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ARTICLE

Detection of gene–environment interaction in pedigree data using genome-wide genotypes

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Heritability may be estimated using phenotypic data collected in relatives or in distantly related individuals using genome-wide single nucleotide polymorphism (SNP) data. We combined these approaches by re-parameterizing the model proposed by Zaitlen *et al* and extended this model to include moderation of (total and SNP-based) genetic and environmental variance components by a measured moderator. By means of data simulation, we demonstrated that the type 1 error rates of the proposed test are correct and parameter estimates are accurate. As an application, we considered the moderation by age or year of birth of variance components associated with body mass index (BMI), height, attention problems (AP), and symptoms of anxiety and depression. The genetic variance of BMI was found to increase with age, but the environmental variance displayed a greater increase with age, resulting in a proportional decrease of the heritability of BMI. Environmental variance of height increased with year of birth. The environmental variance of AP increased with age. These results illustrate the assessment of moderation of environmental and genetic effects, when estimating heritability from combined SNP and family data. The assessment of moderation of genetic and environmental variance will enhance our understanding of the genetic architecture of complex traits.
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INTRODUCTION

Gene–environment (GxE) interaction is an important issue in genetics with potentially important empirical implications.[1] In human genetics, the moderation of genetic effects by environmental risk factors has been considered with respect to, for example, psychiatric disorders (for reviews see refs 2–6), transcriptomics,[7] and body mass index (BMI).[8,9] GxE interaction studies have often been criticized for lack of statistical power,[10] poor choice of candidate genes, or genetic markers,[11,12] and poor replication.[13] To judge whether GxE interaction studies may inform complex trait genetics, and may play a role in explaining missing heritability,[5] more knowledge is required about the extent to which GxE interaction contributes to phenotypic variance. Studies that employ genetically informative designs (eg, the twin design),[14,15] and studies including genome-wide genotype data provide a means to evaluate GxE interaction effects. One example of the latter approach involves establishing whether the effect of a polygenic risk score is moderated by an environment variable.[16–18] Such polygenic scores are usually based on a weighed linear combination of single-nucleotide polymorphisms (SNPs) which satisfy some significance level in a GWAS. An alternative approach to quantify the effect of a set of measured SNPs on a phenotype is to fit a random effects model using genetic relatedness matrix restricted maximum likelihood (GREML), as implemented in the GCTA software package.[19] GCTA was developed to estimate 'chip based' heritability in large groups of distantly related individuals, and allows estimation of GxE interaction, given a dichotomous environmental exposure. Vinkhuyzen and Wray[16] recently discussed the current options for GxE interaction research with dichotomous exposures, both for polygenic risk scores and GCTA analyses.

Here, we propose a model that can include a continuous, ordinal, or nominal moderator of genetic and environmental variance components. In addition, we extend the model to inclusion of data of closely related subjects, such as twin pairs, or members of extended pedigrees. To this end, we re-parameterized the model proposed by Zaitlen *et al.*[1] This allows an evaluation of GxE interaction in terms of the moderation of the genetic variance attributable to the measured SNPs, the total additive genetic variance, and the residual (environmental) variance. Including distantly related and closely related subjects increases the power to resolve the moderator's effect on genetic and environmental variance components.

We conducted simulation studies to investigate the performance of the model. First, we simulated data given several null hypotheses to establish that the type-1 error rates for the significance tests used are correct. Second, we established that the estimated variance components were accurate when phenotypic data are normally distributed. GREML is usually based on the assumption of phenotypic multivariate normality. Speed *et al.*[20] previously tested the performance of the model given non-normality due to non-normally distributed environmental effects, they reported little evidence for bias. Here we explored the effects of phenotypic positive skewness on the type-1 error rates and parameter estimates. Positive skewness is often observed in test scores obtained from questionnaires used to asses psychopathology due to an abundance of items with low endorsement rates. We applied our model to BMI and height, and to attention problems (AP) and anxious depression (AnxDep). We estimate the contribution of common genetic variants, all genetic effects, and the environment subject to moderation by age

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or birth year (for height). We considered birth year the appropriate moderator for height as the variance in adult height does not change with age. The largest variance in adult height is attributable to the mean increase in height throughout the 20th century. The distributions of BMI and height are normal, whereas AP and AnxDep are positively skewed.

METHODS

Statistical methods

Genome-wide genetic similarities between individuals based on measured SNPs can be used to estimate the variance attributable to these measured SNPs (for the derivation, see ref. 19). The associated model is provided in Equations 1a–1d.

$$Y = X\beta + Wu + e \tag{1a}$$

$$Y \sim N(\mu, V) \tag{1b}$$

$$V = \mathrm{GRM}_{n*n} * \sigma^2_{\mathrm{snp}} + I_{n*n} * \sigma^2_e \tag{1c}$$

$$\mu = X\beta \tag{1d}$$

In Equation 1a, Y (n × 1) is a random vector of phenotypic scores as observed in n individuals, X (n × m) is the matrix of m fixed covariates, and β (m × 1) is the vector of fixed effects. The matrix W (n × p) is the matrix of p standardized SNPs, u (p × 1) is a zero mean vector of random effects (regression coefficients), and e is the n × 1 vector of zero mean residuals. The phenotype Y is assumed to be a random multivariate normal vector with mean vector μ and covariance matrix V (Equation 1b). The GRM is a matrix of pairwise genetic similarities computed as WW'/p. The parameter σ^2_{snp} is an estimate of the variance explained by the SNPs included in W and SNPs in strong LD with SNPs included in W and I is an identity matrix. The GCTA software can be used to fit the model above, with extensions allowing for dichotomous GxE moderation, among other options.[19]

