Introducing M-GCTA a software package to estimate maternal (or paternal) genetic effects on offspring phenotypes

Zhen Qiao[1], Jie Zheng[2,3], Øyvind Helgeland[4,5], Marc Vaudel[4], Stefan Johansson[4,6], Pål R. Njølstad[4,7], George Davey Smith[2,3], Nicole M. Warrington[1,8], David M. Evans[1,2,3*]

[1]University of Queensland Diamantina Institute, University of Queensland, Brisbane, Queensland, Australia.

[2]Medical Research Council Integrative Epidemiology Unit, University of Bristol, Bristol, UK

[3]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

[4]KG Jebsen Center for Diabetes Research, Department of Clinical Science, University of Bergen, Bergen, Norway.

[5]Department of Genetics and Bioinformatics, Health Data and Digitalization, Norwegian Institute of Public Health, Oslo, Norway.

[6]Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway.

[7]Department of Pediatrics and Adolescents, Haukeland University Hospital, Bergen, Norway.

[8]K.G. Jebsen Center for Genetic Epidemiology, Department of Public Health and Nursing, NTNU, Norwegian University of Science and Technology, Norway

Running Head: M-GCTA software package

*Corresponding Author:

David M. Evans

University of Queensland Diamantina Institute, Translational Research Institute, 4102, Brisbane, Australia. Telephone: +61 7 34437051; Email: d.evans1@uq.edu.au

MRC Integrative Epidemiology Unit, University of Bristol, BS8 2BN, UK

Telephone: +44 (0)117 3310094; Fax: +44 (0)117 3310123; Email: dave.evans@bristol.ac.uk

Abstract

There is increasing interest within the genetics community in estimating the relative contribution of parental genetic effects on offspring phenotypes. Here we describe the user-friendly M-GCTA software package used to estimate the proportion of phenotypic variance explained by maternal (or alternatively paternal) and offspring genotypes on offspring phenotypes. The tool requires large studies where genome-wide genotype data are available on mother- (or alternatively father-) offspring pairs. The software includes several options for data cleaning and quality control, including the ability to detect and automatically remove cryptically related pairs of individuals. It also allows users to construct genetic relationship matrices indexing genetic similarity across the genome between parents and offspring, enabling the estimation of variance explained by maternal (or alternatively paternal) and offspring genetic effects. We evaluated the performance of the software using a range of data simulations and estimated the computing time and memory requirements. We demonstrate the use of M-GCTA on previously analyzed birth weight data from two large population based birth cohorts, the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother and Child Cohort Study (MoBa). We show how genetic variation in birth weight is predominantly explained by fetal genetic rather than maternal genetic sources of variation.

Key Words: M-GCTA; Maternal effects; Paternal effects; G-REML; Heritability; SNP heritability

Introduction

Maternal genetic effects may be defined as the causal influence of maternal genotypes on offspring phenotypes over and above that which results from the transmission of genes from mother to child (Mather and Jinks 1982; Wolf and Wade 2009). This definition of maternal genetic effects focuses on the effect of the maternal genome and is distinct from contributions due to mitochondrial inheritance and genetic effects due to imprinting. Maternal genetic effects can affect offspring phenotypes through the intrauterine environment or postnatal influences via the environment that the mother provides for her offspring. The importance of maternal genetic effects has long been recognized by quantitative geneticists (Wolf and Wade 2009). However, in human studies maternal effects have largely been treated as environmental sources of resemblance between relatives, and/or as a factor that may contaminate estimates of the heritability and/or common environment depending on the study design (Falconer and Mackay 1996; Meyer 1992).

The impact of maternal genetic effects on offspring phenotypes can be quantified in studies of experimental animals, using controlled breeding and cross-fostering designs, such as factorial designs involving reciprocal crosses, half-sibling and full-sibling hybrid designs (Bernando 1996; Lynch and Walsh 1998). In humans, maternal effects are typically estimated using study designs involving the children of monozygotic and dizygotic twins (Corey and Nance 1978), or using studies involving half-siblings (York et al. 2013). In these studies, the covariance between different pairs of relatives allows decomposition of the phenotypic variance into maternal genetic effects and other variance components of interest. For example, using a study design involving the offspring of monozygotic and dizygotic twins, Magnus et al. (1984a) reported a distinct maternal genetic effect on offspring birth weight (3% to 20% of the phenotypic variance), which nevertheless was smaller than the estimated contribution from the fetal genome (50% to 69.4% of the phenotypic variance) (Magnus 1984a; Magnus 1984b).

