A FAST, ACCURATE TWO-STEP LINEAR MIXED MODEL FOR GENETIC ANALYSIS APPLIED TO REPEAT MRI MEASUREMENTS

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Abstract: Large-scale biobanks are being collected around the world in efforts to better understand human health and risk factors for disease. They often survey hundreds of thousands of individuals, combining questionnaires with clinical, genetic, demographic, and imaging assessments; some of this data may be collected longitudinally. Genetic associations analysis of such datasets requires methods to properly handle relatedness, population structure and other types of biases introduced by confounders. Most popular and accurate approaches rely on linear mixed model (LMM) algorithms, which are iterative and computational complexity of each iteration scales by the square of the sample size, slowing the pace of discoveries (up to several days for single trait analysis), and, furthermore, limiting the use of repeat phenotypic measurements. Here, we describe our new, non-iterative, much faster and accurate Two-Step Linear Mixed Model (2sLMM) approach, that has a computational complexity that scales linearly with sample size. We show that the first step retains accurate estimates of the heritability (the proportion of the trait variance explained by additive genetic factors), even when increasingly complex genetic relationships between individuals are modeled. Second step provides a faster framework to obtain the effect sizes of covariates in regression model. We applied Two-Step LMM to real data from the UK Biobank, which recently released genotyping information and processed MRI data from 9,725 individuals. We used the left and right hippocampus volume (HV) as repeated measures, and observed increased and more accurate heritability estimation, consistent with simulations.

Keywords: SNP, Heritability, Repeated Measures, Empirical Kinship, Imaging Genetics, Genome Wide Association Studies (GWAS), Big Data

1. Introduction

Genome-wide association studies (GWAS) of complex human traits can enable an understanding of the trait’s underlying biological architecture, for example revealing genetic risk factors for disease. However, large-scale populations on the order of tens to hundreds of thousands of individuals are often needed to reliably detect the relatively small effects of single nucleotide polymorphisms. This need for large sample sizes has launched widespread interest in biobank studies that collect data from large populations that may include siblings or other relatives; therefore, some degree of known or cryptic relatedness may exist between individuals included in the study. In order to include all participants while accurately accounting for the fact that some samples may not be fully independent, a linear mixed model (LMM) is often used to improve
association power and accuracy and avoid an inflation of results, which would occur if wrongfully assuming independence in the population.

Active efforts exist for the development of LMM methods, yet current methods continue to have limitations that prevent them from being practical for GWAS of hundreds or thousands of phenotypes (traits). Factored spectrally transformed linear mixed models [2], and genome-wide efficient mixed-model association [3] are two widely used exact models for LMM. However, these iterative optimization methods are computationally intensive, on the $O(N^2)$ for each iteration. Bayesian mixed models [4], have been proposed to overcome some of this complexity, and each iteration takes on the order of $O(N)$; these methods do not estimate narrow-sense heritability, and they require the sample size to be sufficiently large to attain model accuracy. Because of the iterative optimization strategy, the computation time for most existing methods increases rapidly as the sample sizes become larger, to ensure convergence. This all makes them une feasible for scaling on large datasets or analyzing many phenotypes, which is essential requirement for such methods in this coming biobank era. We note that large-scale genetics problems have motivated the development of faster methods to be developed [7, 8], yet to the best of our knowledge, these methods assume only minimal or no genetic relationships among individuals.

Here, we build on and fill a gap in existing work and develop a novel approach to avoid time-consuming iterative steps. We propose an efficient, unbiased and robust Two-Step Linear Mixed Model method (2sLMM), which not only can be used to incorporate the complex genetic relationships among individuals within a large population, but also repeated measures from the same individuals. This two-step method can be used to analyze the heritability of a trait with multiple measurements, while also being to identify genetic loci that may be significantly associated with the trait’s population variance through association testing. The 2sLMM consists of two steps: 1) to estimate heritability of the trait ($h^2$) and then the effect size of independent variables, $\beta$. Here, we use dimensionality reduction methods for preprocessing and moment-matching regression [8, 11] to estimate the variance components due to genetic and environmental effects. Using the genetic relationship matrix derived from genome-wide genotypes of single nucleotide polymorphisms (SNPs), this heritability is commonly referred to as SNP-based heritability; 2) in the second step, we use Generalized Least Squares (GLS) regressions.

While the time complexity for dimensionality reduction is the same as Singular Value Decomposition (SVD), the time complexity for our estimation steps is simply $O(N)$ without any iterations, scaling only linearly with sample size, offering an efficient framework to analyze repeated measurements. Consider if each subject had one repeat measurement, then the sample size would double, and the computational burden for many other algorithms would quadruple, per iteration. We show our 2sLMM, for estimating heritability and fixed effects, is capable of modeling complex genetic relationships in population studies with relatedness and repeated measurements. This framework could dramatically reduce the estimation time from hours or days [3, 4, 9], to approximately 5 minutes per trait, when analyzing large scale genetics populations with a large number of traits. Our method is motivated primarily by applications for neuroimaging genetics, where GWAS can be performed on a large number of traits, representing volume, or area of various regions, even over a million voxels within a brain image [5]. Large scale biobank initiatives such as UK Biobank, are making it possible to do such image wide analyses in approximately 10,000 individuals at once [27], are methods are currently confining such analyses to unrelated individuals and analysis of single, non-repeated measurements. Here,
we demonstrate our method on brain volumes derived from MRI measurements. As there is little evidence for genetic lateralization between left and right hemispheres [28], we further use these measures as repeated measurements from approximately 10,000 individuals scanned as part of the UK Biobank [17], to demonstrate the robustness in our method.

