A Comparison of GMAC and ADDIS

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1. Overview

Both MRPC-LOND and MRPC-ADDIS techniques inferred a large number of trans mediated trios. The trans-mediation model has been previously identified, but is not the commonly ackowledged mode of mediation. Since this result is surprising relative to the existing literature, we sought to apply another method for inferring mediation on a subset of GTEx trios analyzed herein by MRPC. The Genomic Mediation analysis with Adaptive Confounding (GMAC) algorithm allows for a unique selection of a subset of potential confounders, $\mathbf{X_{ij}}$ from a larger covariate pool, $\mathbf{H}$, for each trio. By taking advantage of the Principle of Mendelian Randomization, the authors filter $\mathbf{H}$ by removing common child and intermediate confounding variables (e.g variables associated with the eQTL as well as the cis/trans genes). Post-filtering, GMAC preforms a mediation test on the edge between the cis gene and trans gene via the regression of the trans-gene T_j on the cis-eQTL L_i, cis-gene C_i, and the set of adaptively selected confounders $\mathbf{X_{ij}}$:

$$T_j = \beta_0 + \beta_1 C_i + \beta_2 L_i + \mathbf{\Gamma X}_{ij} + \epsilon \tag{1}$$

The mediation statistic is the observed t-value of the cis-gene coefficient β_1. A null distribution for no-mediation is constructed by iteratively permuting the values of the cis-transcript within each genotype and repeating the above regression. The authors argue that the permutation of the cis-transcript within the genotypes of the cis-eQTL removes the association between the cis and trans gene transcripts while preserving the higher order associations with the cis-eQTL. The resulting mediation test compares the observed relationship between the trans and cis gene to a null distribution constructed from a model with no association and assuming that possible confounding has been well adjusted via the selected covariates.

It is important to note that the above mediation test describes only the association between cis-gene and trans-gene transcripts ($C_i \leftrightarrow T_j$) and does not consider possible effects between the cis-eQTL and the cis-gene transcript ($L_i \rightarrow C_i$), or the cis-eQTL and trans-gene transcript ($L_i \rightarrow T_j$).

2. Methods

2.1 Applying GMAC to GTEx Trios

To compare the GMAC and MRPC algorithms, we applied the GMAC algorithm to the top five GTEx tissues by sample size. Following with the creators of GMAC, we used the full set of principle components retained from the PCA of the expression matrix as the covariate pool, and three additional known confounders: the PCR used, the platform used, and sex of the individual in each sample (Yang et al. 2017).

Consistent with Yang et al. (2017), the analysis was preformed using a common child and intermediate variable filtering FDR of 10% and a confounder selection FDR of 5% for each trio. Each trio supplied to

GMAC consisted of the cis-QTL and the PEER normalized cis and trans gene transcripts with the highest association to the eQTL. To mitigate missing values in the eQTL matrix, multiple imputation of the matrix of unique cis-eQTLs was preformed via multiple correspondence analysis (MCA) prior to its use in GMAC (Josse, Husson, and others 2016). The analysis was preformed twice on each trio, first with the cis gene as the mediator and second with the trans gene as the mediator. This allowed for GMAC inferred trios to be decomposed into the three groupings used under MRPC: 1) Cis-gene mediation, 2) Trans-gene mediation, 3) both (undirected).

2.2 Comparison of GMAC and MRPC Results

After applying GMAC to each tissue, the false discovery rate among the retained mediation p-values was controlled at the more liberal rate of 10% (Yang et al. 2017). Each trio determined to have significant mediation after FDR filtering was compared with the regulatory network type inferred by MRPC-ADDIS. MRPC-ADDIS can infer three types of regulatory networks that contain an edge between the cis and trans gene (M1, M2, or M4). Since GMAC considers only the presence of the edge and not its direction, trios inferred to be one of M1, M2, or M4 under ADDIS, that were also significant under GMAC, were considered consistent (e.g $C_i \rightarrow T_j$; $T_j \rightarrow C_i$; $C_i \leftrightarrow T_j$ are synonymous under GMAC).

