S1 Supplementary Material

S1.1 Propositions

Quadratic forms

If $\mathcal{X}$ is a vector of random variables with mean μ and (nonsingular) covariance matrix Σ, then the quadratic form $\mathcal{X}^T A\mathcal{X}$ is a scalar random variable:

$$E(\mathcal{X}^T A\mathcal{X}) = tr(A\Sigma) + \mu^T \Sigma\mu \tag{S1}$$

$$Var(\mathcal{X}^T A\mathcal{X}) = 2tr(A\Sigma A\Sigma) + 4\mu A\Sigma A\mu \tag{S2}$$

See ref. [1, Appendix 3, pp. 843] for more details.

Linear transform of random vector

If B is a constant matrix and $\mathcal{X}$ is a vector of random variables with mean μ and covariance matrix Σ, then $B\mathcal{X}$ is a vector of random variables:

$$E(B\mathcal{X}) = BE(\mathcal{X}) \tag{S3}$$

$$Var(B\mathcal{X}) = BVar(\mathcal{X})B^T \tag{S4}$$

The proof makes use of definitions of mean and variance.

Eigen-value decomposition (EVD)

If K is the covariance matrix of size $n \times n$, that means K is symmetric and positive semi-definite. Furthermore, EVD of K is

$$K = QDQ^T = QDQ^{-1} \tag{S5}$$

where Q is an $n \times n$ orthogonal matrix of eigen-vectors and D is a $n \times n$ diagonal matrix of eigen-values (λ_K^i with i from 1 to n).

EVD for the matrix inverse to K is

$$K^{-1} = QD^{-1}Q^T \tag{S6}$$

EVD for the matrix such as $V = aK + bI$, where a and b are scalars, I is the $n \times n$ identity matrix, is

$$V = aK + bI = aQDQ^T + bI = aQDQ^T + bQIQ^T = Q(aK + bI)Q^T \tag{S7}$$

Trace operator and eigen-value decomposition (EVD)

For the covariance matrix K and the matrix $V = aK + bI$, we have the following series of equations in relation to the trace operator.

$$\begin{aligned} tr(K) &= \sum_{i=1}^{n} \lambda_K^i \\ tr(K^{-1}) &= \sum_{i=1}^{n} (\lambda_K^i)^{-1} \\ tr(V) = tr(aK + bI) &= \sum_{i=1}^{n} (a\lambda_K^i + b) \\ tr(V^{-1}) = tr((aK + bI)^{-1}) &= \sum_{i=1}^{n} (a\lambda_K^i + b)^{-1} \\ tr(V^{-1}K) = tr((aK + bI)^{-1}K) = tr((aI + bK^{-1})^{-1}) &= \sum_{i=1}^{n} (a + b(\lambda_K^i)^{-1})^{-1} \end{aligned} \tag{S8}$$

In the last equation we used the following equality.

$$\begin{aligned} V^{-1}K = (aK + bI)^{-1}K &= (aK + bI)^{-1}(K^{-1})^{-1} \\ &= K^{-1}(aK + bI)^{-1} = (aI + bK^{-1})^{-1} \end{aligned} \tag{S9}$$

S1.2 Analytical derivation of multiplier γ_β for families

The effective sample size (ESS) multiplier for family-based association studies is given in Equation 33 of the main text. We write down this result again.

$$\gamma_\beta = \frac{tr\left((K\sigma_a^2 + I\sigma_r^2)^{-1}K\right)}{N} \tag{S10}$$

We can further rewrite the numerator using the relationship between the trace operator and eigen-value decomposition (EVD) of matrix $(K\sigma_a^2 + I\sigma_r^2)^{-1}K$ given in Equation S8 of this Supplementary Material.

$$\gamma_\beta = \frac{1}{N} \sum_{i=1}^{N} \frac{\lambda_i}{\lambda_i \sigma_a^2 + \sigma_r^2} \tag{S11}$$

The assumption of y being standardized leads to $\sigma_r^2 = 1 - \sigma_a^2$:

$$f(\sigma_a^2, \lambda) = \gamma_\beta = \frac{1}{N} \sum_{i=1}^{N} \frac{\lambda_i}{(\lambda_i - 1)\sigma_a^2 + 1} \tag{S12}$$

Splitting K into blocks of sub-matrices K_s

The sample generally consists of N_s families such that there is no between-family genetic relatedness. Then the kinship matrix K can be represented as a block matrix.

$$K = \begin{pmatrix} K_{s_1} & 0 & \dots & 0 \\ 0 & K_{s_2} & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & K_{s_{N_s}} \end{pmatrix}$$

Hence, the multiplier can be evaluated separately for each family using the kinship matrices K_{s_k} for each family, where k is from 1 to N_s and s_k is the dimension of square matrix K_{s_k}.

For family-based designs such as related pairs (siblings or twins) the blocks are the same and the block matrix K has the form.

$$K = \begin{pmatrix} K_s & 0 & \dots & 0 \\ 0 & K_s & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & K_s \end{pmatrix} \tag{S13}$$

The matrix K_s for sibling pairs is:

$$K_s = \begin{pmatrix} 1 & 0.5 \\ 0.5 & 1 \end{pmatrix}$$

The matrix K_s for monozygotic twins is:

$$K_s = \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix}$$

In a general case, we consider s related pairs with the relatedness coefficients r, where s is a positive integer and r is from 0 (unrelated) to 1 (monozygotic twins).

$$K_s = \begin{pmatrix} 1 & r & \dots & r \\ r & 1 & \dots & r \\ \dots & \dots & \dots & \dots \\ r & r & \dots & 1 \end{pmatrix} \tag{S14}$$

Eigenvalues of K_s

Let λ_i denote s eigenvalues of K_s matrix in Equation (S14). These eigenvalues can be analytically calculated by representing the matrix K_s as a weighted sum of two matrices (one of which is a diagonal matrix).

$$K_s = \begin{pmatrix} 1 & r & \dots & r \\ r & 1 & \dots & r \\ \dots & \dots & \dots & \dots \\ r & r & \dots & 1 \end{pmatrix} = \begin{pmatrix} r & r & \dots & r \\ r & r & \dots & r \\ \dots & \dots & \dots & \dots \\ r & r & \dots & r \end{pmatrix} + \begin{pmatrix} 1-r & 0 & \dots & 0 \\ 0 & 1-r & \dots & 0 \\ \dots & \dots & \dots & \dots \\ 0 & 0 & \dots & 1-r \end{pmatrix}$$

If λ_1 is the first eigenvalue and λ_{-1} are the remaining $(s-1)$ eigenvalues, then we use the results in Equation S7 and obtain.

$$\begin{aligned} \lambda_1 &= rs + (1-r) \\ \lambda_{-1} &= 0 + (1-r) = 1-r \end{aligned} \tag{S15}$$

Therefore, the sum of eigenvalues of K_s is further simplified.

$$\sum_{i=1}^{s} \lambda_i = \lambda_1 + (s-1)\lambda_{-1} = [rs + (1-r)] + [(s-1)(1-r)]$$

Related pairs	No. pairs, s	Relatedness, r	First eigenvalue, λ_1	Other eigenvalues, λ_{-1}
Monozygotic twins	s	1	s	0
Siblings	s	1/2	(s+1)/2	1/2
Cousins	s	1/4	(s+3)/4	3/4

Table S1: The eigenvalues of the matrix K_s (a submatrix of K) with respect to the relatedness distribution.

Computing γ_β for the related pairs through EVD of K_s

To further simplify the analytical form of multiplier in Equation (S12), we reformulate it using the block-wise representation of K given in Equations (S13) and (S14).

$$f(\sigma_a^2, \lambda) = \frac{1}{s} \sum_{i=1}^{s} \frac{\lambda_i}{(\lambda_i - 1)\sigma_a^2 + 1} \tag{S16}$$

Finally, we get the result in Equation (31) of the main text by summing the weighted eigenvalues of K_s derived in (S15). Here we write the Equation (31) again.

$$\begin{aligned} \gamma_\beta(\text{Related pairs}) &= \frac{1}{s}\left(\frac{rs+1-r}{(rs+1-r)\sigma_a^2 + \sigma_r^2} + \frac{(s-1)(1-r)}{(1-r)\sigma_a^2 + \sigma_r^2}\right) \\ &= \frac{1}{s}\left(\frac{rs+1-r}{(rs-r)\sigma_a^2 + 1} - \frac{(s-1)(1-r)}{(r\sigma_a^2 + 1}\right) \\ &= \frac{1}{s}\left(\frac{(s-1)r+1}{(s-1)r\sigma_a^2 + 1} - \frac{(s-1)(1-r)}{r\sigma_a^2 + 1}\right) \end{aligned}$$

Minima of the function $\gamma_\beta(\sigma_a^2)$

To minimize the function f from Equation (S16) with respect to σ_a^2 and get its extrema, we only have to find the solution:

$$\frac{\partial f(x,\lambda)}{dx} = 0$$
$$-\frac{1}{s}\sum_{i=1}^{s}\frac{\lambda_i^2-\lambda_i}{((\lambda_i-1)x+1)^2} = 0$$

Note that $f(0,\lambda) = f(1,\lambda) = 1$ except for the case of twins. For the case of twins, we have $f(0,(s,0,\ldots,0)) = 1$ and $\lim_{x\to 1} f(x,(s,0,\ldots,0)) = \frac{1}{s}$.

Case 1: Siblings (s, r = 1/2) The solution is:

$$\begin{aligned}
&\frac{(\frac{s+1}{2})^2 - \frac{s+1}{2}}{((\frac{s+1}{2}-1)x+1)^2} = (s-1)\frac{1}{4(1-\frac{1}{2}x)^2}\\
&\frac{s^2-1}{((s-1)x+2)^2} = \frac{s-1}{(2-x)^2}\\
&(s-3)sx^2 + 8sx - 4s = 0\\
&x = \frac{2(\sqrt{s+1}-2)}{s-3} \text{ if } s \neq 3\\
&x = \frac{1}{2} \text{ if } s = 3
\end{aligned} \tag{S17}$$

Case 2: Cousins (s, r = 1/4) The solution is:

$$x = \frac{4(s-1)(\sqrt{3(s+3)}-4)}{3s^2-10s+7} \tag{S18}$$

Case 3: Twins (s, r = 1) Because only one eigenvalue is not null, the derivative of function f with respect to σ_a^2 is:

$$\frac{\partial f(\sigma_a^2,\lambda)}{d\sigma_a^2} = -\frac{1}{s}\frac{s^2-s}{((s-1)\sigma_a^2+1)^2} < 0 \tag{S19}$$

The monotonic decrease of the function $\gamma_\beta(\sigma_a^2)$ for twins is observed on Supplementary Figure S7.

