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Penalized multivariate linear mixed model for longitudinal genome-wide association studies

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Abstract

We consider analysis of Genetic Analysis Workshop 18 data, which involves multiple longitudinal traits and dense genome-wide single-nucleotide polymorphism (SNP) markers. We use a multivariate linear mixed model to account for the covariance of random effects and multivariate residuals. We divide the SNPs into groups according to the genes they belong to and score them using weighted sum statistics. We propose a penalized approach for genetic variant selection at the gene level. The overall modeling and penalized selection method is referred to as the penalized multivariate linear mixed model. Cross-validation is used for tuning parameter selection. A resampling approach is adopted to evaluate the relative stability of the identified genes. Application to the Genetic Analysis Workshop 18 data shows that the proposed approach can effectively select markers associated with phenotypes at gene level.

Background

The Genetic Analysis Workshop 18 (GAW18) data consists of multiple longitudinal traits and dense genome-wide single-nucleotide polymorphism (SNP) markers. A commonly used approach for identifying markers associated with traits is to conduct single-variant analysis and then adjust for multiple comparisons on each trait. However, for complex polygenic traits, single-variant analysis methods may not be appropriate as they fail to take into account the accumulated and/or joint effects of multiple genetic variants on the traits. In addition, analyzing each trait separately does not take into account the correlation among traits, and thus can be ineffective. To overcome these limitations, we developed a joint analysis approach referred to as the penalized multivariate linear mixed model (PMLMM). This approach takes into account covariance of both random effects and residuals and uses a group minimax concave penalty (MCP) approach [1] for variant selection at the gene level. A resampling approach is adopted to evaluate

the relative stability of the identified genes. Our analysis of the GAW18 data indicates that the proposed approach can effectively select markers associated with multiple traits at the gene level.

Methods

Consider a genetic association study with longitudinal measurements on N subjects, p genetic variants, and q environmental exposure covariates. Here a genetic variant can be a single SNP marker or a score representing a group of SNPs. For subject i, suppose that there are n_i longitudinal measurements on m traits. Let Y_i be the $n_i \times m$ trait matrix for subject i. Let Y be the $n \times m$ trait matrix for all the N subjects, where $n = \sum_{i=1}^{N} n_i$. The transpose of Y is $Y\prime = (Y_1', \cdots, Y_N')$. Let X_i be the $n_i \times p$ matrix consisting of the genetic variant scores of subject i. Let Z_i be the $n_i \times q$ covariate matrix. We center all the measurements to have sample means equal to zero. When $m = 1$, this setting simplifies to that in Schelldorfer et al [2].

Consider the multivariate linear mixed model

$$Y_i = X_i B + Z_i C_i + E_i, \quad i = 1, ..., N, \tag{1}$$

where B is a $p \times m$ matrix representing the effects of p genetic variants on m traits, and C_i is a $q \times m$ matrix

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representing the subject specific effects of the covariates Z_i for the ith subject. We treat C_i as random effects. Assume that (a) $E_i \sim \text{MN}_{n_i \times m}(0, \sum_1, I_{n_i})$, that is, E_i is row-independent with column covariance matrix Σ_1, and each E_i is independent for $i = 1, ..., N$; (b) $C_i \sim \text{MN}_{q \times m}(0, \sum_2, D)$, where Σ_2 is the column covariance matrix and D is the row covariance matrix, and each C_i is independent for $i = 1, ..., N$; (c) each E_i and C_i is independent; and (d) $\Sigma_1 = \Sigma_2 = \Sigma$.

Then $Z_i C_i + E_i \sim \text{MN}_{n_i \times m}(0, \sum, Z_i D Z_i' + I_{n_i})$ and $Y \sim \text{MN}_{n \times m}(XB, \sum, V)$ where $V = Diag(V_1, ..., V_N)$ and $V_i = Z_i D Z_i' + I_{n_i}$, where I_{n_i} is an $n_i \times n_i$ identity matrix. A more detailed description of this model can be found in Liu et al [3].