2.3 Simulations

To further understand the conflicting behavior between MRPC-ADDIS and GMAC, we simulated the mediation test - the test for the β_1 coefficient in the presence of all adaptively selected confounders, $\mathbf{X}_{ij}$, as described by the regression in **eq (1)**. We simulated the trans gene of each trio using the linear relationship:

$$T_j^* = \hat{\beta}_0 + \hat{\beta}_1 C_i + \hat{\beta}_2 L_i + \hat{\mathbf{\Gamma}} \mathbf{W}_{ij} + \epsilon \tag{2}$$

where T_j^* is the simulated trans gene, the coefficients are replaced by their estimates from the regression in **eq (1)**, and $\mathbf{W}_{ij}$ is a subset of $\mathbf{X}_{ij}$ representing the "highly" significant GMAC confounders from **eq (1)** ($p < 0.001$). Note that if the GMAC inferred mediation type was trans gene mediation only then the cis gene was simulated and the mediation test was preformed on the β_1 coefficient from $C_i = \beta_0 + \beta_1 T_j + \beta_2 L_i + \mathbf{\Gamma} \mathbf{X}_{ij} + \epsilon$.

We refer to the trans-gene generating function in **(2)** as the small truth model (STM) as the simulated trans gene comes from a model that is a subset of of the model described in **(1)**. Therefore, the simulated mediation test can be decomposed as the test on the β_1 coefficient from:

$$T_j = \beta_0 + \beta_1 C_i + \beta_2 L_i + \mathbf{\Gamma}_1 \mathbf{W}_{ij} + \mathbf{\Gamma}_2 \mathbf{M}_{ij} + \epsilon \tag{3}$$

where $\mathbf{M}_{ij}$ represents the additional confounders in $\mathbf{X}_{ij}$ that are not included in $\mathbf{W}_{ij}$.

Table 1: Descriptive statistics for the distribution of missing values across the eQTL's for each tissue used in GMAC

	Adipose Subcutaneous	Artery Tibial	Muscle Skeletal	Skin Sun Exposed	Whole Blood
Min.	0.000000	0.000000	0.000000	0.000000	0.000000
1st Qu.	0.000000	0.000000	0.000000	0.000000	0.000000
Median	0.000000	0.000000	0.000000	0.000000	0.000000
Mean	0.006365	0.006625	0.006560	0.006701	0.006103
3rd Qu.	0.003442	0.003425	0.002833	0.003306	0.002985
Max.	0.156627	0.159247	0.158640	0.160331	0.155224

3. Results

3.1 Comparing GMAC and MRPC

In light of the surprising number of trans-gene mediation trios inferred by MRPC, we sought to compare our results with GMAC by applying the GMAC method to the top five GTEx tissues by sample size. It is important to note that the test for mediation used by GMAC describes only the association between cis-gene and trans-gene transcripts ($C_i \leftrightarrow T_j$) and does not consider the possible effects between the cis-eQTL and the cis-gene ($L_i \rightarrow C_i$), or the cis-eQTL and trans-gene ($L_i \rightarrow T_j$). Therefore, since GMAC considers only the presence of the mediation edge, trios inferred to be one of M1, M2, or M4 under ADDIS, that were also significant under GMAC, were considered consistent (e.g $C_i \rightarrow T_j$; $T_j \rightarrow C_i$; $C_i \leftrightarrow T_j$ are synonymous under GMAC).

At the 10% false discovery rate, GMAC identified $2,160$ trios with an edge between the cis and trans genes out of $55,446$ total trios tested across the five selected tissues: Adipose subcutaneous, Tibial artery, Muscle skeletal, Sun exposed skin, and Whole blood. Of the trios with mediation edges, 653 were identified as the cis gene mediating the trans gene, 245 as trans gene mediating the cis gene and 1,345 as both (29.1%, 10.9%, and 60% respectively). As can be seen from **Table 2**, the consistency in inferred mediation edges between the two methods varied between 35% and 46% of the trios across tissues.

To uncover the computational differences between MRPC and GMAC, we focused on trios with conflicting results between the two methods (trios inferred M0 or M3 under MRPC). The primary differences we observed between the two algorithms for these trios were that the inclusion of a larger set of confounding variables by GMAC often had the effect of strengthening the association between the cis and trans genes. That is, let $\mathbf{Z}_{ij}$ denote the set of confounding variables used under MRPC and $\mathbf{X}_{ij}$ the larger set used by GMAC such that the columns $\{z_{ij}\} \subset \{x_{ij}\}$. Because the confounding variables under GMAC are selected such that they have a significant association with both the cis and trans genes, the partial correlation between the cis gene and trans gene tends to strengthen as the column dimension of $\mathbf{Z}_{ij}$ approaches the column dimension of $\mathbf{X}_{ij}$. The result for GMAC is that under the mediation test, relatively weak associations ($0.1 \leq \rho \leq 0.2$) can be deemed significant. Conversely, for MRPC, as the size of the network increases, the method becomes increasingly conservative. Therefore, when $\mathbf{Z}_{ij} = \mathbf{X}_{ij}$ MRPC tends to infer the null model unless the association between two nodes in the network is substantial.