S1.3 The relationship matrices K, K_D and K_I

To study the gene-environment interaction effect δ on a quantitative trait y, Equation (8) in the main text outlines the model, $y \sim \mathcal{N}(w\beta + d\tau + v\delta, \Sigma_y)$, where y, w, d are observed $N \times 1$ vectors of the standardized trait, genetic variant and exposure, respectively; v is a vector of the interaction between the genetic variant and exposure obtained by element-wise multiplication of w and d; β, τ, δ are the effect sizes; Σ_y is the $N \times N$ covariance

matrix of trait across N individuals. The matrix $\Sigma_v \equiv K_D$ is a covariance matrix of the interaction variable v.

Table 2 in the main text, according the ref. [2], suggests to include two types kinship matrices K and K_I into Σ_y: $\Sigma_y = \sigma_a^2 K + \sigma_{ai}^2 K_I + \sigma_r^2 I$. Inclusion of the matrix K_I controls for the family structure when testing for the gene-environment interaction effect and protects from spurious associations (false positives).

Overall, the relationship matrices K, K_D and K_I define the behavior of test statistic in association studies of gene-environment interactions. So we would like to show how these matrices look like for a particular example of the nuclear family.

Nuclear Families

Consider a single nuclear family of 5 individuals, 2 parents and 3 offspring. The kinship matrix K is:

$$K = \begin{pmatrix} 1 & 0 & 0.5 & 0.5 & 0.5 \\ 0 & 1 & 0.5 & 0.5 & 0.5 \\ 0.5 & 0.5 & 1 & 0.5 & 0.5 \\ 0.5 & 0.5 & 0.5 & 1 & 0.5 \\ 0.5 & 0.5 & 0.5 & 0.5 & 1 \end{pmatrix}$$

Consider next a binary environmental exposure d, which is drawn such that the first two individuals (parents) are unexposed and the last three individuals (offspring) are exposed to the environment. Thus, the frequency of binary exposure is $f = 0.6$.

$$d = \begin{pmatrix} 0 & 0 & 1 & 1 & 1 \end{pmatrix}$$

The matrix K_I is computed by element-wise multiplication of K and a special masking matrix M, which defines whether a pair of individuals have the same exposure status (d).

$$M = \begin{pmatrix} 1 & 1 & 0 & 0 & 0 \\ 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 1 \end{pmatrix}$$

$$K_I = M \circ K = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0.5 & 0.5 \\ 0 & 0 & 0.5 & 1 & 0.5 \\ 0 & 0 & 0.5 & 0.5 & 1 \end{pmatrix}$$

Equations (20) and (21) in the Methods section of main text introduce the matrices E, D and K_D. These matrices are further used to derive the test statistic in association studies of gene-environment interactions. See Equations (32) and (33) for the result of derivation.

We next show how the matrices E, D and K_D look like for our example of nuclear family and binary exposure. One can see further that the matrices are scaled by factor

$f(1-f)$, because the genetic and environmental exposure variables are standardized according to our association model, Equation (8).

The matrix E is simply defined as $E = diag(d)$.

$$E = \frac{1}{\sqrt{f(1-f)}} \begin{pmatrix} -f & 0 & 0 & 0 & 0 \\ 0 & -f & 0 & 0 & 0 \\ 0 & 0 & (1-f) & 0 & 0 \\ 0 & 0 & 0 & (1-f) & 0 \\ 0 & 0 & 0 & 0 & (1-f) \end{pmatrix}$$

The matrix D is defined using the fact that $D_{i,j} = E_{i,i}E_{j,j}$ for i, j from 1 to N.

$$D = \frac{1}{f(1-f)} \begin{pmatrix} f^2 & f^2 & -f(1-f) & -f(1-f) & -f(1-f) \\ f^2 & f^2 & -f(1-f) & -f(1-f) & -f(1-f) \\ -f(1-f) & -f(1-f) & (1-f)^2 & (1-f)^2 & (1-f)^2 \\ -f(1-f) & -f(1-f) & (1-f)^2 & (1-f)^2 & (1-f)^2 \\ -f(1-f) & -f(1-f) & (1-f)^2 & (1-f)^2 & (1-f)^2 \end{pmatrix}$$

Further simplifying the notation, we obtain.

$$D = \begin{pmatrix} f/(1-f) & f/(1-f) & -1 & -1 & -1 \\ f/(1-f) & f/(1-f) & -1 & -1 & -1 \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \end{pmatrix}$$

The matrix K_D, which is the covariance matrix Σ_v of the interaction variable v in Equation (8), has the form.

$$\Sigma_v = K_D = D \circ K = \begin{pmatrix} f/(1-f) & 0 & -0.5 & -0.5 & -0.5 \\ 0 & f/(1-f) & -0.5 & -0.5 & -0.5 \\ -0.5 & -0.5 & (1-f)/f & 0.5(1-f)/f & 0.5(1-f)/f \\ -0.5 & -0.5 & 0.5(1-f)/f & (1-f)/f & 0.5(1-f)/f \\ -0.5 & -0.5 & 0.5(1-f)/f & 0.5(1-f)/f & (1-f)/f \end{pmatrix}$$

For illustration purposes, we replace f by its value 0.6.

$$E = \frac{1}{\sqrt{0.24}} \begin{pmatrix} -0.6 & 0 & 0 & 0 & 0 \\ 0 & -0.6 & 0 & 0 & 0 \\ 0 & 0 & 0.4 & 0 & 0 \\ 0 & 0 & 0 & 0.4 & 0 \\ 0 & 0 & 0 & 0 & 0.4 \end{pmatrix}$$

$$D = \frac{1}{0.24} \begin{pmatrix} 0.36 & 0.36 & -0.24 & -0.24 & -0.24 \\ 0.36 & 0.36 & -0.24 & -0.24 & -0.24 \\ -0.24 & -0.24 & 0.16 & 0.16 & 0.16 \\ -0.24 & -0.24 & 0.16 & 0.16 & 0.16 \\ -0.24 & -0.24 & 0.16 & 0.16 & 0.16 \end{pmatrix}$$

$$\Sigma_v = K_D = \frac{1}{0.24}\begin{pmatrix} 0.36 & 0 & -0.12 & -0.12 & -0.12 \\ 0 & 0.36 & -0.12 & -0.12 & -0.12 \\ -0.12 & -0.12 & 0.16 & 0.08 & 0.08 \\ -0.12 & -0.12 & 0.08 & 0.16 & 0.08 \\ -0.12 & -0.12 & 0.08 & 0.08 & 0.16 \end{pmatrix}$$

Some elements of K_D are negative, because the genetic and environmental exposure variables are standardized in Equation (8). Figure (3)c in the main text depicts these negative elements by gray color.

Unrelated individuals

We can check whether our family-based derivations of the relationship matrices K, K_D and K_I are consistent with the case of unrelated individuals, for which the kinship matrix is the identity matrix, $K = I$.

The vector d and matrix D are the same for unrelated individuals, but the covariance matrix has a simpler form, $\Sigma_v = diag(D)$.

$$d = \begin{pmatrix} 0 & 0 & 1 & 1 & 1 \end{pmatrix}$$

$$D = \begin{pmatrix} f/(1-f) & f/(1-f) & -1 & -1 & -1 \\ f/(1-f) & f/(1-f) & -1 & -1 & -1 \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \\ -1 & -1 & (1-f)/f & (1-f)/f & (1-f)/f \end{pmatrix}$$

$$\Sigma_v = D \circ I = diag(D) = \begin{pmatrix} f/(1-f) & 0 & 0 & 0 & 0 \\ 0 & f/(1-f) & 0 & 0 & 0 \\ 0 & 0 & (1-f)/f & 0 & 0 \\ 0 & 0 & 0 & (1-f)/f & 0 \\ 0 & 0 & 0 & 0 & (1-f)/f \end{pmatrix}$$

Further, we expect the multiplier γ_δ from Equation (33) to be one for unrelated individuals. Since we have $\Sigma_y = \sigma_r^2 I = I$; $\sigma_r^2 = 1$, we need to show that $tr(\Sigma_v) = N$ for unrelated individuals.

$$\gamma_\delta \approx \frac{tr(\Sigma_y^{-1}(\Sigma_v))}{N} = \frac{tr(diag(D)}{N} = \frac{(1-f)Nf/(1-f) + fN(1-f)/f}{N} = 1$$

S1.4 Data simulations

In the power analysis of testing the marginal genetic effect, we simulate a trait with mean $\mathbb{E}(y) = \beta_g x_g$ on allelic (unstandardized) scale: the allelic effect size $\beta_g = 0.05$ and genetic variant x_g has entries 0, 1, and 2 (the minor allele frequency $p = 0.3$). The effect

size $\beta_g = 0.05$ is allelic and corresponds to β_{allele} in Equation (S20). The genetic variant explains $\approx 0.1\%$ of the trait variance.

Similarly in the power analysis of testing the gene-environment interaction effect, we simulate a trait with mean $\mathbb{E}(y) = \beta_g x_g + \beta_e x_e + \beta_{ge} x_g * x_e$ on unstandardized scale. The genetic variant x_g has entries 0, 1, and 2 (the minor allele frequency $p = 0.3$). The binary exposure x_e has entries 0 and 1 with the exposure frequency $f = 0.6$. All the effect sizes, including the main genetic β_g, main environmental β_e and interaction β_{ge}, are equal to 0.1. The gene-environment interaction (standardized) effect explains $\approx 0.1\%$ of the trait variance.