2. Methods

2.1 Linear Mixed Models for single measurements

The common framework for modeling a trait $Y^*$ according to a genetic component, $g^*$, an environmental component, $e^*$, and fixed effects $X^*$ is as follows:

$$ Y^* = X^*\beta^* + g^* + e^* $$  \hspace{1cm} (1)

here, if there are $N$ individuals, $Y^*$ for a single trait is an $N \times 1$ vector, $X^*$ is an $N \times k$ matrix of $k$ fixed effects, such as age, sex, and in the case of MRI-based volumetric phenotypes, intracranial volume (ICV), which is commonly added as a covariate. Linear mixed models (LMM) are often used to model complex genetic relationships among individuals, including cryptic relatedness, which may be a common occurrence in large-scale population studies such as the UK Biobank. In LMM, the genetic effect $g^*$ and the total error, $e^*$, are often modeled as in [4]:

$$ g^* \sim N(0, \sigma_g^{*2} K^*) $$  \hspace{1cm} (2)

$$ e^* \sim N(0, \sigma_e^{*2} \Sigma^*) $$  \hspace{1cm} (3)

where $\sigma_g^{*2}$ is the additive genetic variance. $K^*$ is an $N \times N$ genetic relationship matrix (GRM), and every entry $K^*[i,j]$ corresponds to the degree of genetic similarity between subject $i$ and subject $j$ [1]. The total error, $e^*$, is modeled as a normal distribution, where $\sigma_e^{*2}$ is the environmental variance and $\Sigma^*$ is an $N \times N$ covariance matrix for total error.

The structure of $\Sigma^*$ is often given based on experimental design or prior information [10]. In independent studies, individual subject errors are independently and identically distributed (i.i.d.) and, therefore, $\Sigma^*$ is an $N \times N$ identity matrix $I^*$.

2.2 Two-Step Linear Mixed Model for repeat measurements

Suppose the number of subjects is $N$; now, for each subject we now have more than one measurement, or repeat measurements, that we will use collectively to model the genetic effect. For $p$ measurements, equation (1) may now be written using matrix notation. Let $Y$, $X$ and $e$ be $Np \times 1$ vectors, representing repeated traits, fixed effects and environmental component.

$$ Y = [y_{11} \ y_{12} \ \cdots \ y_{1p} \ y_{21} \ y_{22} \ \cdots \ y_{2p} \ \cdots \ y_{N1} \ y_{N2} \ \cdots \ y_{Np}]^T $$ \hspace{1cm} (4)

$$ X = [x_{11} \ x_{12} \ \cdots \ x_{1p} \ x_{21} \ x_{22} \ \cdots \ x_{2p} \ \cdots \ x_{N1} \ x_{N2} \ \cdots \ x_{Np}]^T $$ \hspace{1cm} (5)

$$ e = [e_{11} \ e_{12} \ \cdots \ e_{1p} \ e_{21} \ e_{22} \ \cdots \ e_{2p} \ \cdots \ e_{N1} \ e_{N2} \ \cdots \ e_{Np}]^T $$ \hspace{1cm} (6)

Specifically, under the notations of (4), (5) and (6), suppose $g$, $K$, $\Sigma$ are $Np \times 1$ repeated genetic component, $Np \times Np$ repeated GRM and $Np \times Np$ covariance matrix for total error. The Linear mixed model with $p$ measurements is similarly expressed as equation (1), (2) and (3)

$$ Y = X\beta + g + e $$  \hspace{1cm} (7)
the GRM for repeated measures, \( K \) is assumed to be expressed as \( K = K^* \otimes \Delta \), where \( K^* \) is an \( N \times N \) GRM of subjects (no repeated measurements), and \( \Delta \) is a \( p \times p \) definite matrix with \( \Delta [i,j] = 1 \), for all \( i \) and \( j \) [10]. \( \Sigma \) is the covariance matrix for total errors.

The matrix form of repeated LMM is mathematically equivalent to

\[
y_{ij} = x_{ij} \beta + g_{ij} + e_{ij}; \quad i = 1,2,\ldots,N; \quad j = 1,2,\ldots,p
\]

where \( y_{ij} \) is the \( j \)th repeated measurement for \( i \)th subject, \( x_{ij} \) is a \( 1 \times k \) vector of \( k \) fixed effects, as above. \( e_{ij} \) is the \( j \)th total error for \( i \)th subject. For example, \( e_{ij} \) could represent the total effect of environmental effects and individual measurement errors [11].

In order to estimate heritability \( h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2) \), it is assumed that \( \sigma_g^2 \) and \( \sigma_e^2 \) may be estimated as \( \tilde{\sigma}_g^2 \) and \( \tilde{\sigma}_e^2 \), respectively, and therefore, \( \tilde{h}^2 = \tilde{\sigma}_g^2 / (\tilde{\sigma}_g^2 + \tilde{\sigma}_e^2) \). However, going back to equations (8) and (9), we need to model \( K \) and \( \Sigma \) appropriately for the repeated measurements. For repeated GRM, \( K \) in (8), this requires modeling the repeated identities in the matrix \( K^* \), and results in a rank deficient, noninvertible matrix. The effect \( \Sigma \) in (9) now also includes subject specific environmental effects, which would be shared across repeat measurements for subject \( i \), in addition to the measurement errors that correspond to unique errors for each measurement \( j \). In this case, \( \Sigma \) will not be an identity matrix. If there is a correlation between the errors of any \( p \) measures \( i \) and \( j \), then \( \Sigma [i,j] = (1 - \rho) I + \rho I^* \otimes \Delta \), where \( I \) is an \( Np \times Np \) identity matrix, \( I^* \) is a \( N \times N \) identity matrix and \( \rho \) is the correlation coefficient [10]. While this \( \Sigma \) could be modeled as two error terms, in this paper we focus on the LMM case where the repeated measurement correlation coefficient \( \rho \) is negligible. Studies have shown that this assumption performs similarly to the more complex model which accounts for correlated errors [9]. Future work will more accurately account for the non-zero correlation; in the case of our current work, much of this shared error across intra-subject measurements may also be absorbed by the repeated measurement used as a covariate, as we will describe.