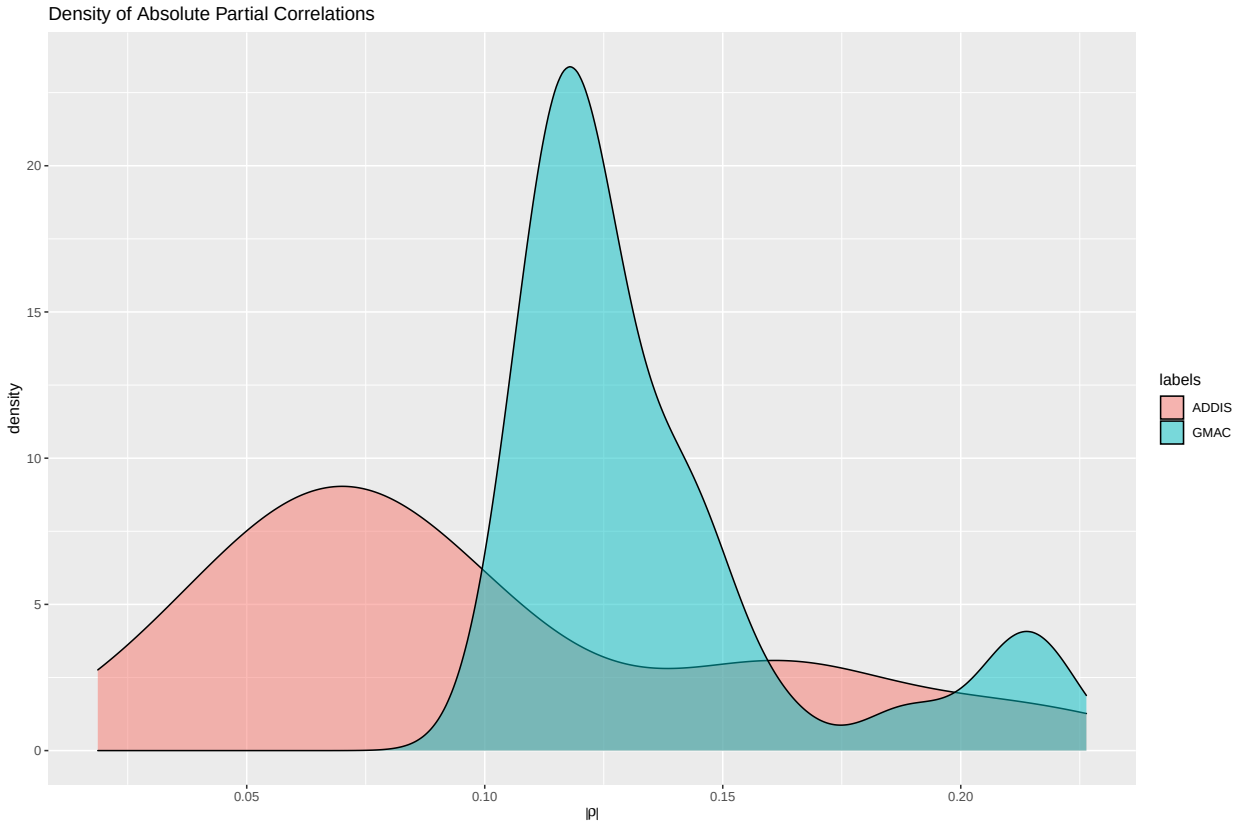
3.2 Simulation Results

Table 2: The breakdown of unqiue trios with inferred significant cis or trans mediation under GMAC across their respective ADDIS inferred regulatory networks. The column "Percentage In Common" is the proportion of significant trios that also contained a mediation edge in the regulatory network inferred under ADDIS