In simulations of unrelated individuals under the polygenic model [3], we set the number of individuals to $N = 1,000$, the number of genetic variants to $M = 2,000$ and the number of causal variants to either $M_c = 200$ (default) or $M_c = 50$. We generate M bi-allelic genetic variants with the minor allele frequency $p = 0.5$, standardize them and store in a $N \times M$ matrix W. We then generate a vector of the genetic effect sizes b (M_c causal variants) from the normal distribution $\mathcal{N}(0, (\sigma_g^2/M_c)I)$, where $\sigma_g^2 = 0.8$ denotes the heritability. Finally, the trait is simulated as $y = Wb + \epsilon$, where the residual noise comes from the normal distribution $\mathcal{N}(0, (1 - \sigma_g^2)I)$. In the next step of fitting LMM to the simulated data, we first construct the GRM using either all or the top associated variants (the LR test statistics), then estimate the variance components, in particular σ_g^2, by REML [3] and finally compute the LMM test statistic. We note that we might not fully recover the true heritability (≈ 0.8, $M_c = 200$) given that the sample size of the simulated datasets is relatively small ($N = 1,000$).

The standardized and allelic effect sizes

We use the standardized marginal genetic effect size β and the standardized gene-environment interaction effect δ in our data simulations and real data analysis of the UK Biobank. The relation to the allelic effect sizes can be derived through the minor allele frequency of the genetic variant, p, and, for example, the frequency of the binary environmental exposure, f [4, Appendix B].

$$\beta = 2p(1-p)\beta_{allele} \tag{S20}$$

$$\delta = 2p(1-p)f(1-f)\delta_{allele} \tag{S21}$$

The variance explained by the genetic variant and gene-environment interaction variable is readily expressed through the standardized effect sizes, β^2 and δ^2, respectively.

S1.5 Simulation results for the Unrelated+GRM scenario

Before studying the relative power between the Unrelated and Unrelated+GRM scenarios on simulated data, we sought to examine the impact of several LMM configurations that differ by the variant selection for the GRM.

As described in the Methods section of the main text, we simulated a trait under the polygenic model on $N = 1,000$ unrelated individuals, $M = 2,000$ (unlinked) genetic

variants and $M_c = 200$ causal variants that explain 80% of the trait variance (the heritability is denoted with σ_g^2). We first performed association study by the LR model, from which we ranked variants by their association statistic. We then examined three sets of $M_s = 200$ selected variants ($M_s = M_c$): the random variants (Random), the top LR-based associated variants (Top) and the causal variants (Causal).

When fitting the LMM to estimate the heritability, we observed that the three models revealed different estimates of the heritability, $\hat{\sigma}_g^2$: 9% for the Random set, 65% for the Top set and 80% for the Causal set. The accuracy of recovering the true heritability was driven by the sample size, N, the number of selected variants for the GRM, M_s and the number of causal variants captured by the GRM (Supplementary Figure S11). For the three LMM configurations in Figures S9 and S10 discussed below, the number of causal variants included in GRM was equal to 22 for Random, 90 for Top and 200 for Causal.

We then examined how the LMM configurations with different sets of selected variants influence the estimation of the effective size multiplier, γ_β. The LMM association statistics and the multiplier were computed by plugging the estimated heritability, $\hat{\sigma}_g^2$, and the trait covariance, $\hat{\Sigma}_y$, into Equations 3, 4 and 30. Figure S9a shows that the effective size multiplier γ_β, derived using the proposed analytical formulation, accurately approximated the empirical ratios between LR and LMM squared standard errors. Importantly, the approximation worked equally well for all three LMM configurations with different estimates of the heritability and the trait covariance matrix.

We next evaluated the performance of the empirical effective size multiplier γ_β^s [5]. Figure S9b shows that the accuracy of the empirical multiplier γ_β^s was variable across LMM configurations and dependent of sets of variants used to compute the ratios of the test statistics. Given that the choice of the top variants is subjective, we explored two approaches in each LMM configuration: significant variants ($P < 1 \times 10^{-5}$ in LMM) and top variants (significant in LMM, $P < 1 \times 10^{-5}$, and nominally significant in LR, $P < 0.05$).

For the first LMM configuration with the random variants in the GRM (the left panel in Figure S9b), the multiplier γ_β^s is trivially equal to one (LMM $\approx$ LR), because most of the random variants were null and explained nearly zero heritability. For the second LMM configuration with the top associated variants in the GRM (the middle panel in Figure S9b), the empirical multiplier γ_β^s is consistently lower than the effective sample size multiplier γ_β. The reason for this mismatch can be explained by the composition of the top associated statistic for γ_β^s: almost a half of variants are null with the ratios of the test statistic expected to be ones. For the last LMM configuration with all causal variants in the GRM (right panel of Figure S9b), the empirical multiplier γ_β^s largely overestimated γ_β for the set of top associated *causal* variants. There were particular causal variants with the low effect sizes (Supplementary Figure S12), which were significant only in the LMM: the residual variance was remarkably reduced, as $\approx$ 80% heritability was explained by the GRM. This overestimation was partially mitigated if nominally insignificant variants in LR ($P > 0.05$) were filtered out.

If one uses median instead of mean to estimate the ratio of the test statistic for γ_e, then the estimator is less affected by the outlier variants, which were nominally insignificant in the LR model with $P_{LR} > 0.05$ (see Supplementary Figure S10).

S2 Supplementary Tables

Relationship	ϕ	Inference criteria
Monozygotic twin	$\frac{1}{2}$	$\phi > \frac{1}{2^3}$
Parent–offspring	$\frac{1}{4}$	$\phi \geq \frac{1}{2^{5/2}}$ & $\phi < \frac{1}{2^{3/2}}$ & IBS0 ≤ 0.0012
Full sibling	$\frac{1}{4}$	$\phi \geq \frac{1}{2^{5/2}}$ & $\phi < \frac{1}{2^{3/2}}$ & IBS0 > 0.0012
2nd Degree	$\frac{1}{8}$	$\phi \geq \frac{1}{2^{7/2}}$ & $\phi < \frac{1}{2^{5/2}}$
3rd Degree	$\frac{1}{16}$	$\phi \geq \frac{1}{2^{9/2}}$ & $\phi < \frac{1}{2^{7/2}}$

Table S2: The criteria for inference of the pairwise relationships based on the estimated kinship coefficients (ϕ), as recommended by the authors of KING; see Table 1 in ref. [6]. The IBS0 coefficients are additionally used to distinguish between parent–offspring and full-sibling pairs [7]. These two types of related pairs have the same expected kinship coefficient 1/4, and any such pair with IBS0 ≤ 0.0012 is called parent-offspring.

Relationship	No. pairs	No. individuals	γ_β*	σ_a^{2*}
Monozygotic twin	179	358	0.500	1.000
Parent–offspring	6,273	11,202	0.922	0.560
Full sibling	22,664	41,512	0.929	0.531
2nd Degree	11,115	20,196	0.982	0.511
All above (<2nd Degree)	40,231	68,910	0.939	0.537

Table S3: The relative power of GWAS in related samples (up to the second degree) from the UK Biobank. *The last two columns report the minimum value of the ESS multiplier γ_β across the range of heritability (σ_a^2) values, [0, 1].

S3 Supplementary Figures

References

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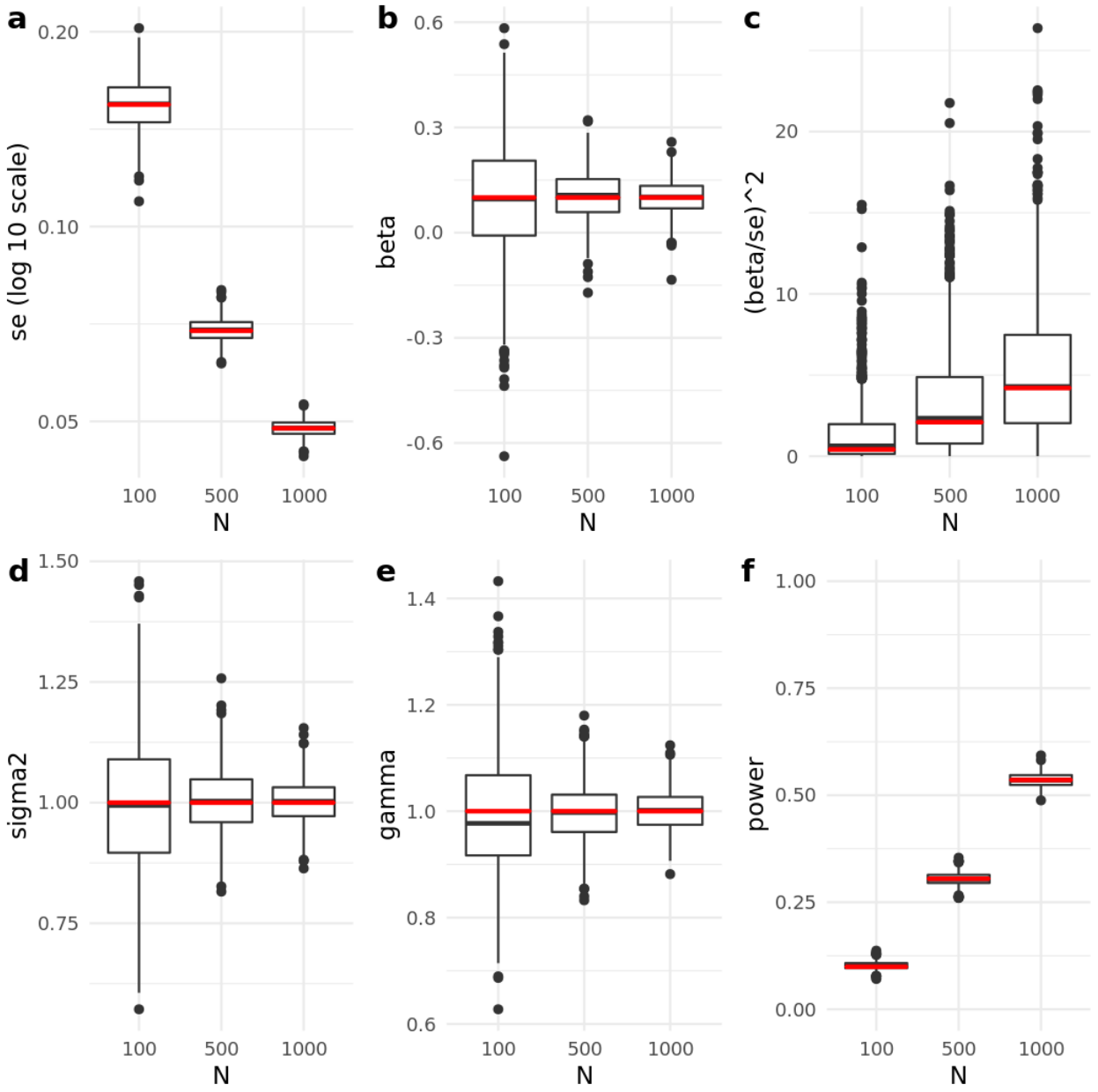

Figure S1: The results of simulations for testing the marginal genetic effect in unrelated individuals by linear regression (LR). A quantitative trait was simulated 1,000 times at each value of the sample size N according to a data model $y \sim \mathcal{N}(w\beta, \Sigma_y = \sigma_r^2 I)$ (see Supplementary Material). The same model was used for association testing. Boxplots at panels (a-d) show the distribution of standard error of $\hat{\beta}$, $\hat{\beta}$, χ^2 statistic and $\hat{\sigma}_r^2$, respectively; true values of model parameters are depicted by red lines. Panel (e) shows the distribution of empirical ESS multiplier estimated as $1/[var(\hat{\beta})N]$ (see Equation (17)) for every simulation; the red lines correspond to the analytical multiplier $\gamma_\beta = tr(\sigma_r^2 I)/N = 1$ calculated with true model parameters used to simulate data (see Equation (27)). Panel (f) reports observed power at the nominal level of $\alpha = 0.05$, where each point is a simulation with 1,000 variants. The total number of points for each value of N is also 1,000, and the red lines give analytical estimates of the expected power.