With the advent of genome-wide genotyping technology, genome-wide association studies (GWAS) have provided the ability to identify specific genetic loci in mothers that influence offspring phenotypes independent of offspring genotype. For example, investigators have used a combination of conditional association analysis of genotyped mother-offspring pairs (Horikoshi et al. 2016; Beaumont et al. 2018) and more recently structural equation modelling of genotyped mothers who report their own and their offspring's phenotype (Warrington et al. 2018a) to identify maternal genetic effects at individual loci on offspring birth weight. However, maternal loci that influence offspring phenotypes typically have small

effect sizes and thus may be difficult to identify in anything but the largest GWAS (Moen et al. 2019).

In 2010, Yang et al. introduced the G-REML framework in human genetic studies in which genome-wide genetic similarity between unrelated individuals was used to partition phenotypic variance into genetic components of variation explained by tagged SNPs present on genome-wide arrays and residual sources of variation (Yang et al. 2010). This method allowed investigators to estimate the proportion of variance in the phenotype explained by all the genotyped SNPs on microarrays (so-called "SNP heritability"), and suggested that much of the missing heritability (Maher 2008) was in fact hiding in the form of variants of small effect scattered across the genome. We subsequently extended the basic G-REML framework to estimate the proportion of trait variance due to maternal genetic effects via a procedure we called maternal genome-wide complex trait analysis (M-GCTA) (Eaves et al. 2014). Specifically, M-GCTA used genome-wide SNP data from mother-offspring pairs to partition the phenotypic variance in the offspring phenotype into components attributable to the mothers' genome, the child's genome, the covariance between the two, and residual sources of variation (Eaves et al. 2014). Our results suggested that the presence of maternal genetic effects could inflate estimates of SNP heritability from procedures based on G-REML that did not take into account this putative source of variation (Yang et al. 2010; Speed et al. 2017). Although not discussed in the original article, we note that exactly the same method can also be used to estimate variation in offspring phenotype due to paternal genetic effects (although not maternal and paternal genetic effects simultaneously). The M-GCTA method has been applied to different perinatal phenotypes including birth length, birth weight, and gestational weight gain during pregnancy (Eaves et al. 2014; Horikoshi et al. 2016; Warrington et al. 2018b). Although the M-GCTA method attracted much attention across the scientific community (Hewitt 2015; Timpson et al. 2018), it has not been widely adopted by researchers. One reason is that application of the method requires thorough understanding of what might be perceived as a complicated linear mixed model and also the construction of several large genetic relatedness matrices (GRMs), which can be difficult and error prone in practice.

In this manuscript, we introduce the user-friendly M-GCTA software package, which implements the M-GCTA statistical model, and includes an extension to estimate the variance due to paternal genetic effects (i.e. instead of maternal genetic effects, but not both simultaneously). Our software automatically constructs GRMs indexing genetic similarity across the genome between parents and offspring, thus allowing for the estimation of either maternal or paternal genetic effects. Several options for data cleaning and quality control are

coded in the software, including the ability to detect and automatically remove one individual from each pair of related individuals. In order to assess the performance of M-GCTA, we applied the program to simulated data and compared the results with estimation in the GCTA software package using custom derived genomic relatedness matrices (Yang et al. 2011), and also by structural equation modelling using the OpenMx library and the R software package, version 3.5.1 (Boker et al. 2011; Eaves et al. 2014). We estimated the CPU time and memory requirements of the M-GCTA software by applying it to simulated datasets consisting of different numbers of genetic markers and mother-offspring pairs. We also applied M-GCTA to birth weight data from two large population based cohort studies that contain genotyped mothers and their offspring; the Norwegian Mothers and Child Cohort Study (MoBa) (Magnus et al. 2016) and the Avon Longitudinal Study of Parents and Children (ALSPAC) (Boyd et al. 2013; Fraser et al. 2012). We show that offspring birth weight is influenced by fetal genetic factors and indirectly by the maternal genome to a lesser extent. Finally, we discuss the challenges in interpretation, limitations, assumptions and opportunities for future application of M-GCTA.

Methods

M-GCTA Model

Following Eaves et al. (2014), the path model underlying the M-GCTA method is illustrated in Figure 1 (Eaves et al. 2014). Although we have specified this model with respect to mothers and their offspring, we emphasize that the model could equally apply to fathers and their offspring where these relationships are of interest and genome-wide data are available. In Figure 1, and following the convention of structural equation modelling, observed (measured) variables are denoted by square boxes and latent (unmeasured) factors by circles. Single headed arrows represent causal relationships and double-headed arrows indicate correlational relationships. The variable P denotes the measured offspring phenotype (which is influenced by both offspring and maternal genotypes).