Our goal is to obtain fast, accurate and robust estimators \( \tilde{h}^2 \) and \( \tilde{\beta} \). This may include the fixed effect of a SNP, \( \tilde{\beta}_{SNP} \), when tested individually or when tens of millions of SNPs are tested in genome-wide association studies (GWAS).

The 2sLMM method consists two steps to estimate \( h^2 \) and \( \beta \). In the first step, we use a dimensional reduction method to compute SVD-transformation matrices \( S_1 \) and \( U \) for preprocessing, and use moment matching regression to estimate \( \sigma_g^2 \), \( \sigma_e^2 \) and \( h^2 \); in the second, we use the Generalized Least Squares (GLS) method to estimate \( \beta \). We describe these steps in a diagram in Figure 1 and in the following sections.
Here, dimensionality reduction preprocessing is necessary to obtain an efficient, unbiased and consistent and Best Linear Unbiased Estimator (BLUE) $\sigma_\varepsilon^2$. $\sigma_\varepsilon^2$ can then be estimated only when $\sigma_\varepsilon^2$ is known; it is also efficient, unbiased and consistent. Based on the statistical properties of OLS, $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_\varepsilon^2)$ is the most appropriate estimator.

2.2.1 Step 1 part 1: SVD dimensionality reduction preprocessing

First, find a SVD-transformation matrix $S_1$, such that

$$ S_1^T X = 0, \quad S_1^T g = 0, \quad S_1^T S_1 = I \quad (11, 12, 13) $$

If the conditions stated in equations (11), (12) and (13) are satisfied, then the original LMM (7) is equivalent to the following new linear mixed model, where $\sigma_\varepsilon^2$ is the only unknown parameter.

$$ S_1^T Y = S_1^T e \quad (14) $$

$$ S_1^T e \sim N (0, \sigma_\varepsilon^2 I) \quad (15) $$

The existence of $S_1$ is proved by Theorem 1 (Supplementary Materials) and can be computed using Fast Singular Value Decomposition (Fast SVD) [12].

2.2.2 Step 1 part 2: Estimate $\sigma_\varepsilon^2$

After SVD dimensionality reduction preprocessing, we estimate $\sigma_\varepsilon^2$ using moment matching regression [8, 11], which is mathematically equivalent to LD regression [13] under some constrains.

The SVD-transformed phenotypes are $S_1^T Y$. To estimate $\sigma_\varepsilon^2$, we regress the 2nd moment of transformed phenotypes $S_1^T Y Y^T S_1$, onto the variance component of $S_1^T e$, the matrix I:

$$ vec(S_1^T Y Y^T S_1) = \sigma_\varepsilon^2 vec(I) + \varepsilon_1 \quad (16) $$

where $vec()$ is a vectorization function -- with an input of any matrix it simply stacks the columns; $\varepsilon_1$ is the regression error. The OLS estimator of (16) is numerically solved as

$$ \hat{\sigma_\varepsilon^2} = trace(S_1^T Y Y^T S_1)/trace(I) \quad (17) $$

The unbiased, consistent and efficient properties of $\hat{\sigma_\varepsilon^2}$ are summarized in Theorem 2 and Theorem 3 (Supplementary Materials). The variance of $\sigma_\varepsilon^2$ is $\frac{(\sigma_\varepsilon^2)^2}{N}$ [24], which is approximated by the asymptotic variance based on the consistency of $\sigma_\varepsilon^2$ (Theorem 4, Supplementary Materials).

2.2.3 Step 1 part 3: Estimate $\sigma_g^2$ and $h^2$

$\sigma_g^2$ and $h^2$ can also be estimated using SVD-moment matching regression. In order to estimate $\sigma_g^2$, we compute a SVD-transformation matrix $U$, such that

$$ U^T X = 0, \quad U^T U = I \quad (18, 19) $$

We then apply the matrix $U$ to both sides of the original linear mixed model (7)
\[ U^TY = U^T g + U^T e \] (20)

where \( U^T g \sim N (0, \sigma_g^2 U^T K U) \), and \( U^T e \sim N (0, \sigma_e^2 I) \). Given \( \hat{\sigma}_g^2 \), we use the moment-matching regression [8, 11] and consider a simpler, but still accurate linear regression for (20), where \( \epsilon_2 \) is the regression error:

\[ \text{vec}(U^TY^TU) - \hat{\sigma}_g^2 \text{vec}(I) = \sigma_g^2 \text{vec}(U^T K U) + \epsilon_2 \] (21)

Then an unbiased, consistent and efficient OLS estimator \( \hat{\sigma}_g^2 \) is derived

\[ \hat{\sigma}_g^2 = \text{trace}(U^T K U V U^T Y U - \hat{\sigma}_g^2 U^T K U) / \text{trace}(U^T K U U^T K U) \] (22)

The variance of \( \hat{\sigma}_g^2 \) is \( \frac{(\sigma_g^2)^2}{\sqrt{pN}} \) [24], where \( p \) is the number of repeat measurements (Theorem 4, Supplementary Materials).