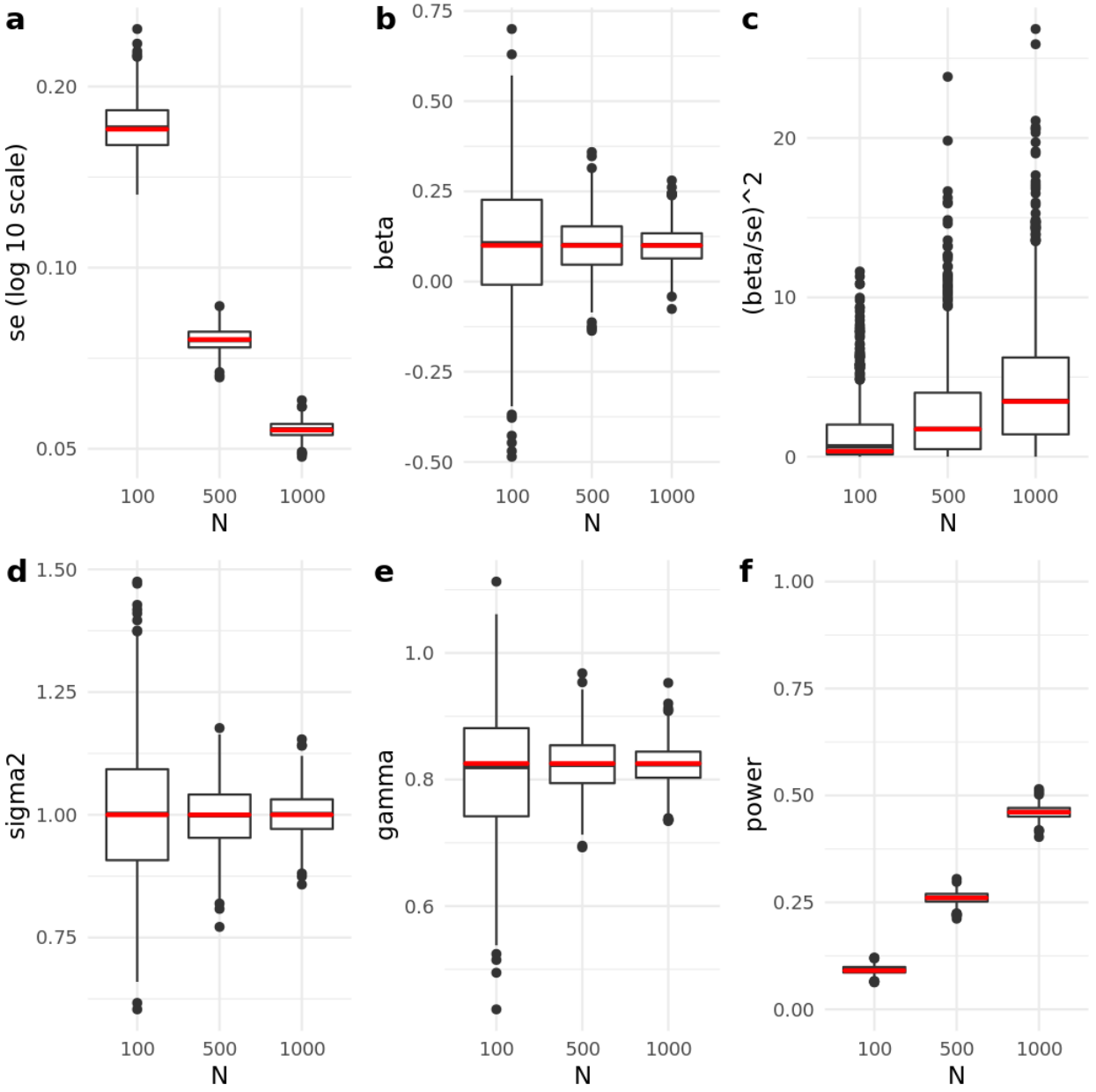

Figure S2: The results of simulations for testing the marginal genetic effect in related individuals (nuclear families of two parents and three offspring) by linear mixed model (LMM).

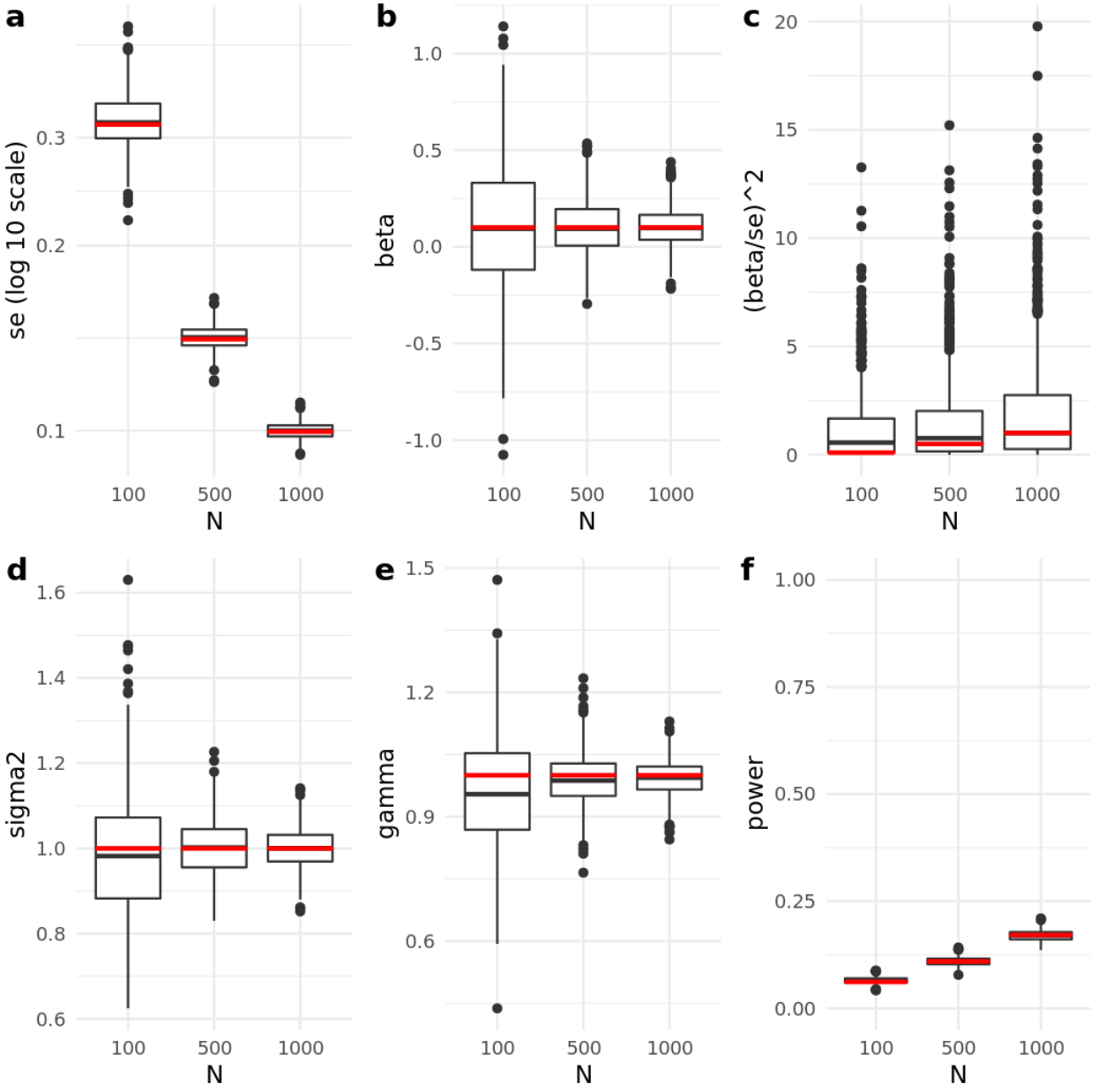

Figure S3: The results of simulations for testing the gene-environment interaction effect in unrelated individuals by linear regression (LR).