The continuous moderation model

The continuous moderation model allows the magnitude of the variance components to vary with respect to a moderator M. Parameter β_{snp} quantifies the effect of moderation by M of the variance explained in Y by common SNPs. Parameter σ_{snp} quantifies the effect of measured SNPs on Y given $\beta_{snp} = 0$. Parameter β_e quantifies the effect of moderator M of the residual variance of Y. Parameter σ_e quantifies the effect of residual variance given $\beta_e = 0$.

$$\begin{aligned} V(Y|M) = V_{n*n} = {} & (\sigma_{snp} + \beta_{snp}.M_{1*n})^t * (\sigma_{snp} + \beta_{snp}.M_{1*n}).\,\mathrm{GRM}_{n*n} \\ & + (\sigma_e + \beta_e.M_{1*n})^t * (\sigma_e + \beta_e.M_{1*n}).\,I_{n*n} \end{aligned} \tag{2}$$

In Equation 2, '*' indicates a matrix product and '.' indicates element-wise multiplication. This model allows for moderation of the genetic and residual (co)variances by M. Equation 2 is a variation on the moderation model introduced by Purcell in the context of twin studies (2002).[14] Note that we assume that the moderator M is also included in the matrix X, that is, as a fixed covariate with a main effect on the phenotype. We have presented the moderator M as continuous, but it may be discrete (ordinal or nominal). Given a binary moderator M, coded $\sqrt{0.5}$ and $-\sqrt{0.5}$, the model in Equation 2 is equivalent to the GxE approach used in GCTA.

Related individuals in the sample

It is a standard practice in GCTA to exclude genetically closely related individuals to avoid confounding of the total heritability and the SNP heritability. An extension proposed by Zaitlen *et al.*[1] allows for inclusion of closely related individuals to estimate the variance due to the SNPs, as well as the total additive genetic variance. In Equation 3 below, the matrix $\mathrm{GRM}^{\mathrm{IBS}}$ is equivalent to the GRM in Equations 1 and 2, but now includes closely related individuals. Closely related in our analysis is defined as genetic relatedness greater than 0.05, as in Zaitlen *et al.*[1] The matrix $\mathrm{GRM}^{\mathrm{IBS}>0.05}$ equals the matrix $\mathrm{GRM}^{\mathrm{IBS}}$ in which all relatedness coefficients below 0.05 set to zero. Note that the values of these coefficients in closely related individuals tend towards the expected proportion of alleles shared identically by decent (~IBD; we denote the expected proportion with pi-hat) (ie, full siblings are characterized by pi-hat = 0.5 IBD, and ~0.5 in the $\mathrm{GRM}^{\mathrm{IBS}>0.05}$). Using an IBD matrix or IBS > 0.05 matrix yields very similar results.[1]

$$V_{n*n} = \mathrm{GRM}^{\mathrm{IBS}}_{n*n} * \sigma^2_{snp} + \mathrm{GRM}^{\mathrm{IBS}>0.05}_{n*n} * (\sigma^2_{\sim\mathrm{IBD}} - \sigma^2_{\mathrm{snp}}) + I_{n*n} * \sigma^2_e \tag{3}$$

In Equation (3), parameter σ^2_{snp} reflects the variance explained by SNPs, the term $(\sigma^2_{\sim\mathrm{IBD}} - \sigma^2_{\mathrm{snp}})$ represents the difference between the total additive genetic variance $\sigma^2_{\sim\mathrm{IBD}}$ and the variance explained by SNPs, and σ^2_e reflects the variance attributable to residual effects. Inspection of the parameter correlation matrix derived from the Hessian revealed very strong negative parameter correlations between σ^2_{snp} and $(\sigma^2_{\sim\mathrm{IBD}} - \sigma^2_{\mathrm{snp}})$, which complicates any moderation of these terms. To ensure low-parameter correlations, and to allow for separate moderation of σ^2_{snp} and $\sigma^2_{\sim\mathrm{IBD}}$, we re-parameterized the Zaitlen *et al.* model (Equation 3) as shown in Equation 4.

$$V_{n*n} = \mathrm{GRM}^{\mathrm{IBS}<0.05}_{n*n} * \sigma^2_{\mathrm{snp}} + \mathrm{GRM}^{\mathrm{IBS}>0.05}_{n*n} * \sigma^2_{\sim\mathrm{IBD}} + I_{n*n} * \sigma^2_e \tag{4}$$