Tissue	M0	M1	M2	M3	M4	Other	Total GMAC Inferred	Percentage In Common
AdiposeSubcutaneous	100	41	4	122	135	2	404	0.4455
ArteryTibial	83	22	5	135	110	0	355	0.3859
MuscleSkeletal	116	30	6	173	123	2	450	0.3533
SkinSunExposed	111	33	10	162	127	0	443	0.3837
WholeBlood	109	46	16	162	172	3	508	0.4606

Table 3: Breakdown of trios with inferred mediation under GMAC across both cis and trans mediation types. The column "Unique Both" represents the intersection of the columns "Total Cis Mediated" and "Total Trans Mediated"

Tissue	Total Trios	Total Cis Mediated	Total Trans Mediated	Unique Cis Only	Unique Trans Only	Unique Both	Unique Total
AdiposeSubcutaneous	11850	374	282	122	30	252	404
ArteryTibial	11471	334	226	129	21	205	355
MuscleSkeletal	10257	401	314	136	49	265	450
SkinSunExposed	13045	404	333	110	39	294	443
WholeBlood	8823	451	398	110	57	341	508



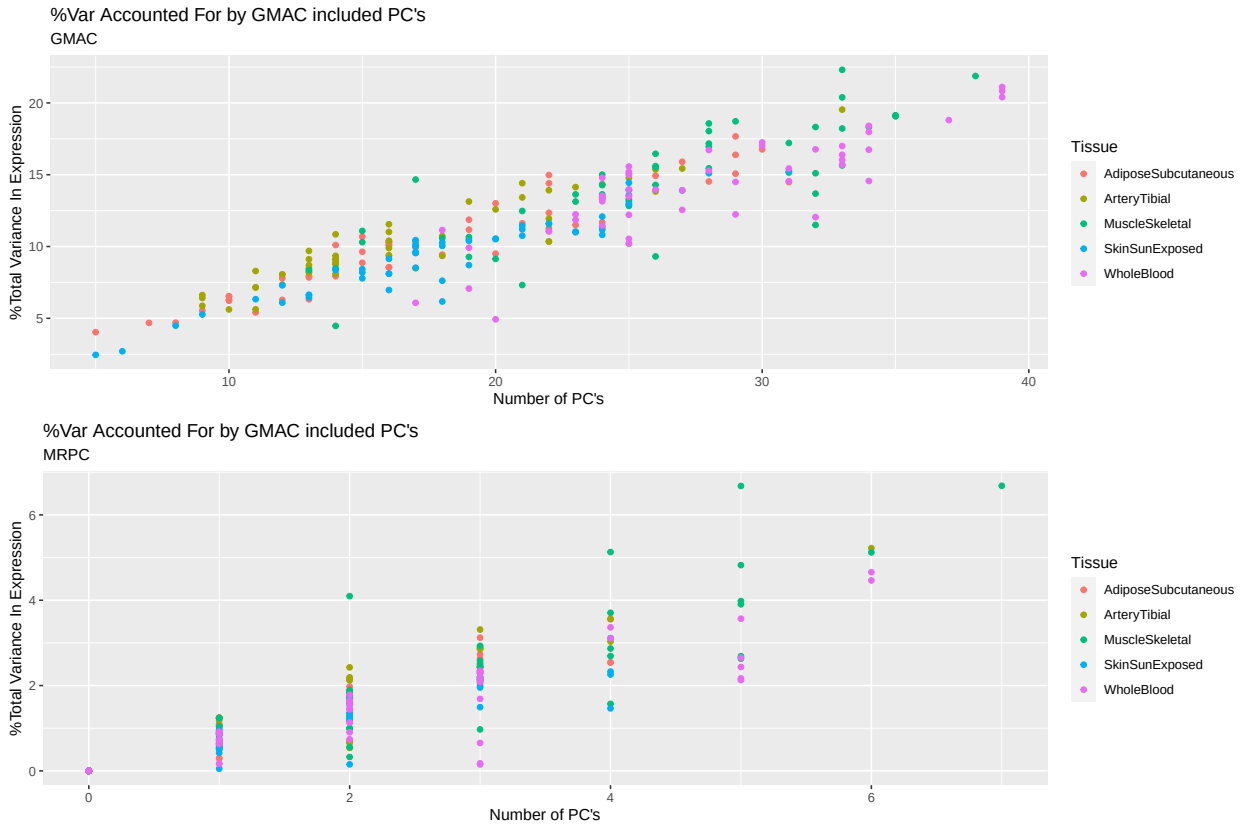


Figure 1: scatter plots for the number of PC's included by GMAC (top) and MRPC (bottom) and the percentage of total variation in expression the PC's summarize