From Dawid [4], the negative log-likelihood function is:

$$-\ell(B, V, \textstyle\sum) = \text{constant} + \frac{n}{2}\log|\textstyle\sum| + \frac{m}{2}\log|V| + \frac{1}{2}\text{tr}(\textstyle\sum^{-1}(y - XB)'V^{-1}(y - XB)) \quad (2)$$

Hastie et al. [5] suggest using $\hat{\Sigma} = y'y/n$ for estimating $\sum$. We estimate D by using the estimates from m univariate linear mixed models and subsequently get the estimate $\hat{V}$ of V as $\hat{V}_i = Z_i \hat{D} Z_i' + I_{n_i}$. Given $\hat{\sum}$ and $\hat{V}$, we can transform the negative likelihood function into a weighted least squares criterion for estimating B, which is $\text{tr}(\hat{\sum}^{-1}(y - XB)'\hat{V}^{-1}(y - XB))$. For variant selection, we adopt the group MCP approach [6]. The overall penalized objective function is

$$Q(B) = \text{tr}(\hat{\textstyle\sum}^{-1}(y - XB)'\hat{V}^{-1}(y - XB)) + \sum_{j=1}^{p} \rho(||B_j||_2; \lambda, \gamma), \quad (3)$$

where B_j is the jth row of B and $\rho(t; \lambda, \gamma) = \lambda \int_0^{|t|} (1 - x/(\gamma\lambda))_+ dx$ is the MCP with tuning parameter λ and regularization parameter γ [7].

For computation, we use a group coordinate descent algorithm [1]. The group MCP involves a regularization parameter and a tuning parameter. Generally speaking, smaller values of γ are better at retaining the unbiasedness of the MCP penalty for large coefficients, but they have the risk of creating objective functions that have problems with nonconvexity [8], are difficult to optimize, and yield solutions that are discontinuous with respect to λ. Simulation studies by Breheny and Huang [8] suggest that $\gamma = 6$ is a reasonable choice. Therefore, we fix it to be 6 in our analyses. We search for the optimal value of λ using 5-fold cross-validation.

Results
The GAW18 data set consists of dense genome-wide markers with longitudinal measurements on systolic and diastolic blood pressure (SBP and DBP) and other covariates. Other measurements include gender, age, year of examination, use of antihypertensive medications, and tobacco smoking at up to 4 time points. In this study, we analyze the 157 unrelated individuals using SBP and DBP as traits and other medical and demographic covariates as random effects. Gene annotations for SNP data are obtained from http://www.scandb.org. SNPs in each gene are scored using weighted sum statistics to generate gene-level measurements [9]. After quality control, we have the genetic scores of 10,400 genes for further analysis. SBP, DBP, and genetic scores are standardized to have zero means and unit variances. This procedure removes the estimation of intercepts and makes the genes comparable.

We apply the proposed PMLMM to identify genetic variants that are associated with both SBP and DBP at the gene level. As a benchmark, we also analyze each trait separately using a penalized linear mixed model (PLMM) approach. Table 1 shows the genes identified using PMLMM. Table 2 summarizes the overlaps of genes selected using the different approaches. Although there is overlap, PMLMM and PLMM identify significantly different sets of genes. We evaluate the relative stability of identification of each gene using a resampling approach and calculate the observed occurrence index (OOI) [10]. A larger value of OOI indicates that the corresponding identified gene is more stably identified. Table 1 also shows OOI results. The identified genes have reasonably high OOIs.

Discussion
In this study, we analyze the GAW18 data and develop a PMLMM approach. A multivariate linear mixed model is used to model variance components among traits and longitudinal measurements. A penalization approach is adopted for variant selection. In the estimation procedure, it can be considered heuristic to use $\sum$ and $\hat{V}$ as proposed. Assumptions (a) to (c) are standard in mixed models, but the assumption that $\Sigma_1 = \Sigma_2$ may be restrictive. Because our study is to identify multitrait-associated markers at the gene level, the restriction on variance components does not affect the selection result significantly. We are currently developing a similar approach to update variance components with more relaxed assumptions on Σ_1 and Σ_2. An iterative algorithm can be implemented to solve for B, $\sum$, and V. In variant selection, our method is designed to search for genes associated with all the traits considered. When different sets of genetic variants are suspected to be associated with different phenotypes, the sparse group penalization approach [11] can be applied.