In Equation 4, the first GRM, $\mathrm{GRM}^{\mathrm{IBS}<0.05}_{n*n}$ includes values only for pairs of distantly related individuals with IBS < 0.05, whereas the other values including the diagonal elements are set to zero. This provides an estimate of variance attributable to SNPs exclusively based on the covariance between distantly related individuals. The second GRM, $\mathrm{GRM}^{\mathrm{IBS}>0.05}_{n*n}$ contains only values for pairs of individuals that are closely related, with IBS > 0.05, reflecting all genetic variance as a function of approximate IBD. Note that this model requires the presence of closely related individuals to reliably estimate $\sigma^2_{\sim\mathrm{IBD}}$. The re-parameterized model (Equation 4) and the Zaitlen *et al.* model (Equation 3) produce the same estimates of $\sigma^2_{\sim\mathrm{IBD}}, \sigma^2_{\mathrm{snp}}$, and σ^2_e. This equivalence was established empirically by simulating data for a wide range of $\sigma^2_{\sim\mathrm{IBD}}, \sigma^2_{\mathrm{snp}}$, and σ^2_e, under the Zaitlen *et al.* model, and subsequently fitting both models, and obtaining the same −2*log-likelihood and parameter estimates (Supplementary Table S1). We note that in the unlikely scenario that $\sigma^2_{\mathrm{snp}} = 0$ or $(\sigma^2_{\sim\mathrm{IBD}} - \sigma^2_{\mathrm{snp}}) = 0$, the equivalence does not hold. However, if $\sigma^2_{\mathrm{snp}} = 0$ or $(\sigma^2_{\sim\mathrm{IBD}} - \sigma^2_{\mathrm{snp}}) = 0$ is true, separate moderation of σ^2_{snp} or $\sigma^2_{\sim\mathrm{IBD}}$ is undesirable given the absence of the variance component to be moderated. Furthermore, note that σ^2_{snp} is not added to the total variance of a trait. This is due to our reparameterization of the Zaitlen *et al.* model, given that we estimate σ^2_{snp} in the matrix diagonal and entries for covariance between closely related subjects is set to 0, σ^2_{snp} no longer influences the total phenotypic variation.

We extended Equation 4 to include moderation, as shown in Equation 4a. Parameter $\beta_{\sim\mathrm{IBD}}$ in Equation 4a reflects the change in additive genetic variance as a function of the moderator M. Parameter β_{snp} reflects moderation of genetic variance attributable to SNPs as a function of M.

$$\begin{aligned} V(Y|M)_{n*n} = {} & (\sigma_{\mathrm{snp}} + \beta_{\mathrm{snp}}.M_{1*n})^t * (\sigma_{\mathrm{snp}} + \beta_{\mathrm{snp}}.M_{1*n}).\,\mathrm{GRM}^{\mathrm{IBS}<0.05}_{n*n} \\ & + (\sigma_{\sim IBD} + \beta_{\sim IBD}.M_{1*n})^t * (\sigma_{\sim\mathrm{IBD}} + \beta_{\sim\mathrm{IBD}}.M_{1*n})\,\mathrm{GRM}^{\mathrm{IBS}>0.05}_{n*n} \\ & + (\sigma_e + \beta_e.M_{1*n})^t * (\sigma_e + \beta_e.M_{1*n}).\,I_{n*n} \end{aligned} \tag{4a}$$

In fitting model 4a, we assume that we have a sufficient number of related individuals to accurately estimate $\sigma^2_{\sim\mathrm{IBD}}$. Although the main innovation presented here is continuous moderation of the variance attributable to SNPs, our model allows for moderation of the other variance components. It is important to consider moderation of all variance components. First, moderation of each of the variance components influences standardized variance components as these are expressed as a ratio. Accordingly, moderation of variance components featured in the denominator of a ratio results in moderation of the entire ratio. Second, allowing moderation of all genetic variance components yields an internally consistent model, as moderation of the variance of a trait explained by common SNPs should be accompanied by moderation of the total additive variance of the trait.

Model estimation

All models were fitted in R (Vienna, Austria) using full information maximum likelihood optimization with exact derivatives. The implementation in R includes two FORTRAN routines to speed up calculation of the likelihood function and derivatives. Optimization using exact derivatives is done using the

optim() function available in R. Scripts to perform the optimization and the required FORTRAN routines are available online (https://sites.google.com/site/mgnivard/multigrm). We tested the significance of parameters by means of the likelihood ratio test. We adopted a significance level of 0.05 for each test. The four outcome phenotypes were regressed on the first 6 principal components that reflect the population structure.[21] The standardized residuals were further analyzed to reduce the number of fixed effects in the final model, and thus reduce computational burden. Fixed effects covariates entered into all analyses were sex and standardized age, and age squared. For height, year of birth rather than age was included as a covariate.