Finally, the heritability estimator is obtained through \( \hat{h}^2 = \hat{\sigma}_g^2 / (\hat{\sigma}_g^2 + \hat{\sigma}_e^2) \), and variance of estimated heritability is calculated by Delta method [25]

\[ \text{var}(\hat{h}^2) = \frac{(\hat{\sigma}_g^2)^2}{(\hat{\sigma}_g^2 + \hat{\sigma}_e^2)} \text{var}(\hat{\sigma}_g^2) + \frac{(\hat{\sigma}_e^2)^2}{(\hat{\sigma}_g^2 + \hat{\sigma}_e^2)} \text{var}(\hat{\sigma}_e^2) \] (23)

### 2.2.2 Step 2: Estimate \( \beta \)

\[ \hat{\beta} = (X^T \hat{\Sigma}^{-1} X)^{-1} X^T \hat{\Sigma}^{-1} Y \] (24)

\[ \text{cov}(\hat{\beta}) = (X^T \hat{\Sigma}^{-1} X)^{-1} (\hat{\sigma}_g^2 + \hat{\sigma}_e^2) \] (25)

in which \( \hat{\Sigma} \) is the OLS estimator of variance-covariance matrix [15]. In particular,

\[ \hat{\Sigma} = \hat{h}^2 K + (1 - \hat{h}^2) I \] (26)

\( \hat{\beta} \) is obtained using GLS method, so \( \hat{\beta} \) is also unbiased, consistent and efficient, which is an important consideration in large scale big data applications such as imaging genetics.

### 2.3 Simulation methods

To determine the accuracy and efficiency of our 2sLMM method, we simulated several conditions similar the real neuroimaging genetics dataset we use in the next section (from UK Biobank), and compared our estimates to those obtained from Ge et al.’s moment-matching regression for SNP-based Heritability Estimation (MMHE) [8, 11], whose application scope now includes repeated measurements (Repeated MMHE) [11].

Suppose the total number of simulations is \( N_{sim} \), and the number of subjects is \( N \). We perform simulation analysis for the proposed LMM with repeat measurements:

\[ Y = X\beta + g + e \] (7)

\[ g \sim N (0, \sigma_g^2 K) \] (8)

\[ e \sim N (0, \sigma_e^2 \Sigma) \] (9)

where, \( \Sigma \) is an identity matrix \( I \) and the number of repeated measurements \( p \) is 2. To make the simulated GRM (the matrix \( K^* \)) close to a real GRM from a family-based population, we first randomly generated a 2-trial binomial distributed \( N \times m \) genotype matrix \( Z \), where the number of SNPs, \( m \) is 100,000, and probability of success for each trial is 0.2. Here, the probability of
success for each binomial trial corresponds to the minor allele frequency (MAF). In particular, every column of $Z$ needs to be the normalized. $K$ is obtained by $K = K^* \otimes \Delta$, $K^* = Z^T Z / m$.

We designated the true values for our simulation based on our real data example (see next section). Suppose fixed effects $X$ in (7) include an intercept, age, sex, and a scaled version of intracranial volume (scaled ICV), $X = [1 \ X_{age} \ X_{sex} \ X_{scaledICV}]$.

Motivated by the distribution of data in the UK Biobank, we sampled $X_{age}$ from a normal distribution with mean 60 and variance 10. $X_{sex}$ is a binary variable, coded here as 0 for female and 1 for male. $X_{sex}$ was simulated using a discrete uniform distribution, with only two values, 0 and 1. $X_{scaledICV}$ was drawn from a normal distribution with mean 1.2 and variance 0.05, $X_{scaledICV} \sim N(1.2, 0.05)$. For robustness, we also performed a follow-up test modeling in noise: $X_{scaledICV}$ was drawn from a contaminated normal distribution, in which 91% of simulated $X_{scaledICV}$ were from $N(1.2, 0.05)$ as before, while the remaining 9% are drawn from another normal distribution with a much higher variance, $N(1.2, 3)$ such that now, $X_{scaledICV} \sim 0.91N(1.2, 0.05) + 0.09N(1.2, 3)$. The design of contaminated normal distribution follows from [16].

Using random sampling with replacement, 100 simulations were conducted for 10,000 subjects. For each simulated sample, both our 2sLMM method and MMHE [11] were run. True values of estimators are assigned as $h^2 = 0.4$, sum of errors $\sigma^2_e + \sigma^2_g = 170,000, \beta_0 = 6,600, \beta_{age} = -15, \beta_{sex} = 5, \beta_{scaledICV} = -1,400$.

**2.4 Analysis with real data**

The UK Biobank is a large scale epidemiological study, collecting genotypes, brain MRIs, as well as numerous other variables from adults aged 45-75 in the UK. Information regarding brain imaging analysis from the UK Biobank is detailed in [17]. Summary volumetric data has been made available in their database and was accessed here as part of application #11559. As of July 2017, a sample size of 9,725 individuals had non-missing data on age at scan, sex, left and right subcortical volumes, and scaled intracranial volume (scaledICV). Raw genotypes were downloaded and used to create the GRM in the raremetalworker (RMW) software package (https://genome.sph.umich.edu/wiki/RAREMETALWORKER), which calculates the GRM according to [2]; we did not use the X chromosome in our GRM calculation. The resulting GRM matrix is full rank and invertible.

9,725 subjects in the UK Biobank were analyzed using our 2sLMM method for a repeat analysis study of hippocampal volume heritability. To date, large-scale genome-wide association initiatives from large scale consortia, including the ENIGMA consortium, use a bilaterally averaged measure of hippocampal volume as the trait of interest [18, 19, 20]. This avoids possible left/right mismatches across different cohorts, and also reduces noise compared to running each left or right volumes separately. However, if the lateralized volumes are considered as repeated measures, rather than averaged, the effective sample size may be higher, possibly leading to more stable estimates than the current averaging techniques. To demonstrate these repeat measurements, 9,725 left and 9,725 right measurements are considered separately as individual elements of trait vector $Y$. Fixed effects $X$ in (7) included the scaled ICV to control for head size, age, sex and an intercept. Due to the limited exclusion criteria, many health conditions are represented in UK Biobank; for the current study, we did not eliminate or control for any conditions and performed our estimates on the more diverse phenotypic pool.
Using this data, we first estimate the heritability of hippocampal volume using bilaterally averaged measures from all 9,725 subjects, resulting in 9,725 data points. Second, we estimate the heritability of hippocampal volume using each of the lateral measures from all 9,725 subjects as unique samples as described above, resulting in 19,450 data points.