Conclusions
We have presented a penalized multivariate linear mixed model (PMLMM) for detecting pleiotropic genetic

Table 1 Genes identified by PMLMM: estimates for SBP and DBP, and OOI

Gene	SBP	DBP	OOI	Gene	SBP	DBP	OOI
MMEL1	0.002	−0.002	0.333	TMEM41B	0.027	0.033	0.403
CD52	0.085	0.060	0.697	ARNTL	0.024	0.006	0.247
DPH2	0.071	−0.032	0.323	SPTY2D1	0.025	−0.007	0.507
C8A	0.018	0.032	0.563	CHST1	−0.008	−0.031	0.540
DNAJB4	−0.028	−0.022	0.333	MRE11A	−0.042	−0.007	0.623
HS2ST1	0.002	0.006	0.307	ENOX1	−0.068	−0.032	0.647
PROK1	0.006	0.010	0.373	LOC100132760	−0.041	0.048	0.693
THBS3	−0.004	0.001	0.337	SPRY2	−0.004	−0.005	0.297
C1orf182	2E-04	0.045	0.490	GABRG3	−0.027	0.006	0.573
TGFBR2	−0.033	−0.030	0.627	THBS1	0.023	0.028	0.353
LOC100129194	0.005	0.011	0.217	CSPG4	0.098	−0.012	0.880
LMOD3	−0.013	−0.028	0.493	C15orf27	0.047	−0.014	0.490
LOC653712	0.017	0.001	0.450	LOC100128570	0.026	0.019	0.283
LAMP3	0.034	0.008	0.627	HOMER2	0.024	0.013	0.250
EIF2B5	−0.014	−0.014	0.417	ADAMTS17	0.002	0.002	0.270
EHHADH	0.003	−0.052	0.677	SLC16A11	0.015	−0.004	0.170
SFRS12	0.005	0.007	0.290	ALDH3A1	0.002	0.021	0.657
C5orf32	0.019	−0.029	0.560	FLJ44815	0.019	−0.005	0.210
ZNF346	0.001	−0.024	0.553	TANC2	0.003	−0.001	0.187
LOC100128901	−0.069	0.006	0.627	PDE6G	−0.056	−0.012	0.377
OGDH	0.123	0.038	0.777	C19orf6	0.013	0.048	0.577
NSUN5	0.035	−0.014	0.660	TMEM146	−0.001	−0.063	0.550
PPP1R3A	−0.034	−0.041	0.453	STX10	4E-04	0.015	0.333
MEST	−2E-04	−3E-04	0.197	RLN3	0.024	−0.034	0.603
NOM1	0.029	−0.001	0.630	CYP4F11	0.018	0.005	0.127
FLJ41200	0.013	0.005	0.333	LOC728326	0.048	−0.040	0.547
LRRC19	−0.015	−0.040	0.480	ZNF585A	0.028	0.082	0.720
CCIN	0.006	−0.004	0.437	SUPT5H	0.020	−0.014	0.233
LOC100130911	0.004	0.024	0.467	FLJ10490	0.004	2E-04	0.327
PTCH1	0.004	−1E-04	0.300	ZNF331	0.027	0.003	0.330
DFNB31	0.057	−0.008	0.710	BACE2	−0.116	−0.074	0.940
OR52D1	0.023	−0.017	0.490	KRTAP10-12	0.002	0.003	0.233

Table 2 Overlap of selected genes between PMLMM and PLMM

	PMLMM	PLMM*	PLMM†
PMLMM	64	24	16
PLMM[1]		40	0
PLMM[2]			29

*PLMM on SBP.
†PLMM on DBP.

associations among multiple traits in the presence of pedigree structures. The proposed approach combines the advantages of mixed models that allow for elegant correction for pedigree-based family data and integrative analysis of multiple traits. Compared with PLMM which considers one trait at a time, the proposed PMLMM can achieve better performance when the pleiotropic effect is appropriately modeled.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
All authors were involved in study design. JL conducted the numerical work. All authors were involved in manuscript preparation, and read and approved the final manuscript.

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