Simulations

Type-1 error. It is well known that the distribution of the test statistic associated with bounded variance components deviate from the standard central χ^2 distribution. The approximate null distribution is a $0.5\ X^2_{df=0}/0.5\ X^2_{df=1}$ mixture distributions, but the exact distribution of the test statistic is dependent on the Eigen spectrum of the design matrix (GRM).[22] In GCTA, this 0.5:0.5 mixture distribution is used. To establish that this assumed distribution is reasonable, we simulated data sets, based on the empirical GRMs as observed in the NTR data set. First, data were simulated with $\sigma_{snp}=0$, $\sigma_{\sim IBD}=0.2$ and $\sigma_e=0.8$ and $\beta_{snp}=\beta_{\sim IBD}=\beta_e=0$, and we tested the type-1 error associated with the null hypothesis $\sigma_{snp}=0$. Assuming the mixture null distribution, we expected the type-1 error rates to be 0.025 and 0.05 (ie, the nominal significance level divided by 2). In our second scenario, we simulated a data set where: $\sigma_{snp}=0.2$, $\sigma_{\sim IBD}=0.4$ and $\sigma_e=0.6$ and $\beta_{snp}=\beta_{\sim IBD}=\beta_e=0$. Here we tested the type-1 error associated with the (omnibus) null hypotheses $\beta_{snp}=0$, $\beta_{\sim IBD}=0$ and $\beta_e=0$ at significance levels 0.1 and 0.05. The moderation parameters are not bounded and therefore should follow the standard distribution $X^2_{df=3}$. To gauge the effect of violations of phenotypic normality, the simulated normal data were transformed by mapping the data onto the empirical distribution of AnxDep scores, while retaining the rank of the data. We then repeated our test type-1 error based on the empirically distributed data.

Parameter accuracy given distributional violations

In fitting the model, we usually assume that the data are multivariate normally distributed. However, in practice data are often non-normal. To test the effects of non-normality on parameter estimates, we simulated sets of multivariate normal data, in which the SNP heritability was increased from 0 to 0.5 in 0.01 steps, and the residual heritability was increased from 0 to 0.5 in 0.01 steps for each step. Forty data sets were simulated per step. The simulated normal data were transformed by mapping the data onto the empirical distribution of AnxDep, while retaining the rank of the data. We chose the distribution of AnxDep because its distribution is typical of the distributions arising from questionnaires concerning psychopathology. That is, such data are positively skewed (the AnxDep data has a skewness of 1.62, and the AP data a skewness of 0.91). We estimated the parameters both in the normal and the skewed data, and evaluated the effects of non-normality by a regression of the estimates based on the normal data and non-normal data on the true values. Unbiased estimates will give rise to a slope parameter equal to 1. We separately tested the bias in the SNP heritability variance component, and genetic variance not attributable to SNPs.

Subjects, genotypes and measures

Phenotype data were collected from participants in the Netherlands Twin Register (NTR)[23] by mailed or online surveys, or during home visits. Adult participants received surveys in 10 consecutive waves over the past 25 years. Adolescent participants received self-report questionnaires, from the age of 14 onwards. AnxDep and AP scores were obtained from the Youth or Adult Self Report.[24] as part of the Achenbach system for empirical assessment.[24] AnxDep and AP were defined as described in previous work.[25,26] Height and BMI were assessed during a home visit for the NTR biobank projects.[27] and by self-report. AnxDep scores were available in 6881, AP scores in 6618, BMI in 6395, and height in 6409 individuals. As our simulations show that a skewed distribution influences the parameter accuracy in the proposed models, we carried out a square-root transformation of the AP and AnxDep scores to reduce the skewness of the observed distributions. AP had a skewness of 0.91 before transformation and −0.30 after square-root transformation, AnxDep had a skewness of 1.62 before transformation and 0.25 after square-root transformation. *Genotypes:* DNA samples were obtained in different projects of the NTR.[27,28] Genotyping in the different projects was performed on the Affymetrix 6.0 chip (Affymetrix, Santa Clara, CA, USA). Genotypes were called, cleaned and processed in a single pipeline to ensure consistency across projects. SNPs that were genotyped in less than 95% of individuals were removed. Individuals with a contrast quality control (CQC) score below 0.40, who had less than 90% of SNPs successfully genotyped, or had excess genome-wide heterozygosity/inbreeding levels ($F<-0.10$ or $F>0.10$) were removed. Individuals of non-European descent were excluded. The resulting sample included genotypes of 8485 individuals. A genetic relatedness matrix (GRM) was computed on the basis of all autosomal SNPs with a minor allele frequency >0.01, and Hardy–Weinberg Equilibrium test P-value $>1\times10^{-6}$ using GCTA 1.24.2.[19] Informed consent was obtained from all participants. The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB-2991 under Federal-wide Assurance-3703; IRB/institute codes, NTR 03-180).

RESULTS

Type-1 error

We simulated 1000 data sets in which the SNP-heritability variance component was zero. To establish the type-1 error rate, we tested the hypothesis that the variance component was zero using a naive chi.[2] 1) test of the likelihood ratio with significance levels set at 0.1 and 0.05, but expect type-1 error rate of 0.05 and 0.025, respectively, as the variance estimate is bounded by zero. This test produced the type-1 error rates of 0.057 and 0.029. These values are consistent with expectation, that is, they do not deviate significantly (given $\alpha=0.01$) from the expected values of 0.025 and 0.05. Type-1 error rates calculated using the skewed data produced type-1 error rates of 0.035 and 0.059, neither of which deviated significantly ($\alpha=0.01$) from the expected values of 0.025 and 0.05.