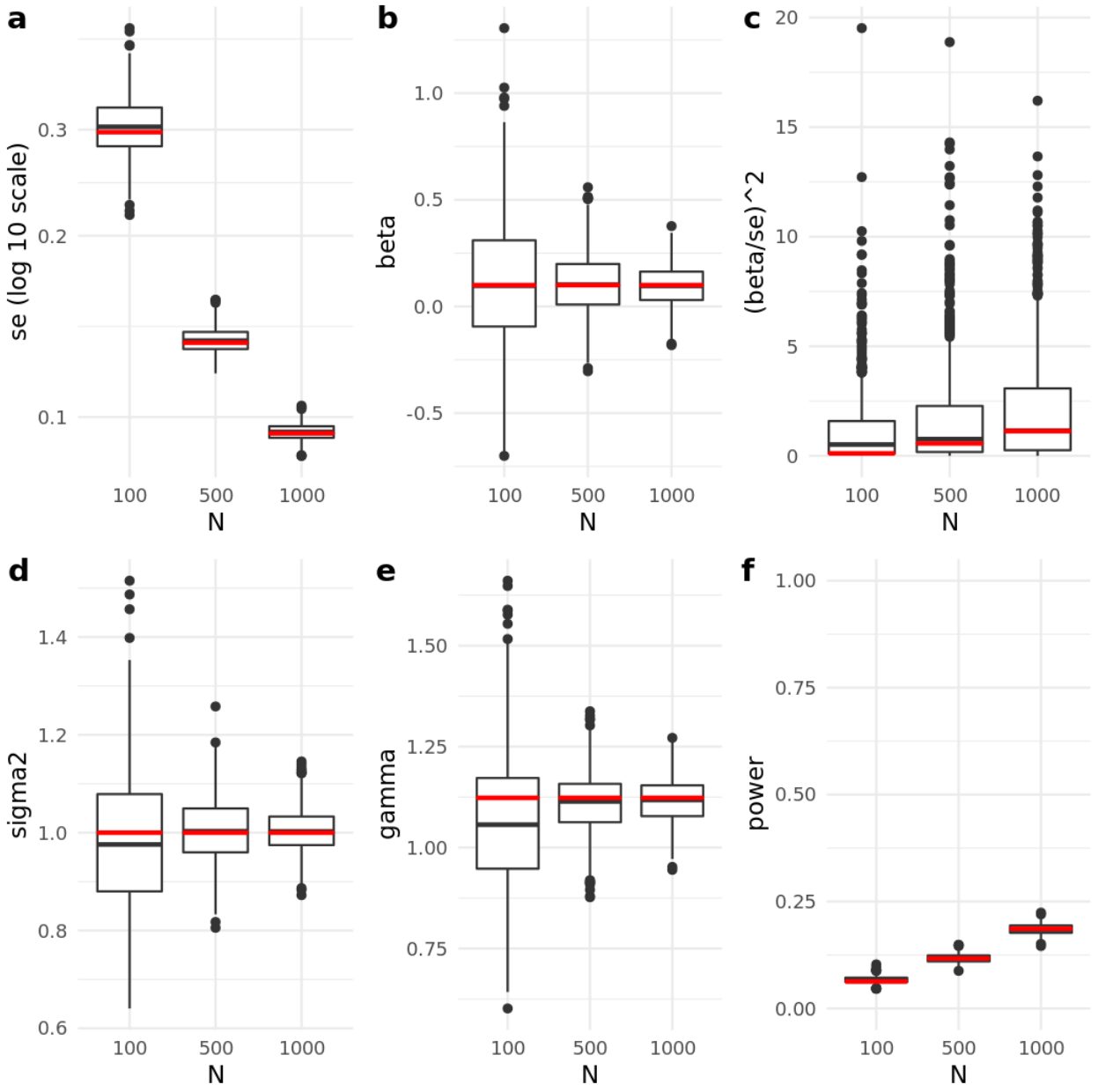

Figure S4: The results of simulations for testing the gene-environment interaction effect in related individuals by the linear mixed model (LMM) with two genetic variance components.

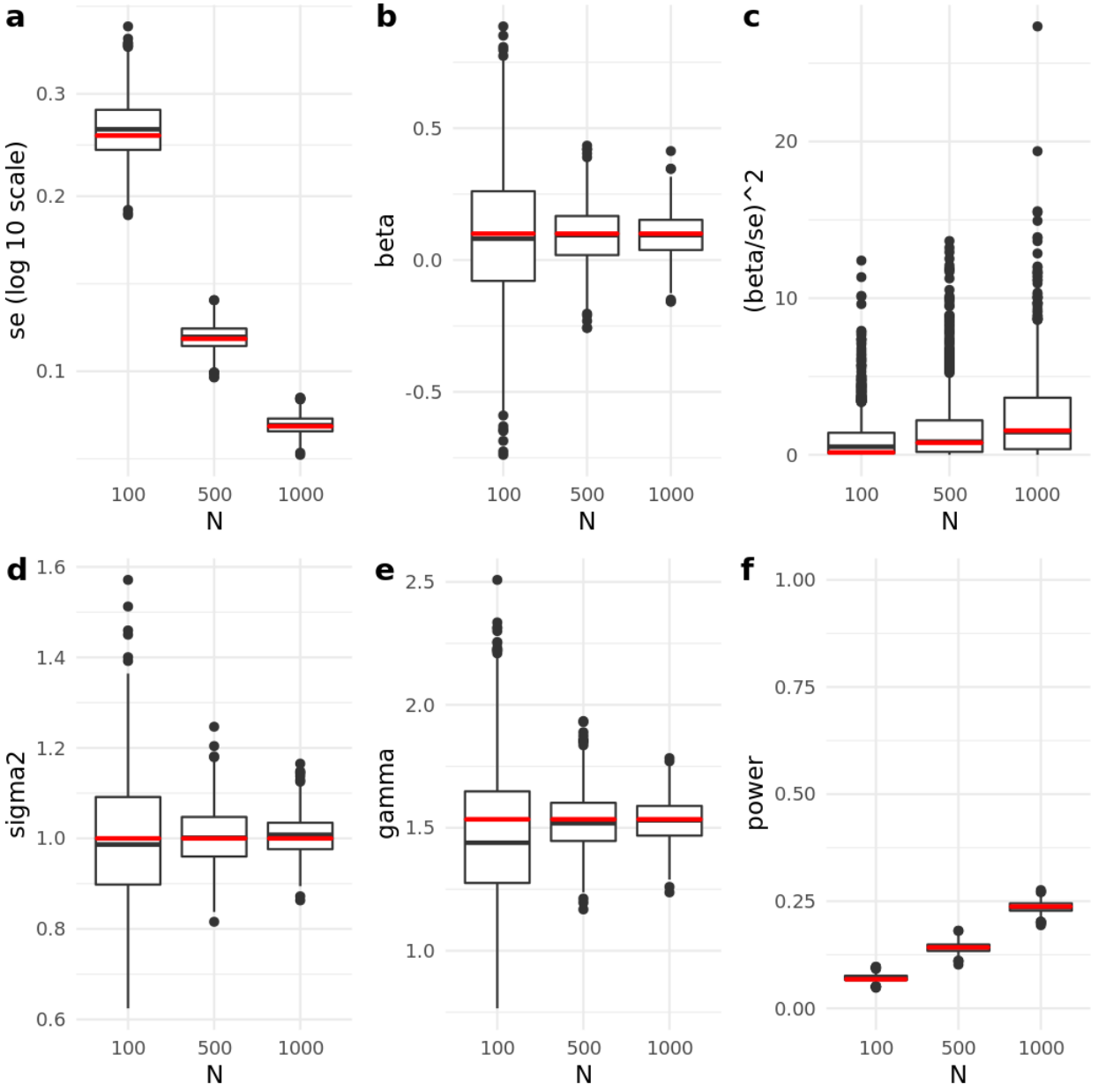

Figure S5: The results of simulations for testing the gene-environment interaction effect in related individuals by the linear mixed model (LMM) with one genetic variance component.

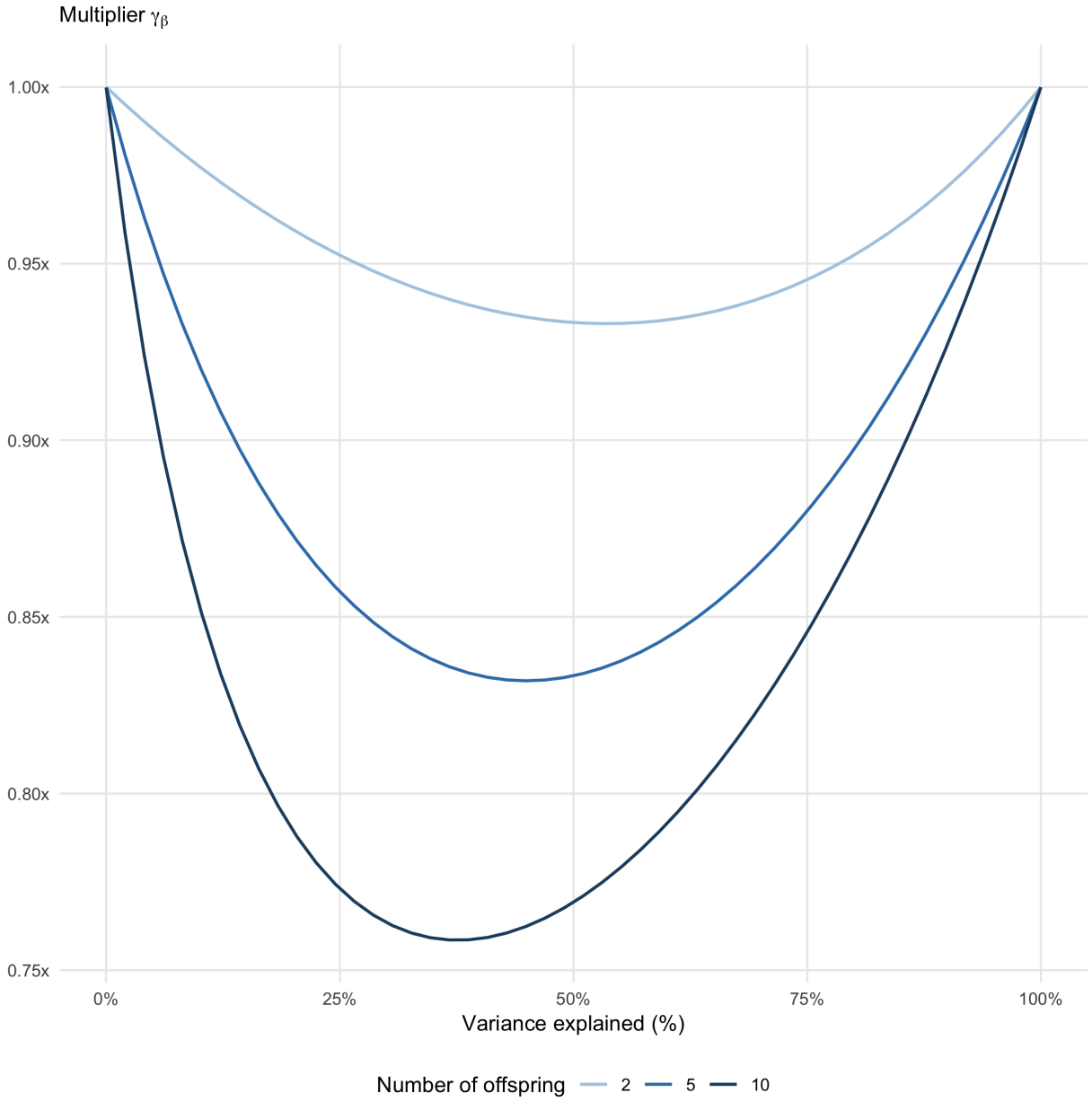

Figure S6: The effective size multiplier γ_β, analytically computed for nuclear families (2 parents and offspring), varies with the proportion of variance explained by family relationships (heritability σ_a^2) and family structure (the number of offspring in nuclear families).