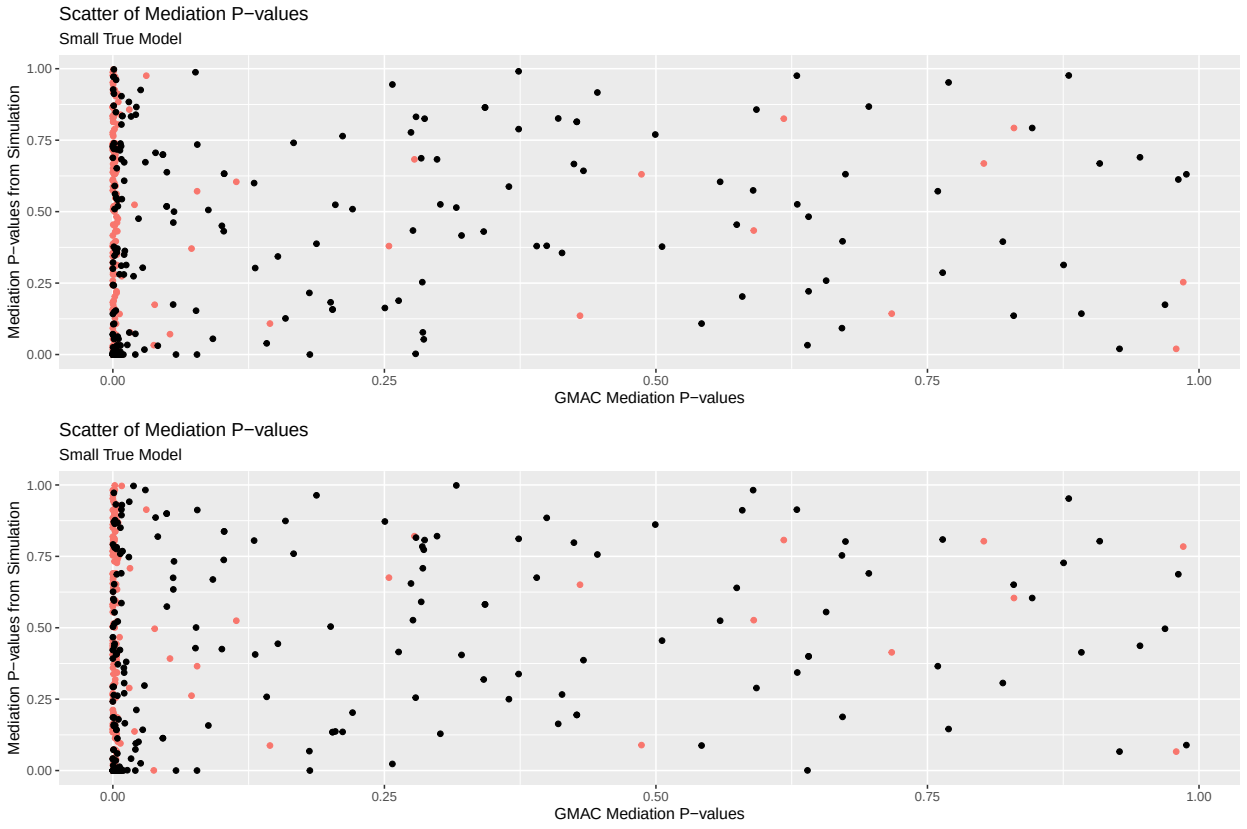


Figure 2: Scatter plots of the mediation test p-values obtained from the permutation test (red) and the regression of the trans gene as described in eq (1) (black) across the simulated p-values using as described in Methods 2.3

References

Josse, Julie, François Husson, and others. 2016. "MissMDA: A Package for Handling Missing Values in Multivariate Data Analysis." *Journal of Statistical Software* 70 (1): 1–31.

Yang, Fan, Jiebiao Wang, Brandon L Pierce, Lin S Chen, François Aguet, Kristin G Ardlie, Beryl B Cummings, et al. 2017. "Identifying Cis-Mediators for Trans-eQTLs Across Many Human Tissues Using Genomic Mediation Analysis." *Genome Research* 27 (11): 1859–71.