We simulated 1000 data sets in which moderation of the SNP, additive genetic, and environmental variance component was absent. We estimated a model with and without the 3 moderation parameters, and tested the likelihood ratio (a 3 df χ^2 test) using a significance levels of 0.05 and 0.10. The observed type-1 error rates were 0.047 and 0.097, which do not deviate significantly ($\alpha=0.01$) from the expected values of 0.05 and 0.10. Type-1 error rates for the omnibus test of moderation calculated using the skewed data were 0.181 and 0.266, revealing that distributional violations can result in inaccurate type-1 error rates.

Effects of the distribution on parameter estimates

We simulated 1000 data set with the SNP and additive heritable components increasing in 100 steps of 0.0033, whereas the environmental variance component decreased in 50 steps of 0.0066. We simulated 10 data sets per step. The parameter recovery was found to be good. The slope from the regression of the estimated parameter σ_{snp} on the true parameter is 1.01 (SE = 0.035) and for the regression of the estimated parameter $\sigma_{\sim IBD}$ on the true parameter the slope is 0.97 (SE = 0.012) (Supplementary Figures S1 and S2). However, the parameter estimates obtained in the analysis of the skewed data were biased. Regression of the parameter estimates on the true parameter values yielded a slope of 0.81 (SE = 0.033) for σ_{snp} and a slope of 0.84 (SE = 0.013) for $\sigma_{\sim IBD}$. Regression of the true parameter difference ($\sigma_{\sim IBD}-\sigma_{snp}$, ie the genetic variance not attributable to SNPs) on the estimated difference yielded a slope of 0.93 (SE = 0.04) for the normal data, and 0.88 (SE = 0.039) for the transformed data (Supplementary

Figure S3). We therefore conclude that the particular non-normality considered here resulted in an underestimation of the genetic variance attributable to SNPs and the genetic variance not attributable to SNPs, where the bias in the estimate of the genetic variance attributable to SNPs seemed to be greater.

Heritability and variance explained by SNPs

The variances attributable to total and SNP related genetic effects (Equation 3, see methods) on BMI, Height, AP and AnxDep are given in Table 1. All estimates are significant (P-values < 0.05) except the variance explained by SNPs in AnxDep ($P = 0.078$). SNP heritability for BMI and height were moderate and total heritability was substantial. SNP heritability of AP and AnxDep was low, and the total additive heritability was moderate. The percentage of the genetic variance of BMI, height, AP, and AnxDep explained by the SNPs was 56.2% (SE 8.6%), 59 % (SE 6.9%), 27.5% (SE 14.1%), and 24.3% (SE 14.1%), respectively. The genetic variance of AnxDep and AP may be underestimated, and type-1 error rates would be inflated due to the non-normality of the phenotypic data. As indicated in the methods, AP and AnxDep scores were square-root transformed to reduce the skewness. As the genetic variance explained by SNPs for BMI was somewhat higher than that reported in the literature.[29] we repeated the analysis for BMI in GCTA, based on data of 3119 distantly related individuals only. Here the SNP heritability estimate was 49% (SE 11.4%). Analysis carried out in GCTA based on 6395 individuals (including closely related and distantly related subjects in the GRM, and therefore estimating a variance component dominated by the closely related subjects, approximately equal to $\sigma_{\sim \mathrm{IBD}}$) resulted in a heritability estimate of 75.4% (SE 1.3%). These results are very close to those obtained with our methods.

Genetic variance moderation

Next we fitted the model given in Equation 4a (see methods) to all four variables with age or birth year as the moderator of the variance components (σ^2_{snp}, $\sigma^2_{\sim \mathrm{IBD}}$ and σ^2_e). Moderation of the variance components attributable to SNP effects were not significant for any of the phenotypes (Table 2). For BMI, the total genetic variation was significantly moderated by age. For BMI, height and AP moderation of the residual effects was significant; for AnxDep, no significant moderation by age was seen.

Figures 1a to 3a, show σ^2_{snp}, $\sigma^2_{\sim \mathrm{IBD}}$ and σ^2_e of as a function of age or birth year for BMI, height, and AP. Figures 1b–3b, show the heritability $\left(\frac{\sigma^2_{\sim \mathrm{IBD}}}{\sigma^2_{\sim \mathrm{IBD}}+\sigma^2_e}\right)$ and the proportion of phenotypic variance attributable to SNPs $\left(\frac{\sigma^2_{\mathrm{snp}}}{\sigma^2_{\sim \mathrm{IBD}}+\sigma^2_e}\right)$ as a function of age or birth year. Note the denominator in these ratios does not include σ^2_{snp}, as our model parameterization σ^2_{snp} is estimated separately from the total additive genetic variance $\sigma^2_{\sim \mathrm{IBD}}$. All figures show that the total heritability and the SNP heritability decrease as a function of age, or birth year.