The M-GCTA model parameterizes the similarity between unrelated genotyped mother-offspring pairs as a function of four latent genetic factors. G_{MM} represents maternal genotypes at loci that exert an effect on the offspring phenotype P via path m (i.e. maternal genetic effects). G_{MC} represents these same loci in offspring and the degree to which these loci affect the offspring phenotype is quantified by the path coefficient c. G_{CC} represents offspring genotypes at loci that induce no maternal effect when present in the mother's genome but

contribute directly to the offspring phenotype P via path h. Finally, G_{CM} represents the maternal loci that do not affect the offspring phenotype P. The same latent genetic factors in mothers and offspring are expected to correlate 0.5 with each other. Finally, E represents the effect of residual environmental and untagged genetic factors that contribute to phenotype P through the causal path e.

Assuming all latent variables (i.e., circles) have unit variance, then the path model in Figure 1 represents the following structural equation and variance decomposition:

$$y_j = \mu + mG_{MM_j} + hG_{CC_j} + cG_{MC_j} + eE_j$$

with

$$var(\boldsymbol{y}) = m^2 + h^2 + c^2 + mc + e^2$$

where y_j is the phenotypic value of individual j, μ is the mean phenotype, and m, h, c and e are path coefficients, with m^2, h^2, c^2, and e^2 their corresponding variance components, and G_{MM_j}, G_{CC_j}, G_{MC_j}, and E_j are deviations of each individual on the corresponding latent genetic or residual effects with variance one. We stress that the h^2 and c^2 terms in the variance decomposition above have special meanings that are different from how these terms might be used in other manuscripts (i.e. h^2 and c^2 are often used to represent heritability and variance due to the shared environment respectively in other situations). In contrast, in the present manuscript, h^2 refers to the phenotypic variance explained by SNPs at loci that induce no maternal effect when present in the mother's genome but contribute directly to the offspring phenotype when present in the offspring genome. Likewise, c^2 refers to the phenotypic variance explained by the direct effect of that set of offspring SNPs that when present in the mother's genome exert maternal effects on the offspring phenotype.

In Figure 2, the path model is extended to show the expected relationship between two unrelated mother-offspring pairs (i and j). The correlations between the latent genetic factors are defined as follows: α_{ij} is the genomic relatedness coefficients between mothers of pair i and j, β_{ij} is the relatedness coefficient between the offspring of pair i and j, and δ_{ij} is the relatedness coefficients between the mother of pair i and the child of pair j. The following formula is used to calculate the genetic relatedness coefficient between each pair of individuals:

$$\frac{1}{S}\sum_{k=1}^{S}\frac{(x_{ik}-2p_k)(x_{jk}-2p_k)}{2p_k(1-p_k)}$$

where x_{ik} refers to the number of alleles (0, 1 or 2) for individual i at the k^{th} SNP, p_k is the allele frequency of the k^{th} SNP, and the measure is averaged over S SNPs across the genome. The correlations between the latent genetic variables (α, β, and δ) are assumed to be estimated without bias or error using genome-wide relatedness coefficients between unrelated pairs of individuals (Yang et al. 2011; Eaves et al. 2014). Accordingly, the expected covariance between the offspring's phenotypes from pair i and j is:

$$cov(y_i, y_j) = \alpha_{ij} m^2 + \beta_{ij}(c^2 + h^2) + mc(\delta_{ij} + \delta_{ji})$$

The M-GCTA model can also be expressed in terms of matrix algebra and variance components. Under this formulation, the genomic relatedness coefficients form the elements of three $n \times n$ matrices (where n is the number of mother-offspring pairs) indexing the genomic similarity between mothers ($\boldsymbol{M}$; the elements of which were previously denoted α_{ij}), children ($\boldsymbol{O}$; the elements of which were previously denoted β_{ij}), and between mothers and children ($\boldsymbol{D}$; the elements of which were previously denoted δ_{ij}). In the interest of clarity, the matrices $\boldsymbol{M}$ and $\boldsymbol{O}$ are symmetric, whilst the matrix $\boldsymbol{D}$ is asymmetric.. The matrix formed by summing the $\boldsymbol{D}$ matrix with its transpose $\boldsymbol{D} + \boldsymbol{D}'$) i.e. summing the genomic relatedness coefficients between mother i and child j, and between mother j and child i is also symmetric. By defining the variance of the offspring phenotype explained by maternal genetic effects as $\sigma_M^2 = m^2$, by offspring genetic effects as $\sigma_O^2 = c^2 + h^2$, by twice the covariance between the two as $\sigma_D = mc$, and by the residual environmental effects as $\sigma_e^2 = e^2$, the phenotypic variance-covariance matrix ($\boldsymbol{V}$) can be partitioned as:

$$\boldsymbol{V} = \boldsymbol{M}\sigma_M^2 + \boldsymbol{O}\sigma_O^2 + (\boldsymbol{D} + \boldsymbol{D}')\sigma_D + \boldsymbol{I}\sigma_e^2$$

The variance components can then be estimated using restricted maximum likelihood (REML) (Yang et al. 2011) or full information maximum likelihood in e.g. the OpenMx software package (Boker et al. 2011; Eaves et al. 2014). Whilst we have not included fixed effects in the above description of the M-GCTA model, similar to ordinary GCTA, the M-GCTA software package can estimate fixed effects if included in the model for the means.

2.2 Implementation and application of the M-GCTA software

The M-GCTA software package is an extension of the GCTA software that has been written in C++ (Yang et al. 2011). The M-GCTA software uses source code from the GCTA software package (currently version 1.26.0) that has been modified to fit the M-GCTA statistical model

and perform quality control functions relevant to genome-wide SNP datasets with parents and their offspring. Our program implements the M-GCTA method in two steps. First, the software identifies unrelated mother-/father- offspring pairs and calculates GRMs (i.e. matrices $\boldsymbol{M}$, $\boldsymbol{O}$ and ($\boldsymbol{D}$ + $\boldsymbol{D}$')). The package then uses these GRMs to estimate the variance explained by each component using restricted maximum likelihood. We have implemented the M-GCTA software package as a downloadable application for the Linux/Unix platform. We built the M-GCTA software because application of the M-GCTA statistical method in more generic software packages e.g. OpenMx can be difficult and error prone in practice. The aim of creating the M-GCTA software was to make it easy and straightforward for researchers to apply the M-GCTA statistical method.

M-GCTA identifies maternal and paternal relationships based on information provided by users in PLINK style .fam or .ped files (Purcell et al. 2007). It identifies families according to the family identifier (i.e. the first column of the .fam or .ped file), and recognises father-offspring pairs and mother-offspring pairs based on values provided by the user (i.e. the third and fourth columns of the .fam or .ped file). By specifying the --mat or --pat flag, M-GCTA will extract the relevant parent-offspring pairs and perform the analyses (i.e. no explicit pruning of maternal and or paternal information by the user is required to perform these analyses and both sets of parents can be included in the data files).

Users are required to generate PLINK style pedigree files consisting of mother-offspring duos, father-offspring pairs, parent child trios, or some combination of all of these. Users must only include one offspring per family. If additional offspring are included in the pedigree files then these families will be automatically excluded from analyses, a warning produced, and the identity of the offending families will be written to a text file. It is then up to the user how to deal with these individuals. Details of all the options provided by M-GCTA and recommendations for running the M-GCTA software are described on the M-GCTA github page (https://github.com/uqzqiao/M-GCTA).

Currently, the M-GCTA statistical model can only estimate maternal/paternal (and other) variance components using one parent and child at a time (i.e. either mothers and their child, or fathers and their child). Extending the statistical model to estimate maternal and paternal variance components simultaneously using parent-offspring trios, including more than one child in each family in the analysis, and extending the model to multivariate situations are all current areas of research by our group. We expect that we will include these options in future

releases of the M-GCTA software when the parameterization of these extensions to the basic model are developed.