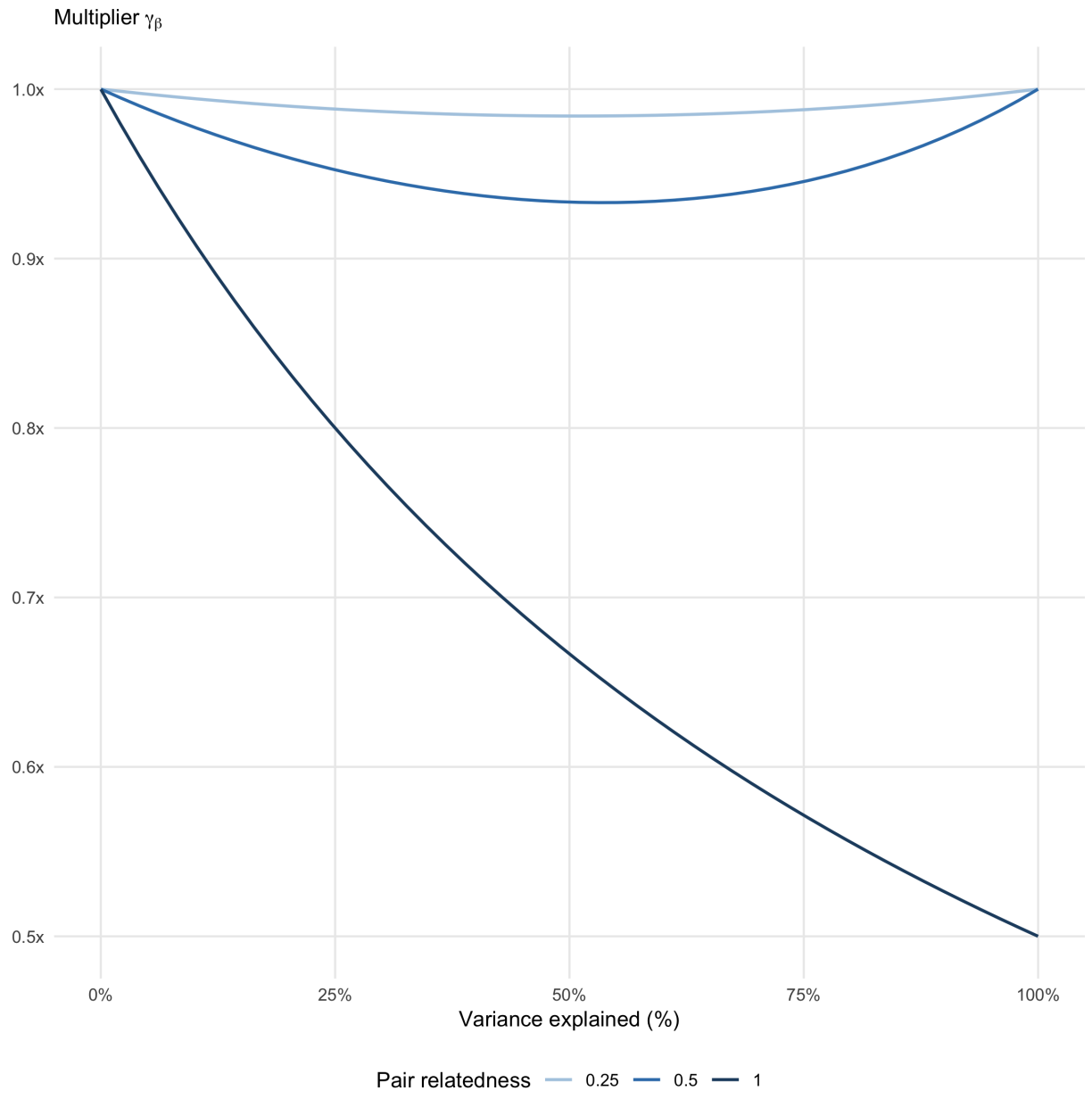

Figure S7: The effective size multiplier γ_β, analytically computed for related pairs, varies with the proportion of variance explained by family relationships (heritability σ_a^2) and family structure (pair relatedness). The relatedness for different pairs (the double kinship coefficient): 0.125 for cousins, 0.5 for siblings, and 1 for monozygotic twins.

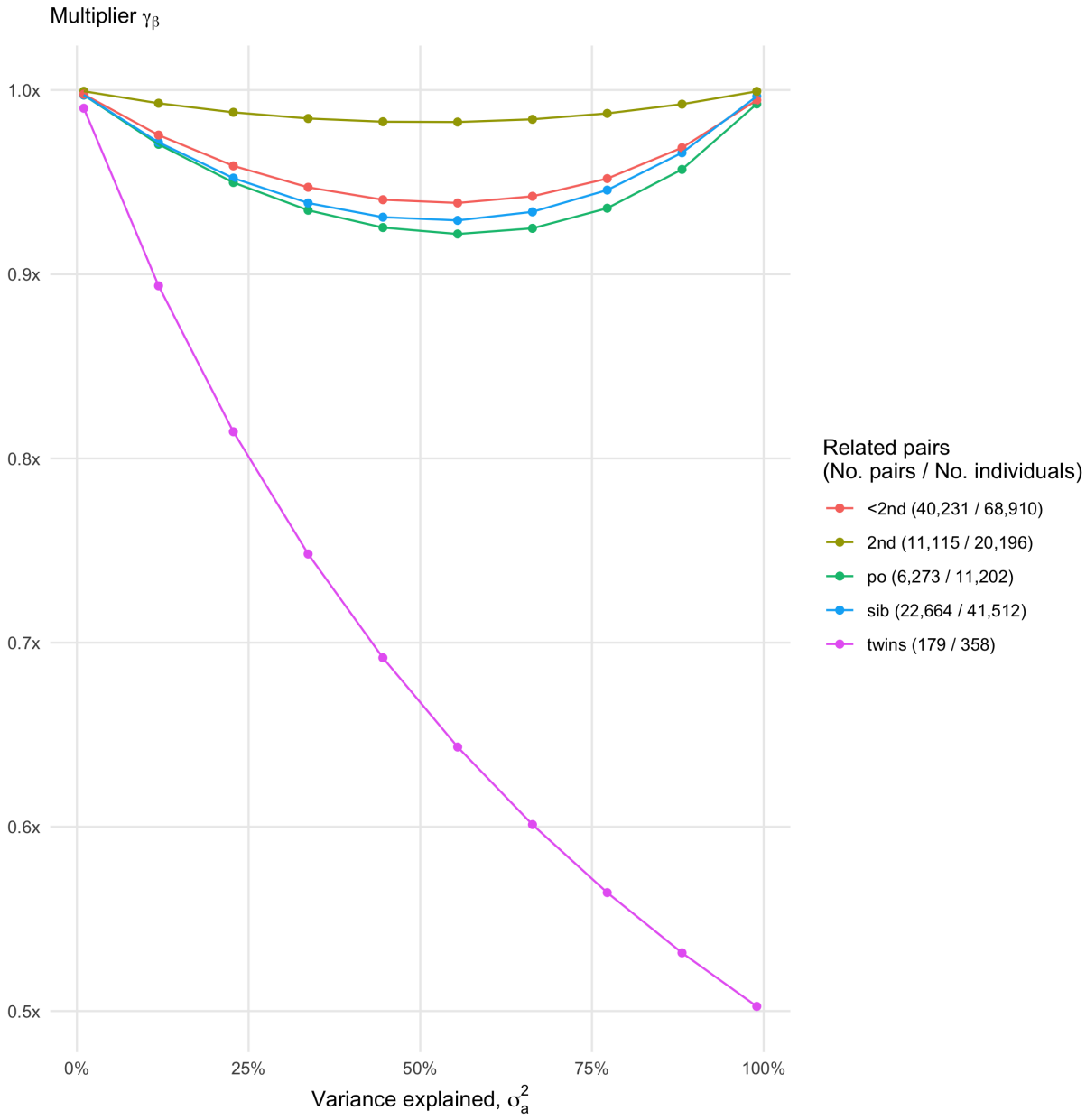

Figure S8: The effective size multiplier γ_β is analytically estimated in 68,910 UK Biobank unrelated individuals (up to 2nd degree; see Supplementary Table S3). The multiplier is a function of the variance explained by family relationships (heritability σ^2_a) and the strength of genetic relatedness (twins, monozygotic twins: 1; sib, sibling pairs: 0.5; po, parent-offspring pairs: 0.5; 2nd, 2nd-order relatives: 0.125).