DISCUSSION

We presented a variance component model which included moderation of SNP genetic, total additive genetic and residual effects. The moderator is assumed to be a measured variable, which may be continuously or discretely distributed. The model can be used in cohorts of unrelated, and/or distantly related, individuals to estimate the moderation of the genetic effects attributable to SNPs. In pedigree data, the model separately estimates the moderation of SNP effects and of total additive genetic effects. By allowing for simultaneous moderation of SNP and total additive genetic variance, we can include data from twin and family cohorts, and estimate the genetic variance attributable to SNPs, and its moderation while retaining all participants in the analyses. We tested the type-1 error associated with a χ^2 test and found that the type-1 error rate is accurate for the test of the unmoderated variance component estimate being larger than zero. For the (omnibus) test of continuous moderation of variance components, the type-1 error rate of the test was also accurate. We further investigated the effects of phenotypic non-normality on the (SNP) heritability estimates. We found that both the variance attributable to SNPs and the residual variance are underestimated by positive skewness of the phenotypic distribution. The variance attributable to SNPs was underestimated more than the additive genetic variance not captured by SNPs. Thus, our simulations showed good type-1 error rates for the χ^2 test of variance components, and good type-1 error rates for the χ^2 test of moderator effects. The type-1 error rates remained good when the data were transformed to match the empirical distribution of the AnxDep scores before square-root transformation. Type-1 error for the test of moderation was accurate if the distributional assumptions were met, violations of the distributional assumptions can induce false positive results.

The moderation models were fitted to data on BMI, height, AP, and AnxDep. Results showed differences between these phenotypes in genetic architecture that were not limited to differences in $\sigma^2_{\sim \mathrm{IBD}}$, σ^2_{snp} and σ^2_e, but also were the results of differences in the degree to which these variance components were moderated by age or year of birth. The results indicated that for AnxDep, neither the genetic nor environmental effects were moderated by age. For AP, BMI, and height the residual variance, σ^2_e, was found to increase with age or birth year. For BMI, age had a positive moderating effect on the additive genetic variance. We found no evidence of moderation of σ^2_{snp}

Table 1 Estimates of the proportion of variance attributable to SNPs, familial genetic effects, and the environment for AnxDep, AP, BMI and height

	AnxDep	*AP*	*BMI*	*Height*
Proportion of phenotypic variance explained by SNPs	9.8% NS (SE = 5.7%)	11.4%* (SE = 5.8%)	41.6%*** (SE = 6.4%)	53.8%*** (SE = 6.3%)
Proportion of genetic variance explained by SNPs	24.3% (SE = 14.1%)	27.5% (SE = 14.1%)	56.2% (SE = 8.6%)	58.9% (SE = 6.9%)
Proportion of phenotypic variance explained by additive genetic influences	40.6%*** (SE = 2.0%)	41.6%***	75.3%*** (SE = 1.3%)	91.3%*** (SE = 0.4%)
Proportion of phenotypic variance explained by residual influences	59.4% (SE = 2.0 %)	56.8% (SE = 2.0%)	24.8% (SE = 1.3%)	9.7% (SE = 0.4%)

*$P<0.05$, ***$P<0.0001$

The heritability is computed as: $\left(\frac{\sigma^2_{\sim \mathrm{IBD}}}{\sigma^2_{\sim \mathrm{IBD}}+\sigma^2_e}\right)$ and proportion of phenotypic variance attributable to SNPs is computed as: $\left(\frac{\sigma^2_{\mathrm{snp}}}{\sigma^2_{\sim \mathrm{IBD}}+\sigma^2_e}\right)$; wherein * denotes significance determined by likelihood ratio testing. SEs for the different ratios were approximated using the delta method.[36] The delta method relies on the parameter correlations found in the Hessian matrix, to obtain the variance for a function of individual parameters.

Table 2 Parameter estimates, −2 log likelihoods, and significance tests in the GxE models

	σ_{snp}	β_{snp}	σ_{IBD}	β_{IBD}	σ_e	β_e	*−2ll*	*Δ − df*	*Likelihood ratio*
AP moderated by age									
Full moderation	0.32	0.096	0.62	−0.002	0.73	0.054	17 830.78	—	—
Drop β_{snp}	0.32	—	0.61	−0.003	0.74	0.054	17 831.78	1	1
Drop β_{IBD} and β_{snp}	0.32		0.61		0.74	0.053	17 831.82	1	0.04
Drop β_{IBD}, β_{snp}, and β_e	0.32		0.62		0.73		17 859.96	1	28.14
AnxDep moderated by age									
Full moderation	0.27	−0.2	0.63	0.03	0.76	−0.005	18 977.33	—	—
Drop β_{snp}	0.31		0.63	0.03	0.76	−0.004	18 978.76	1	1.43
Drop β_{IBD} and β_{snp}	0.31	—	0.63	—	0.76	0.012	18 981.273	1	2.51
Drop β_{IBD}, β_{snp}, and β_e	—	—	0.64	—	0.77	—	18 982.684	1	1.39
BMI moderated by age									
Full moderation	0.62	0.048	0.8	0.045	0.48	0.124	16 480.76		
Drop β_{snp}	0.61		0.8	0.043	0.5	0.125	16 482.06	1	1.3
Drop β_{IBD} and β_{snp}	0.61		0.78		0.52	0.148	16 491.55	1	9.5
Drop β_{IBD}, β_{snp}, and β_e	0.61	—	0.82	—	0.48		16 684.9	1	193.35
Height moderated by birth year									
Full moderation	0.53	0.047	0.69	0.005	0.21	0.02	11 132.06	—	—
Drop β_{snp}	0.53	—	0.69	0.003	0.21	0.02	11 134.03	1	1.97
Drop β_{IBD} and β_{snp}	0.52	—	0.69	—	0.21	0.02	11 134.29	1	0.26
Drop β_{IBD}, β_{snp}, and β_e	0.53	—	0.68	—	0.21	—	11 154.57	1	20.28

β indicates magnitude of change, given a SD change in the moderator.