3. Results

3.1 Simulation results

For a given simulated condition, we plot the resulting estimate of each of the 100 simulations. Simulation Set 1 (Figure 2; Table 1) is generated given $X_{scaledCV} \sim N(1.2, 0.05)$, while simulation Set 2 (Figure 3; Table 2) is the test for robustness, where $X_{scaledCV} \sim 0.91N(1.2, 0.05) + 0.09N(1.2, 0.3)$. For Set 1 and Set 2, the same GRM (single measure or repeated measures) were run using 2sLMM, and Repeated MMHE. Mean, bias, standard deviation (SD) and mean squared error (MSE) are reported in Table 1 and Table 2 for simulated estimators, $\hat{h}^2$, $\hat{\sigma}_g^2$, $\hat{\sigma}_e^2$, $\hat{\beta}_{age}$ of 2sLMM. Repeated MMHE [11] does not provide estimates of $\beta$ values, so we only report the outputs for $\hat{h}^2$, $\hat{\sigma}_g^2$, and $\hat{\sigma}_e^2$.

Simulation Set 1: $N = 1000$, $N_{Sim} = 100$, $p = 2$, $X_{scaledCV} \sim N(1.2, 0.05)$

![Figure 2](image-url)  

**Figure 2.** Heritability estimate simulations ($N = 1000$, $N_{Sim} = 100$). Top: Two-Step Linear Mixed Model (2sLMM); Bottom: Repeated moment-matching heritability estimation (Repeated MMHE [11]). Columns correspond to estimations of $\hat{h}^2$, $\hat{\sigma}_g^2$, $\hat{\sigma}_e^2$, and for our method, $\hat{\beta}_{age}$. The x-axis reflects the number of each of the 100 simulations. The same scales are used for the y-axes for both 2sLMM and MMHE.

| Estimators | True values | Mean  | Bias          | Standard Dev | MSE          |
|-----------|-------------|------|---------------|--------------|--------------|
| $\hat{h}^2$ | 0.40        | 2sLMM | Repeated MMHE | 2sLMM | Repeated MMHE | 2sLMM | Repeated MMHE |
| $\hat{\sigma}_g^2$ | 68000 | 0.39 | -6.67 x 10^-4 | -0.01 | 0.03 | 0.06 | 8.84 x 10^-4 | 1.70 x 10^6 |
| $\hat{\sigma}_e^2$ | 102000 | 65436.22 | -325.53 | -2563.78 | 6585.97 | 12768.58 | 4.35 x 10^7 | 1.02 x 10^6 |
| $\hat{\beta}_{age}$ | -15.00 | 103761.81 | -408.60 | 1761.81 | 4888.26 | 9933.70 | 2.41 x 10^7 | 1.19 |
Simulation Set 2: $N = 1000$, $N_{\text{Sim}} = 100$, $p = 2$, $X_{\text{scaledICV}} \sim 0.91N(1.2, 0.05) + 0.09N(1.2, 0.3)$

![Simulations](image)

Figure 3. Heritability estimate simulations ($N = 1000$, $N_{\text{Sim}} = 100$, $X_{\text{scaledICV}} \sim 0.91N(1.2, 0.05) + 0.09N(1.2, 0.3)$) Top: Two-Step Linear Mixed Model (2sLMM); Bottom: Repeated moment-matching heritability estimation (Repeated MMHE [11]). Columns correspond to estimations of $\hat{h}^2$, $\hat{\sigma}_g^2$, $\hat{\sigma}_e^2$, and for our method, $\hat{\beta}_{\text{age}}$. The x-axis reflects the number of each of the 100 simulations. The same scales are used for the y-axes for both 2sLMM and MMHE.

### Table 2. Simulation Statistics simulations ($N = 1000$, $N_{\text{Sim}} = 100$, $X_{\text{scaledICV}} \sim 0.91N(1.2, 0.05) + 0.09N(1.2, 0.3)$) for 2sLMM and Repeated MMHE

| Estimator | True values | Mean | Bias | Standard Dev | MSE |
|-----------|-------------|------|------|--------------|-----|
| $\hat{h}^2$ | 0.40 | 2sLMM | 0.40 | -4.16 x 10^{-3} | 0.03 |
|           |           | Repeated MMHE | 0.40 | -1.7 x 10^{-3} | 0.15 |
| $\hat{\sigma}_g^2$ | 68,000 | 2sLMM | 67,123 | -877.3 | 6696 |
|           |           | Repeated MMHE | 68,794 | 794.31 | 26635 |
| $\hat{\sigma}_e^2$ | 10,2000 | 2sLMM | 10,2192 | 192.4 | 4457 |
|           |           | Repeated MMHE | 10,1415 | -585.09 | 23995 |
| $\hat{\beta}_{\text{age}}$ | -15.00 | 2sLMM | -15.00 | 1.04 x 10^{-3} | 1.11 |
|           |           | Repeated MMHE | -- | -- | -- |

3.2 Analysis with real data from the UK Biobank

$Y^*$ is the average of left and right hippocampal volumes from each of the 9,725 subjects. Fixed effects $X^*$ in (1) included intercept, age, sex and scaled ICV to control for head size. Using MMHE [8] for a single (not-repeated) measurement, the heritability estimate for a single measurement is $\hat{h}^{*2} = 0.06$ ($\hat{\sigma}_g^{*2} = 8027.81$ and $\hat{\sigma}_e^{*2} = 136015.72$), which despite MMHE being similar to LD score regression under certain conditions [8], the result is far less than the genome-wide summary statistic heritability calculated using LD Score Regression [13] for hippocampal volume in [21] $\hat{h}^{*2} = 0.135$, using GWAS summary data from a comparable sample size ($N \sim 11,600$) from the ENIGMA Consortium [19].