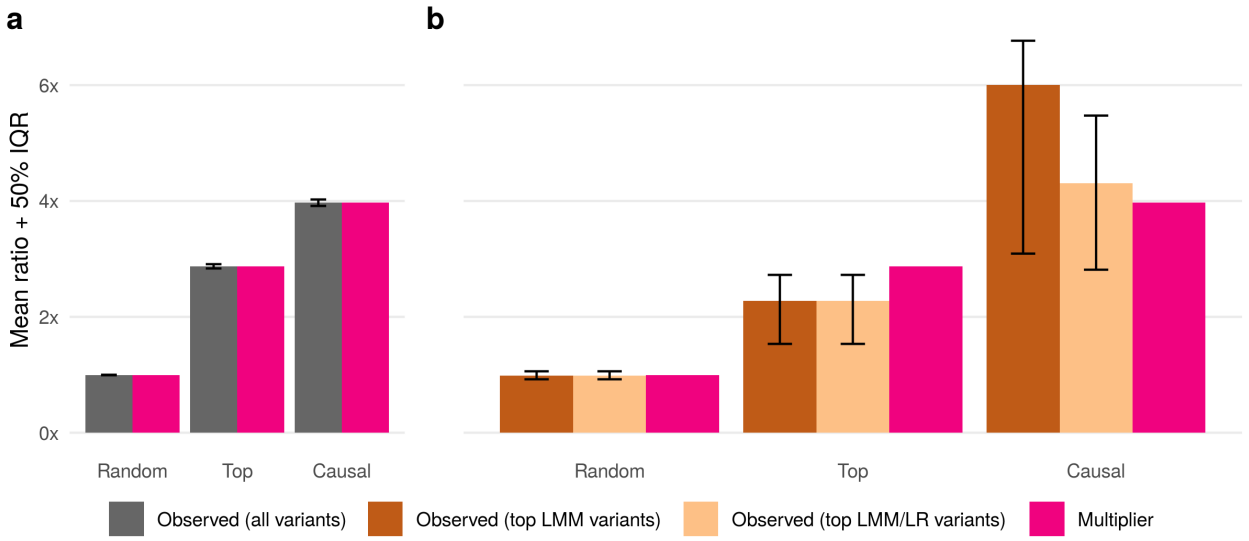

Figure S9: Validation of the analytical multiplier γ_β on simulated data under Unrelated+GRM and Unrelated scenarios (N = 1,000, M = 2,000, M_c = 200 and M_s = 200 (see Supplementary Material). Three sets of M_s = 200 variants are selected to build GRM in LMM: random variants (Random), top LR top associated variants (Top) and causal variants (Causal). (a) The effective size multiplier γ_β (red bars) accurately approximates empirical ratios of squared standard errors (dark gray bars) for every set of M_s variants used in GRM. (b) The empirical multiplier γ_e^s (brown and beige bars) is computed at different sets of variants: significant variants ($P_{LMM} < 1 \times 10^5$ in LMM) and top variants (significant in LMM, $P_{LMM} < 1 \times 10^5$, and nominally significant in LR, $P_{LR} < 0.05$). The multipliers γ_e^s and γ_β match well only in the trivial case when random variants in GRM capture nearly zero heritability (Unrelated $\approx$ Unrelated+GRM). Otherwise, γ_e^s gives biased estimates. Heights of dark gray, brown and beige bars represent mean values, while error bars range from 1st to 3rd quartiles. The multiplier γ_e^s on panel (b) is not reported for sets of all variants (dark gray bars on panel (a)), because the mean statistic is not robust to outliers, which are causal variants with low effect sizes (significant in LMM and insignificant in LR). See also Supplementary Figure S10 for reported median ratios and Supplementary Figure S12 for distribution of tests statistics at causal variants, including causal variants with low effect sizes.

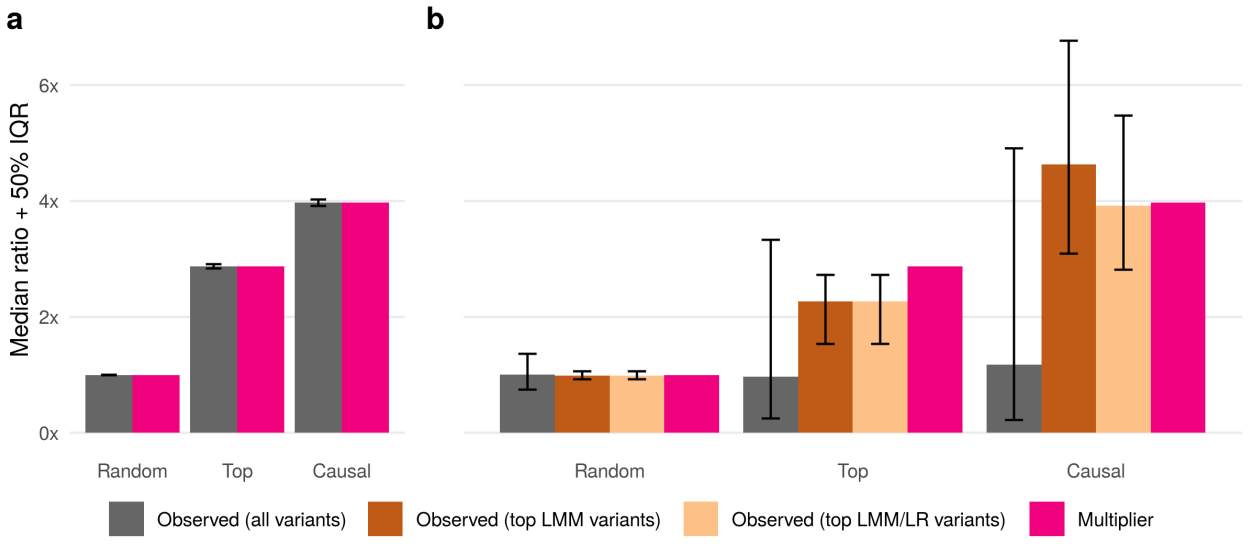

Figure S10: Results on simulated data, reported in Figure S9, using the median rather than mean in computing (a) ratios of squared standard errors and (b) ratios of squared test statistic.

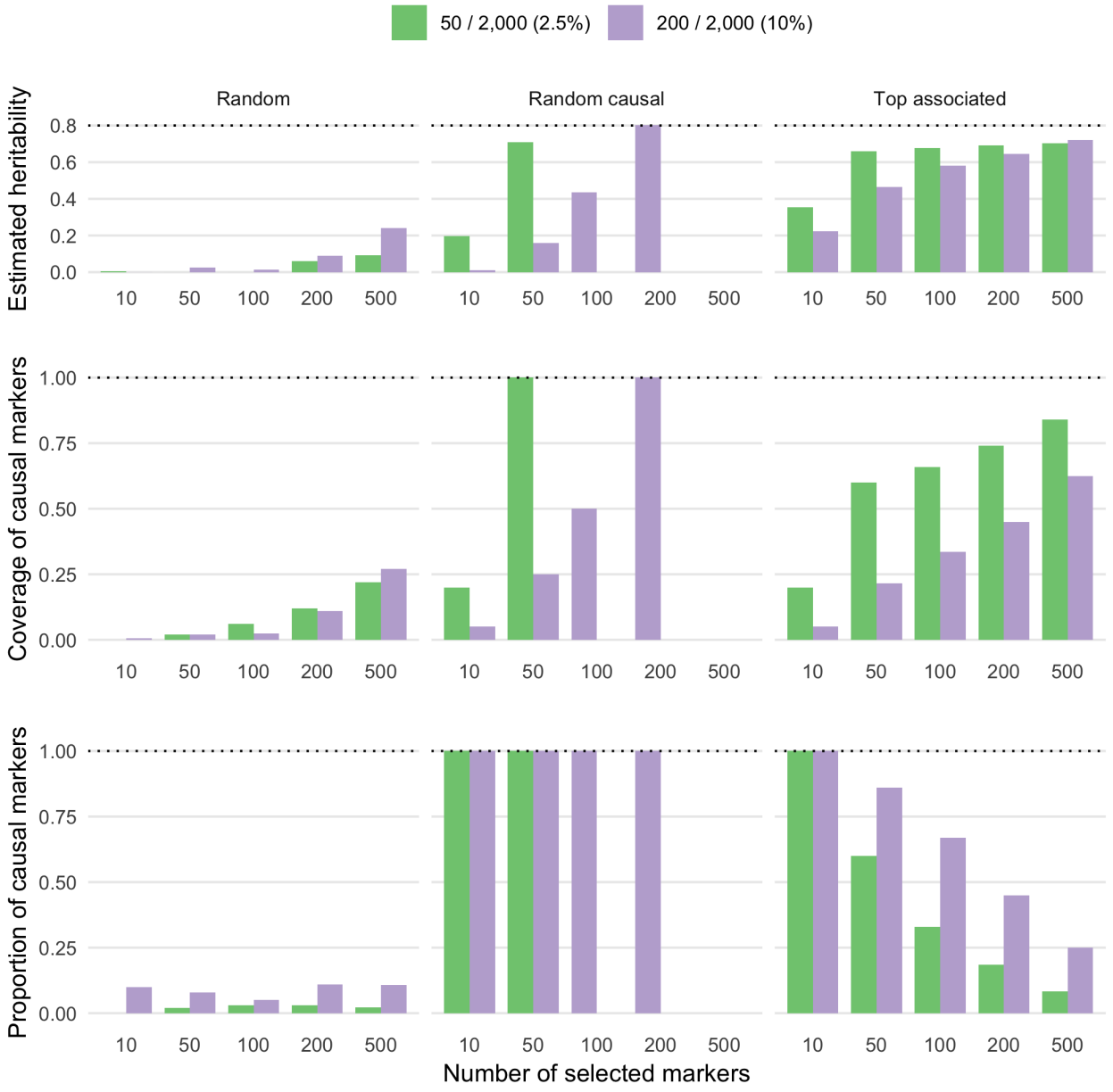

Figure S11: Estimated heritability is reported on simulated data under Unrelated+GRM scenario (N = 1,000, M = 2,000, M_c = 50, 200 and M_s = 10, 50, 100, 200, 500 (see Supplementary Material). Three sets of M_s variants are selected to build GRM in LMM: random variants (Random), random causal variants (Causal) and top LR top associated variants (Top).

