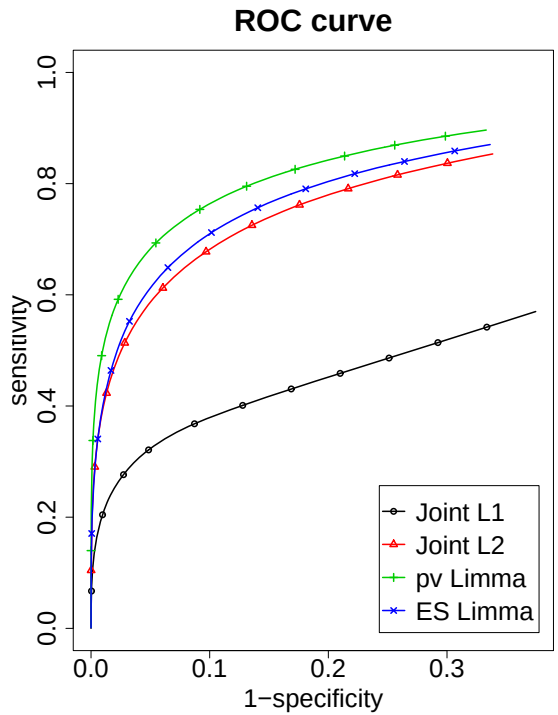


inter-study variability
3 studies with 6 replicates



inter-study variability
10/10 replicates (conditions 1/2) in study 1,
10/8 and 3/9 in studies 2 and 3

Conclusion

- Effect size combination can be improved by shrinkage approaches, especially when the number of replicates in individual studies is low.
- P-value combination is better in terms of sensitivity and AUC while effect size combination is more conservative.

(Marot et al., 2009) **Moderated effect size and p-value combinations for microarray meta-analyses.** Submitted to Bioinformatics.