For hippocampus repeated measurements, we ran both our method and Repeated MMHE [11], and we compare $\hat{h}^2$, $\hat{\sigma}_g^2$, $\hat{\sigma}_e^2$, $\hat{\beta}_0$, $\hat{\beta}_{\text{age}}$, $\hat{\beta}_{\text{sex}}$, $\hat{\beta}_{\text{ICV}}$ running time (implemented with MATLAB and CPU) in Table 3, where $\hat{\beta}_0$ is the estimated $\beta$ for intercepts. The standard error of the heritability estimate $se(\hat{h}^2)$ is estimated using block Jackknife as in [11] (block size = 266)
for Repeated MMHE and 2sLMM, we refer to this as Jackknife $se(\hat{h}^2)$. Additionally, $se(\hat{h}^2)$ is calculated for 2sLMM, which is approximated by the asymptotic $se(\hat{h}^2)$.

As seen in Table 3, even with MMHE, which we have shown may underestimate heritability, we see that using the repeat measurement approach is far more powerful (\(\hat{h}^2 \sim 0.32\) as opposed to \(\hat{h}^2 \sim 0.06\)) and is capable of attributing more of the variance to additive genetic effects.

Our 2sLMM method can capture an even greater portion of the genetic variance \(\hat{h}^2 \sim 0.44\), which is closer to the hippocampal heritability estimates suggested by twin and family studies of approximately 70% [20]. Furthermore, 2sLMM provides effect size estimates for fixed effects.

**Table 3.** Estimation results for repeated measurements of hippocampal volume in UK Biobank. The 2sLMM provides higher estimates of heritability as well as estimates for the \(\beta\) coefficients of fixed effects.

| Estimators and Time | Repeated MMHE | 2sLMM |
|---------------------|---------------|-------|
| $\sigma^2_g$        | 60294.56      | 73722.11 |
| $\sigma^2_e$        | 130520.47     | 93669.50 |
| $\hat{h}^2$         | 0.32          | 0.44   |
| Jackknife $se(\hat{h}^2)$ | 0.007      | 0.005  |
| $se(\hat{h}^2)$     | -             | 0.03   |
| $\beta_0$           | -             | 6612.06 |
| $se(\beta_0)$       | -             | 57.57  |
| $\beta_{age}$       | -             | -14.85 |
| $se(\beta_{age})$   | -             | 0.45   |
| $\beta_{sex}$       | -             | 5.25   |
| $se(\beta_{sex})$   | -             | 8.91   |
| $\beta_{ICV}$       | -             | -1405.52 |
| $se(\beta_{ICV})$   | -             | 36.69  |
| Matlab CPU Time     | 52.65 min     | 33.28 min |

**4. Discussion**

In this paper, we propose a fast, statistically efficient and accurate Two-Step Linear Mixed Model (2sLMM) for population studies with cryptic relatedness and repeated measurements. Speed and accuracy are arguably the most important two criteria to evaluate a statistical model. Most iterative linear mixed models [2-4] based on Restricted Maximal Likelihood (ReML) or Bayesian methods, require many iterations to obtain accurate results.

While fast, non-iterative, linear mixed models exist, under certain conditions some important estimators such as \(\hat{h}^2\) or \(\beta\) for a fixed variable, may be inefficient, underestimated or inaccurate with large sampling errors. This is due to the fact that some of these existing fast LMMs often ignore or just simplify the complex genetic relationships between individuals. In order to estimate \(\beta\) related to fixed effects such as age, sex or more importantly, SNPs, the Two-Step ProbAbel package [15] for example, first ignores genetic effects to estimate non-genetic \(\beta\) values. Non-repeated [8] or Repeated MMHE [11], a moment-matching heritability method works well for population data; this method gives fast and unbiased heritability estimates as shown in Table 3. However, for populations that include family data, the GRM deviates significantly from an
identity matrix, resulting in non-normally distributed, highly correlated and heteroscedastic regression errors in moment-matching methods such as those in [8, 11, 22]. Figure 2 and Figure 3 show that if we use these models that do not support highly related samples, MMHE tends to overestimate $\sigma^2_\epsilon$ and underestimate $\sigma^2_\theta$, resulting in underestimated $\hat{h}^2$. In Figure 3, our robustness test showed that when scaled ICV contains small portion of outliers, Repeated MMHE gives $\hat{h}^2$, $\sigma^2_\epsilon$, $\sigma^2_\theta$ with relatively larger standard deviations, which may explain why Repeated MMHE generates lower heritability than 2sLMM, since the ICV data from UK Biobank contains many outliers. If we estimate $\hat{\beta}$ directly using results from the moment-matching regression, the bias and standard deviations of $\sigma^2_\epsilon$ and $\sigma^2_\theta$ will be further magnified, and the $\hat{\beta}$ will be inaccurate.

In contrast, our fast method projects the original data onto a lower dimensional space, but keeps all the necessary information for estimation. This dimensionality reduction method ensures that the estimated heritability and $\hat{\beta}$ have minimum estimation standard errors, as shown in our simulation studies (Figure 2, Figure 3). In Table 3, Jackknife standard errors are reported for Repeated LMM and MMHE as they are conducted in the original papers [8, 11], indicating both methods are stable. The asymptotic standard error of $\hat{h}^2$, is close to the simulation standard error in Table 1 and Table 2. This reflects the statistical power of our 2sLMM, when applied to large-scale neuroimaging genetics datasets. Table 2 and Figure 3 show that the 2sLMM method is very robust, and insensitive to outliers, ensuring our heritability analysis is not biased by the outliers from the data.