Application of the M-GCTA software to simulated data

In order to compare estimates of maternal and offspring genetic variance components obtained from the M-GCTA software against those obtained from standard GCTA, we performed a simple data simulation. Following Eaves et al. (Eaves et al. 2014), we simulated 1000 mother-offspring pairs and an additional 50 singleton individuals to mimic real life situations where these singleton individuals would need to be detected and removed before analysis. We simulated 250 SNPs that accounted for all of the direct offspring and indirect maternal genetic effects and their covariance on the offspring phenotype. SNPs 1 – 125 were assigned to have direct genetic effects on an individual's phenotype when present in their own genome and SNPs 76 – 200 were assigned to have indirect effects on an individual's phenotype when present in their mother's genome. Thus, SNPs 1 – 75 and SNPs 126 – 200 contributed exclusively to offspring genetic effects and maternal genetic effects, respectively, while SNPs 76 – 125 had both offspring and maternal genetic effects on offspring phenotypes. SNPs 201 to 250 exerted no effects on the offspring's phenotype. We simulated three scenarios. In scenario one, the effects of all shared SNPs (i.e. SNPs 76 – 125) on the offspring's phenotype were in the same direction. In scenario two, the effect of all shared SNPs were in opposite directions (for example, SNPs that had a positive maternal genetic effect had a negative offspring genetic effect). In scenario three, the effects of 30 out of 50 SNPs were in opposite directions whilst the rest of the shared SNPs exerted effects in the same direction. Due to the relatively small sample size, we simulated all increasing alleles having high allele frequencies sampled randomly from a uniform distribution (between 0.4 and 0.6), and each increasing allele exerted a large effect on the simulated phenotype (between 0.45 and 0.55). Across all conditions, we added a random normal variate (mean zero, variance four) to the genotypic value of each individual to simulate residual and environmental effects. We assumed all genetic effects were additive and there were no interactions between maternal and offspring genomes. We conducted 1000 simulations for each scenario, and the mean and the standard deviation of the parameter estimates from the 1000 simulations were reported. The simulated datasets were generated in R (R Core Team 2013), and were then converted to binary PLINK format (Purcell et al. 2007) for downstream analysis. For analysis in GCTA (i.e. in order to benchmark our M-

GCTA software against), we first extracted all mother-offspring pairs (N = 1000), arranged the mothers in order of family identifier, and then their offspring below them in exactly the same order. Having identified the 50 singleton individuals, we directed GCTA to remove them. We created an overall GRM for all individuals (i.e. $2N \times 2N = 2000 \times 2000$) using the GCTA software, and then constructed new GRMs indexing genomic sharing between mothers, offspring, and between mothers and offspring (i.e. the $\boldsymbol{M}$, $\boldsymbol{O}$, and ($\boldsymbol{D} + \boldsymbol{D'}$) matrices) from the appropriate rows of the larger matrix. We then fit the three GRMs in a variance components model using the original GCTA software. For comparison, we applied the M-GCTA software to the same simulated data. We emphasize that the M-GCTA software fits the same underlying variance components model but detects mother-offspring (or father-offspring) pairs in the data, removes singleton individuals from the analysis, and constructs the requisite GRMs automatically for the user without the need for time consuming and error prone scripting.

Finally, we compared the results from GCTA and M-GCTA to those from fitting a structural equation model implemented in R using the OpenMx software package. We parameterized the M-GCTA model two ways in OpenMx. In one formulation we modelled the path coefficients in Figure 1 (i.e. *m*, *h*, *c*, and *e*) and explicitly modelled the constraint $\sigma_D = mc$. In the other, we parametrized the model using a variance components framework in which the above constraint was implicit (i.e. similar to how the model is parameterized in GCTA and the M-GCTA software). We fitted a series of five models. In the full model we estimated all four variance components (σ_M, σ_O, σ_D, σ_E). In the first sub-model we set the covariance between maternal and offspring genetic effects to zero. In the second (and third) sub-models we further constrained the maternal (or offspring) genetic components to zero. Finally, we constrained all variance components responsible for the covariation between mothers and their children to zero (σ_M, σ_O, σ_D). We calculated twice the difference in log-likelihood between the full model and each of the sub-models, and evaluated the difference in relative to the full model by (twice the) difference in log-likelihood chi-square. The R code used for performing the simulations is included in the Supplementary Materials (Supplementary Material 1).

CPU and Memory Requirements

To quantify the computational requirements of the M-GCTA software, we simulated datasets that ranged in size from N = 3,000 to 15,000 mother-offspring pairs and S = 500,000 or 1,000,000 SNP markers. We note that this dataset size is representative of the size of cohorts

of mother-offspring pairs that currently exist around the world (Evans et al. 2019). The datasets were simulated using an approach similar to that described above. For each set of simulated datasets, 37.5% of all simulated SNPs contributed exclusively to offspring genetic effects, 37.5% of SNPs contributed to maternal genetic effects, while the remaining 25% of SNPs exerted offspring and maternal genetic effects on offspring phenotypes in the same direction. We benchmarked the running time and memory use of the M-GCTA software by running simulations on these datasets. Reported run times are the medians of five identical runs using 20 cores of a 3.2 GHz Intel Xeon E5-2667 v3 processor.