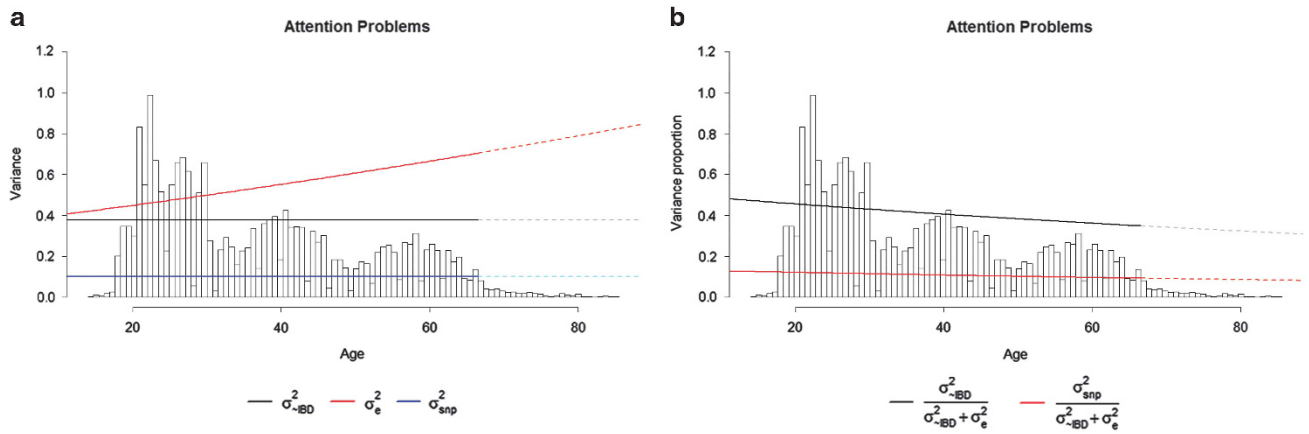

Figure 1 (**a**) σ^2_{snp}, $\sigma^2_{\sim IBD}$, and σ^2_e in AP as a function of age. (**b**) the heritability $\left(\frac{\sigma^2_{\sim IBD}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ and proportion of phenotypic variance attributable to SNPs $\left(\frac{\sigma^2_{snp}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ in AP as a function of age. The histogram displays the distribution of the standardized moderator age.

in any of the phenotypes considered. These differences in results confirm differences in genetic architectures of these phenotypes. As established in twin studies, both AP and AnxDep are moderately heritable,[25,26] whereas BMI and height are strongly heritable.[30,31] The moderation findings for BMI (decreasing heritability with age) agree with a recent meta-analysis into the heritability of BMI[30] and with evidence for GxE at the SNP level. For example, Rosenquist *et al.*[8] reported evidence for an interaction between the FTO gene and birth cohort on BMI. Note the model fitted to the empirical data did not allow for a common environment within family, or for dominant genetic effects, these effects may be of interest and could be accommodated.[32]

The method presented here has some limitations. If related individuals are present in the sample, and the moderator itself is heritable, this needs to be accounted for as shown by van der Sluis *et al.*[33] All GxE models are sensitive to scaling.[34] and GxE interaction effects reported here also are conditional on the scale of the outcome variables; different scaling may yield different results. Where BMI and height have a natural scale (ie, kg/m^2 and m or cm), the scale of the data based on self-report inventories is generally arbitrary. This problem is not limited to the current model and its solution is beyond the scope of this article (potential solutions are discussed elsewhere[15,34]). Estimates of SNP heritability are unbiased if the residual variance is not normally distributed.[20] Our simulation