Furthermore, estimated $\beta$ for age, sex, and ICV (intracranial volume) are reported in Table 3. The score test for significance of $\beta_{\text{age}}$, $\beta_{\text{sex}}$, and $\beta_{\text{ICV}}$ can be obtained with asymptotic $\chi^2$ distributed statistics [15]. As expected, significant effects of age and ICV are observed, while sex differences are not detected to be significant for the hippocampal volume in our study; this may be due to simultaneously controlling for the other factors ICV and age.

This proposed 2sLMM method has some application limitations. Most notably, the method is applied in the case where we are considering independent repeat measurements, which is regarded as the simplest case to quantify the total errors from experiments and environment. In the more precise and complex repeated measurement model, the total error $e$ could incorporate many additive effects, such as intrasubject and intersubject effects, household effects and measurement effects [23]. A fast, efficient and robust statistical method is still an open problem. Despite this limitation, other studies have shown that this assumption performs similarly to the more complex model which accounts for correlated errors in intrasubject measurements [9]. in our case, much of this shared error across the left and right volume measurements may also be absorbed by the repeated ICV measurement fitted in $X$.

In future work, we will evaluate the performance of our model for genome-wide association results, to identify genome-wide significant SNPs for subcortical measures or other genetic related phenotypes. Compared with iterative methods, this non-iterative and accurate method will show much greater time improvement when running on GPU, given GPU optimizes the matrix multiplication far more efficiently than current CPU implementations. Our method will also be further extended to support more complex linear mixed models, to account for additional intra-subject and inter-subject relationships, including common environment, or household effects.
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Supplemental Materials

Theorem 1. There exists a matrix \( U \), such that \( U^T X = 0 \) and \( U^T U = I \). Similarly, there exists a matrix \( W \), such that \( W^T (U^T K U) = 0 \) and \( W^T W = I \). Then \( S_1 \) is defined as \( S_1 = UW \), which satisfies conditions (11), (12) and (13). Furthermore, the dimensionality reduction results, (14) and (15) hold, without impacting the estimate accuracy for \( \sigma_e^2 \).

Proof. \( U \) and \( W \) are related to the left null space for \( X \) and \( U^T K U \). Left null space is a subspace of its corresponding matrix. For any given singular matrix \( A \), any vector \( x \) is in the left null space of \( A \) if it satisfies \( x^T A = 0 \). Consequently, if a matrix \( H \) satisfies \( H^T A = 0 \) and \( H^T H = I \), then all the columns of \( H \) are considered as all the orthogonal bases in the left null space of \( A \). Using the concept of left null space, it is obvious that the \( U \) and \( W \) exist if and only if both \( X \) and \( K \) are singular matrices, or rank deficient. In practice, every column of \( X \) is a kind of fixed effect. In most cases, GWAS data contain hundreds or thousands of subjects, but with less than ten fixed effects to be considered, simultaneously. Thus, the column number of \( X \) is much smaller the row number \( Np \), which is a sufficient condition to ensure that \( X \) is rank deficient.

Second, the GRM for repeated measures, \( K \), is assumed to be expressed as \( K = K^* \otimes \Delta \), where \( K^* \) is the \( N \times N \) GRM of subjects (no repeated measurements), and \( \Delta \) is a \( p \times p \) definite matrix with \( \Delta [i,j] = 1 \), for all \( i \) and \( j \) [10]. Therefore, \( K \) is a singular matrix. Since \( X \) is rank deficient and \( K \) is singular, \( U \) and \( W \) exist based on the definition of the left null space.

Given the existence and properties of \( U \) and \( W \), (11), (12) and (13) must be true. As \( S_1 = UW \), \( S_1^T e \) in (15) is linear transformation of a normal random variable \( e \), such that \( S_1^T e \) is also normal-distributed. Next note that

\[
S_1^T Y = S_1^T X \beta + S_1^T g + S_1^T e = S_1^T e
\]

where \( S_1^T X \beta \) and \( S_1^T g \) are cancelled out because \( S_1^T X = 0 \), and

\[
S_1^T g = W^T U^T g \sim N (0, W^T U^T K U W) = N (0,0)
\]

In sum, (14) and (15) are true.

Theorem 2. \( \hat{\sigma}_e^2 \) is a Best Linear Unbiased Estimator (BLUE) when \( \Sigma \) is an identity matrix.

Proof. Note that the ‘best’ only means that among all the unbiased linear estimators for \( \sigma_e^2 \), the best one has the lowest variance. \( \hat{\sigma}_e^2 \) is a BLUE if only \( \hat{\sigma}_e^2 \) is suitable for Gauss-Markov theorem [14]. The Gauss-Markov theorem confirms that in a linear regression model where errors are uncorrelated, with expectation 0 and equal variance, the BLUE of coefficients is given by the OLS estimator. Therefore, we only need to ensure the expectation and variance of \( \varepsilon_1 \) are both 0.