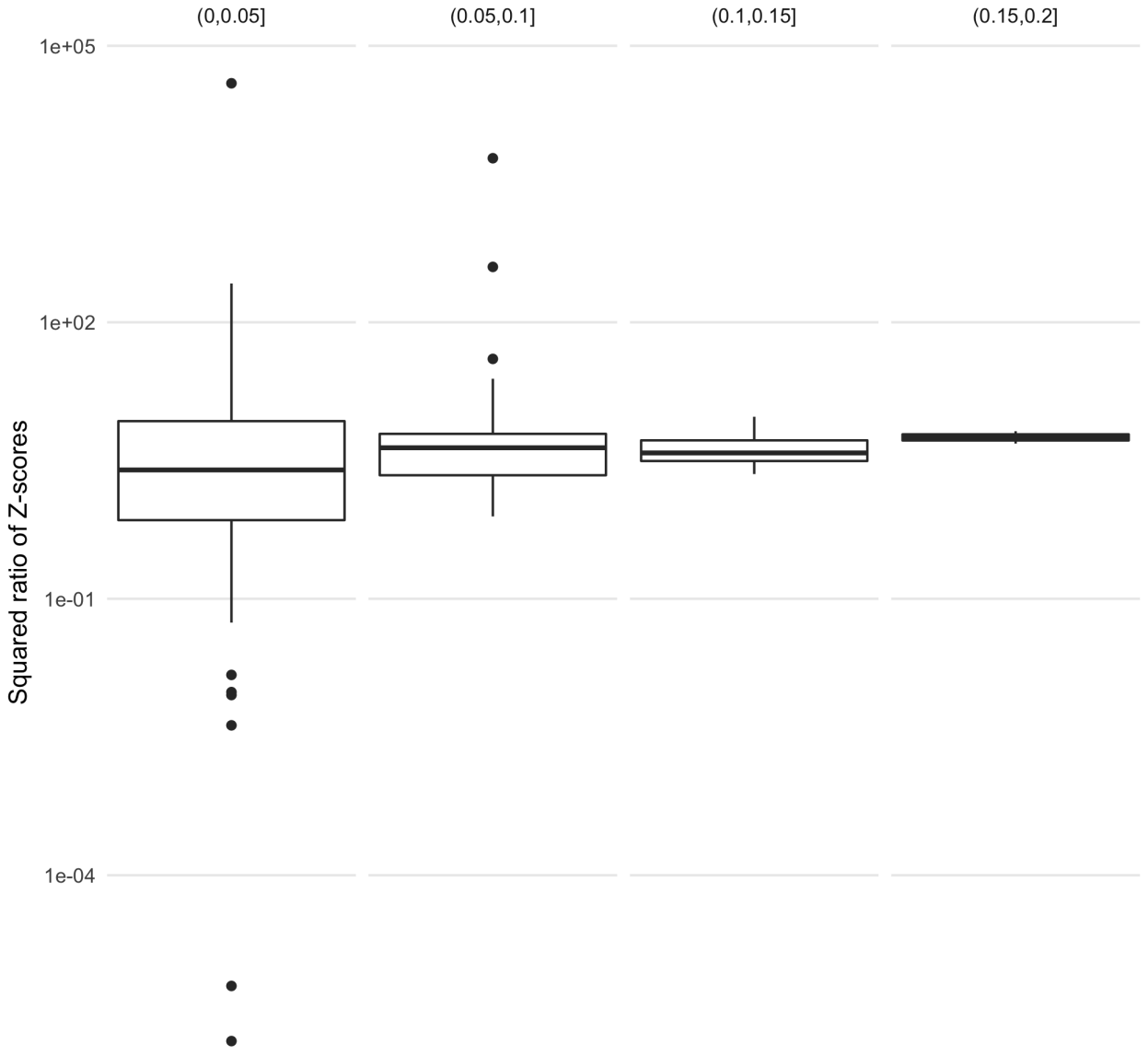

Figure S12: Distribution of LMM and LR test statistics (Z-scores) on simulated data (N = 1,000, M = 2,000, M_c = 200 and M_s = 200 (see Supplementary Material). Ratios of association statistics LMM/LR between Unrelated and Unrelated+GRM scenarios are computed at causal variants and stratified by the effect sizes. Outlier points above the 75% quantile of box plots correspond to causal variants with low effect sizes that are insignificant in LR, but become significant in LMM. These particular variants inflates the empirical multiplier γ_e^s computed on a set of causal variants (Figure S9). All causal variants are included in GRM when producing LMM test statistics.

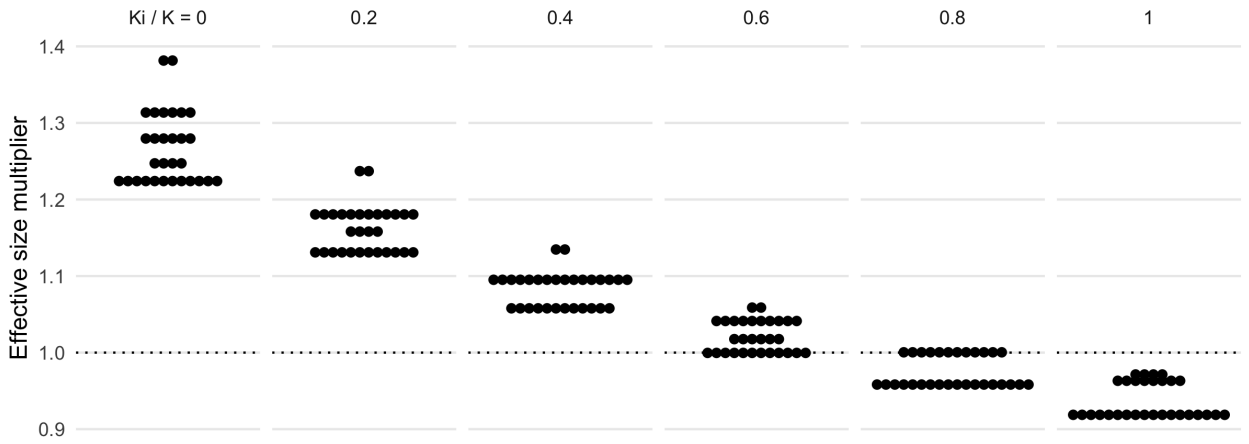

Figure S13: The effective size multiplier for gene-environment interaction effect γ_δ is analytically computed for nuclear families with 2 parents and 3 offspring. All possible realizations of a binary exposure within a family are considered, that results in different exposure frequencies. The distribution of γ_δ is shown as a dotplot for six combinations of variance components in LMM. Recall that the association model to test the gene-environment interaction effect δ is: $y \sim \mathcal{N}(w\beta + d\tau + v\delta, \Sigma_y = \sigma_a^2 K + \sigma_{ai}^2 K_I + \sigma_r^2 I)$. Each panel has its own ratio σ_{ai}^2/σ_a^2, for instance, $\sigma_{ai}^2 = 0$ on the left panel and $\sigma_{ai}^2 = \sigma_a^2$ on the right panel.

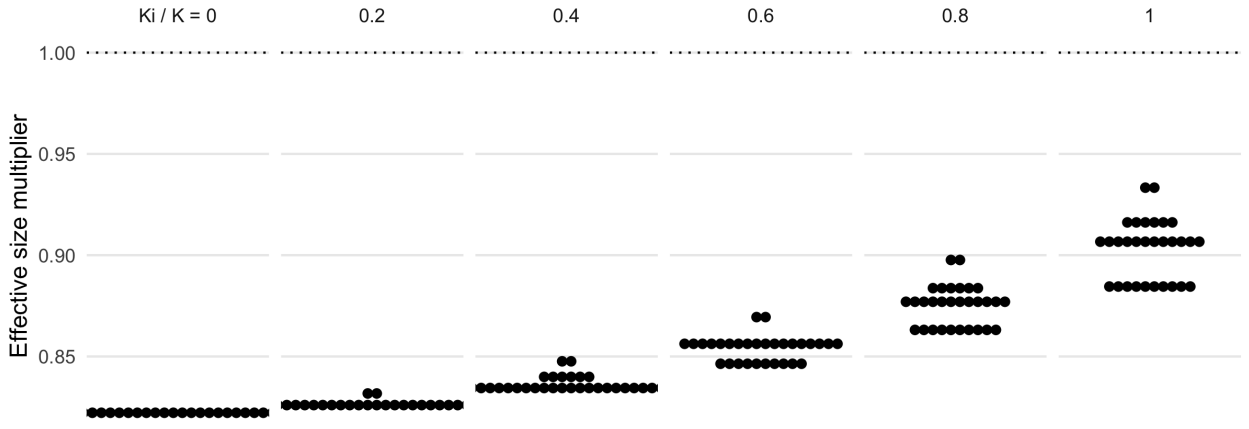

Figure S14: The effective size multiplier for genetic effect γ_β (rather than γ_δ for gene-environment interaction effect) is analytically estimated on the same nuclear family data as in Figure S13.

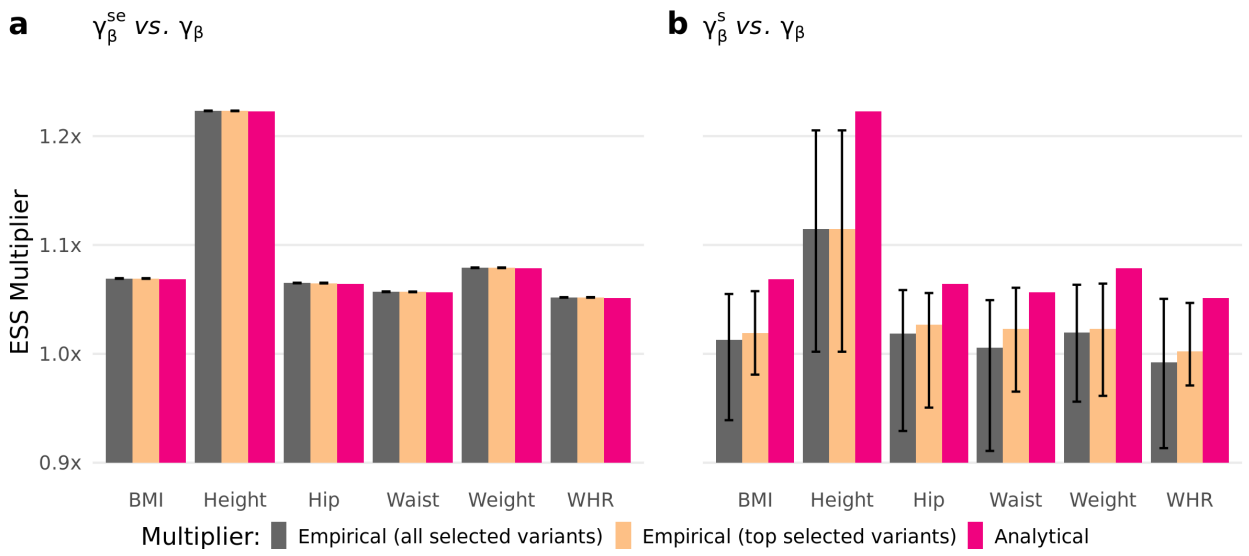

Figure S15: This supplementary figure shows the same results as the main Figure 2 but is based on association test statistics from only Chromosome 1. Those per-trait top 1,000 variants from Chromosome 1 are excluded from the GRM in low-rank LMM (consequently, the estimated heritability used to compute γ_β is smaller than in Figure 2), and association test statistics and standard errors used to compute the empirical estimators γ_β^{se} and γ_β^{s} is available only from Chromosome 1.