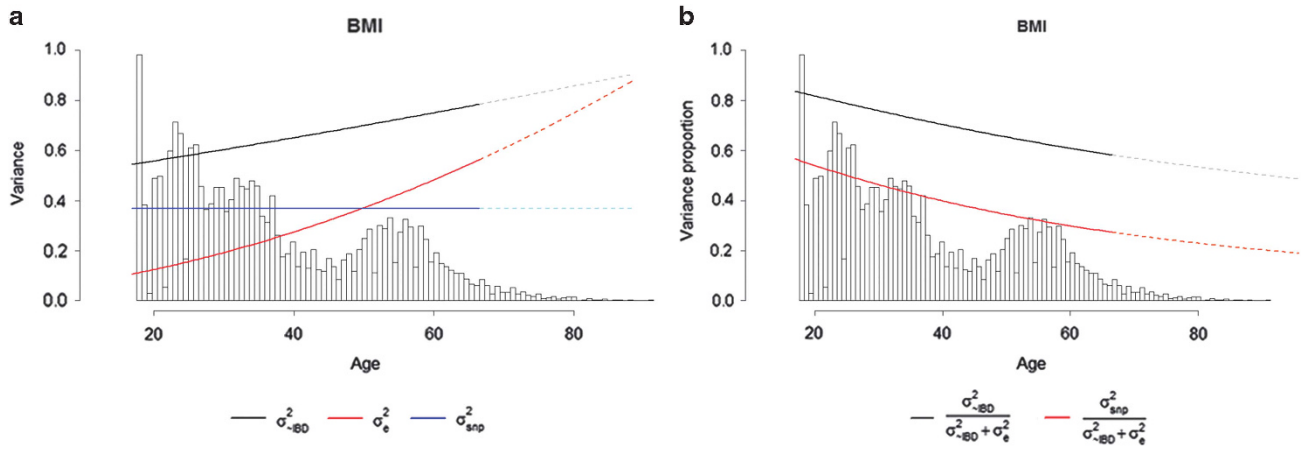

Figure 2 (**a**) σ^2_{snp}, $\sigma^2_{\sim IBD}$, and σ^2_e in BMI as a function of age. (**b**) The heritability $\left(\frac{\sigma^2_{\sim IBD}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ and proportion of phenotypic variance attributable to SNPs $\left(\frac{\sigma^2_{snp}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ in BMI as a function of age. The histogram displays the distribution of the standardized moderator age.

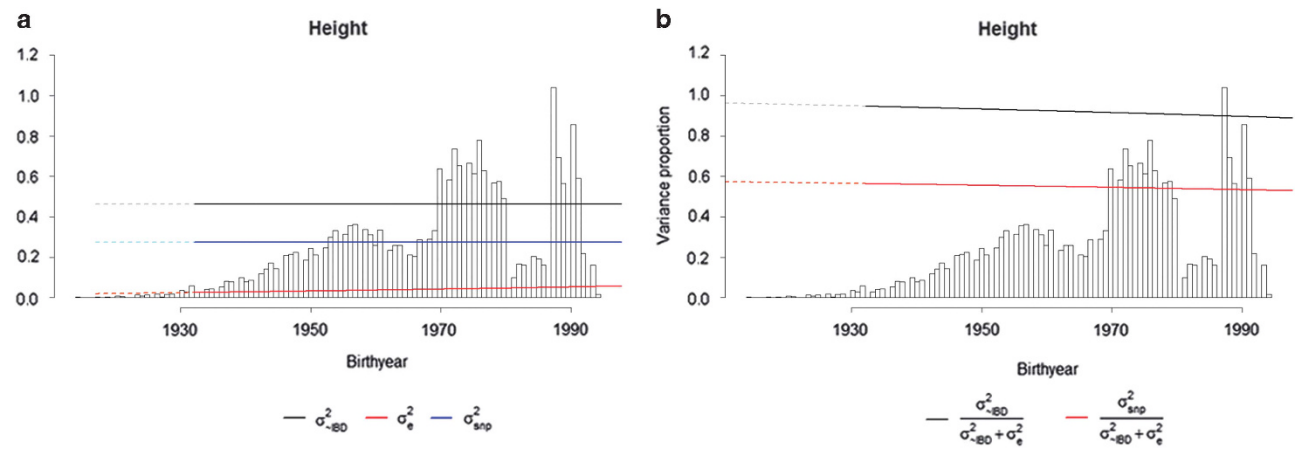

Figure 3 (**a**) σ^2_{snp}, $\sigma^2_{\sim IBD}$, and σ^2_e in height as a function of birth year. (**b**) The heritability $\left(\frac{\sigma^2_{\sim IBD}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ and proportion of phenotypic variance attributable to SNPs $\left(\frac{\sigma^2_{snp}}{\sigma^2_{\sim IBD}+\sigma^2_e}\right)$ in heigh as a function of birth year. The histogram displays the distribution of the standardized moderator birth year.

revealed, however, that even if the underlying variance components are normally distributed, some rating scales give rise to skewed distributions and this may result in parameter bias, and inflated type-1 error for the test of moderation. In view of our simulation results, it is possible that the (genetic) variance attributable to SNPs for AP and AnxDep are underestimated due to the skewness of the AP and AnxDep scores. As we found little evidence of age moderation for AP and AnxDep, the evidence we find (age moderation of the environment for AP) should be interpreted cautiously given the distributions of these phenotypes. To mitigate the distributional violations the AP and AnxDep scores were square-root transformed before analysis to reduce skew. In light of these limitations, presence or absence of age moderation of genetic variance components, for the traits AP and AnxDep need to be replicated across different self-report scales or diagnostic instruments, reducing the reliance on a single (arbitrary) scale.

The present method can include data from distantly and closely related individuals, it can accommodate, categorical, ordinal or continuous moderators, and moderation multiple variance components simultaneously. The proposed method has been successfully applied to detect moderation of the (additive) genetic and environmental effects by age and sex for 400 000+ methylation probes.[35] The analysis of these 400 000 phenotypes showed that large scale implementation is feasible. The addition of multiple genetic effects (GRMs) further allows for the separate moderation of different subsets of the genome. For example, one could limit the SNPs in a GRM to a single biological pathway (ie, SNPs in genes in the serotonin pathway), to a single class of SNPs (ie, coding variants), or to specific regions of the genome (ie, regulatory elements, the exome, etc.). Bearing in mind the assumptions discussed above the moderator can be genetic (GxG ie, a known risk variant), biological (ie, a gene expression; gut microbiota) or environmental (ie, early childhood trauma experiences).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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