Given \( e_1 = S_1^T e \), let us define the \( k^{th} \) value of \( e_1 \) is \( e_{1,k} \), and the \( k^{th} \) value of \( \varepsilon_1 \) is \( \varepsilon_{1,k} \), and the rank of \( Z_1 \) is \( D \).

\[
\therefore Z_1 = S_1^T Y = S_1^T e \text{ in (14)}
\]
\[ \varepsilon_1 = \text{vec}(Z_1 Z_1^T) - \sigma_v^2 \text{vec}(I) = \begin{pmatrix} e_{1,1} & e_{1,1} \\ \vdots & \vdots \\ e_{1,D} & e_{1,1} \end{pmatrix} - \sigma_v^2 \text{vec}(I) = \begin{pmatrix} e_{1,1} & e_{1,1} \\ \vdots & \vdots \\ e_{1,D} & e_{1,1} \end{pmatrix} - E = \begin{pmatrix} e_{1,1} & e_{1,1} \\ \vdots & \vdots \\ e_{1,D} & e_{1,1} \end{pmatrix} \]

\[ \therefore E(\varepsilon_1) = E \begin{pmatrix} e_{1,1} & e_{1,1} \\ \vdots & \vdots \\ e_{1,D} & e_{1,1} \end{pmatrix} - E \begin{pmatrix} e_{1,1} & e_{1,1} \\ \vdots & \vdots \\ e_{1,D} & e_{1,1} \end{pmatrix} = 0 \]

\[ \therefore k^{th} \text{ value of } \varepsilon_1 \text{ is } \varepsilon_{1,k}, \text{ then there exist the corresponding } i(k) \text{ and } j(k) \text{ to } k, \text{ such that } \]

\[ \varepsilon_{1,k} = e_{1,i(k)} e_{1,j(k)} \quad (29) \]

\[ \therefore k - 1 = (j(k) - 1)D + (i(k) - 1), 0 \leq (i(k) - 1) \leq D - 1. \]

This is equivalent to \( i(k) = \text{mod}(k - 1, D) + 1 \), and \( j(k) = (k - i(k))/D + 1 \), where \( \text{mod}(k - 1, D) \) is the modulo, i.e., the remainder after the division of \( k - 1 \) by \( D \).

\[ \therefore \text{cov}(\varepsilon_{1,k_1}, \varepsilon_{1,k_2}) = \text{cov}(e_{1,i(k_1)} e_{1,j(k_1)}, e_{1,i(k_2)} e_{1,j(k_2)}) = E(\varepsilon_{1,i(k_1)} e_{1,j(k_1)}, e_{1,i(k_2)} e_{1,j(k_2)}) \]

Therefore, \( \text{cov}(\varepsilon_{1,k_1}, \varepsilon_{1,k_2}) = 1 \) if and only if \( i(k_1) = j(k_1) = i(k_2) = j(k_2) \). Specifically, \( \text{var}(\varepsilon_{1,i}) = 1 \) for any \( i \). We can now conclude that \( \sigma_v^2 \) is suitable for the Gauss-Markov theorem and \( \sigma_v^2 \) is a BLUE.

**Theorem 3.** \( \sigma_v^2 \) is an unbiased, consistent and efficient estimator.

**Proof.** In statistics, an estimator is said to be unbiased, when the mean of the sampling distribution is equal to the true parameter being estimated; it is said to be a consistent estimator if and only if as the number of data points increases indefinitely, the resulting sequences of estimators will converge to the true parameter. For a linear regression model, if errors have finite variance, and are uncorrelated with the corresponding regressor coefficient, the estimator of any regressor is unbiased and consistent. In terms of regressor errors \( \varepsilon_1 \) in (19) and **Theorem 2**, its variance is equal to 1 which is finite. And it is known that \( \varepsilon_1 \) are uncorrelated with \( \text{vec}(I) \), which proves the consistent and unbiased properties of \( \sigma_v^2 \).

The efficiency of an estimator is that among all unbiased estimators, an efficient estimator has the lowest variance. Efficiency requires that the errors have finite variance and are homoscedastic. First, **Theorem 2** shows that the variance of \( \varepsilon_1 \) are finite. Furthermore, \( E(\varepsilon_1^2|\text{vec}(I)) \) is independent of the regressor \( \text{vec}(I) \), implying that the variance of \( \varepsilon_1 \) is homoscedastic. \[ \square \]
Theorem 4 The variance of $\sigma_{\hat{\varepsilon}}^2$ is $\frac{(\sigma_{\hat{\varepsilon}}^2)^2}{\sqrt{N}}$, which is approximated by asymptotic variance based on the consistency of $\sigma_{\hat{\varepsilon}}^2$. Similarly, the variance of $\sigma_{\hat{\theta}}^2$ is $\frac{(\sigma_{\hat{\theta}}^2)^2}{\sqrt{pN}}$, where $p$ is the number of repeat measurements.

Proof. For variances of $\sigma_{\hat{\varepsilon}}^2$ and $\sigma_{\hat{\theta}}^2$, in general, they are derived from (17) and (22), which can be regarded as the same mixed model:

\[
T = M, M \sim N(0, \sigma_M^2 R) \quad \text{(31, 32)}
\]

\[
\text{vec}(T^T T) = \text{vec}(M) + \varepsilon \quad \text{(33)}
\]

where $\sigma_M^2$ represents genetic variance $\sigma_{\theta}^2$ or environmental variance $\sigma_{\varepsilon}^2$, $D \times D$ matrix $R$ represents Genetic Relationship Matrix, or covariance matrix for total errors. In this paper, $\sigma_M^2$ is solved with Ordinary Least Square, whose result is numerically close to Generalized Least Square. Equation (3.6) and (3.9) in [25] prove that the approximate rate of $\sigma_M^2$ to true $\sigma_{\theta}^2$ is $o(\sqrt{D})$. After detailed calculation of sample variance of $\varepsilon$, the asymptotic variance of $\sigma_M^2$ is $\frac{(\sigma_M^2)^2}{\sqrt{D}}$. Back to our estimates, the reduced dimension space to estimate $\sigma_{\varepsilon}^2$ and $\sigma_{\theta}^2$ is on the order of $N$ and $pN$, leading to variance of $\sigma_{\varepsilon}^2$ is $\frac{(\sigma_{\varepsilon}^2)^2}{\sqrt{N}}$ and $\sigma_{\theta}^2$ is on the order of $\frac{(\sigma_{\theta}^2)^2}{\sqrt{pN}}$, separately. \qed