Application of the M-GCTA software to birthweight data in the ALSPAC Cohort

We applied the M-GCTA software to offspring birth weight data from the ALSPAC birth cohort. ALSPAC is an ongoing transgenerational population-based birth cohort study that initially recruited 14,541 pregnant women resident in the Bristol area of the U.K. (formerly known as Avon) with expected delivery dates between 1 April 1991 and 31 December 1992 (Fraser et al. 2012; Boyd et al. 2013). It resulted in 14,062 live births, of which 13,988 survived to the end of the first year of age. Detailed information has been collected on both mothers and children as early as the eighth gestational week and at regular follow-up intervals, using a combination of questionnaires, clinical assessments, and health and administrative records. Biological samples including DNA have been taken repeatedly, and a wide range of data has been generated including genome-wide array, genome sequencing, and DNA methylation data. Ethical approval has been obtained from the ALSPAC Law and Ethics Committee, and other relevant ethics committees, and written informed consent has been provided by all participants. Birth weight was extracted from routine hospital birth records. Details of the ALSPAC data are available through a fully searchable data dictionary: www.bris.ac.uk/alspac/researchers/data-access/data-dictionary.

Genome-wide genotyping was performed on maternal and offspring samples using the Illumina 660K and HumanHap550 quad SNP genotyping platforms respectively. Detailed quality control and genotyping imputation processes have been described previously (Evans et al. 2013; Fatemifar et al. 2013). After preliminary data management, we obtained a dataset consisting of 8,340 unrelated mothers (i.e., each mother was unrelated with other mothers presented in the dataset) and 8,365 unrelated children, with “best guess” genotypes at 2,425,567 autosomal markers imputed from the HapMap Phase II (release 22) data as the dataset. The

quality control procedures used to generate this dataset are detailed in Supplementary Figure 1.

We first applied the M-GCTA software to this dataset in order to perform data management and quality control. We set a threshold for the genetic relationship between individuals of 0.025 and M-GCTA removed one from each pair of identified cryptically related individuals. This was not applied to the mother-child pairs who will have a genetic relationship of ~0.5 (this was confirmed in the datasets), but rather to test that all the mothers in the sample are unrelated, the children are unrelated and mothers from pair *i* and children from pair *j* are unrelated. This command operated in three consecutive steps. First, singletons and mother-offspring pairs who do not have an offspring phenotype were removed, yielding N = 5,189 mother-offspring pairs. Second, mother-offspring pairs were ordered according to family identifier, and a GRM for all $2N \times 2N$ individuals in the dataset was created. Third, the software iteratively excluded one individual from each pair of cryptically related individuals who had an estimated relationship > 0.025 while minimizing the overall loss in sample size. This process yielded a combined dataset of 4,321 unrelated mother-offspring pairs. Mother-offspring pairs with offspring birth weight measures four standard deviations (SD) away from the sex-specific mean were removed (11 mother-offspring pairs). Birthweight was adjusted for the first twenty principal components, and Z scores were calculated for each sex. Last, we applied M-GCTA on the 4,310 mother-offspring pairs and performed the REML analysis to estimate the effects of maternal genotypes, offspring genotypes and their covariance on offspring birth weight.

Application of the M-GCTA software to birthweight data in the Norwegian Mothers and Children Cohort Study (MoBa)

The M-GCTA software was applied to birth weight data from the Norwegian Mother and Child Cohort Study (MoBa) (Magnus et al. 2016). MoBa is an open-ended cohort study that recruited pregnant women from 1999 to 2008. Approximately 114,000 children, 95,000 mothers, and 75,000 fathers of predominantly Norwegian ancestry were enrolled in the study from 50 hospitals all across Norway. Birth weight was measured at the respective hospitals at birth. In 2012, the project Better Health By Harvesting Biobanks (HARVEST) randomly selected 11,000 triads from MoBa's biobank for genotyping, excluding children matching any of the following criteria: (1) stillborns, (2) deceased, (3) twins, (4) non-existing Medical Birth Registry (MBR) data, (5) missing anthropometric measurements at birth in MBR, (6)

pregnancies where the mother did not answer the first questionnaire, and (7) missing parental DNA samples. In 2016, HARVEST randomly selected a second set of 6,000 triads using the same criteria. Ethical approval has been obtained from the MoBa Ethics Board, and other relevant ethics committees, and written informed consent has been provided by all participants. For the first set of triads, genotyping was performed using Illumina HumanCoreExome-12 v.1.1 and HumanCoreExome-24 v.1.0 arrays for 7,000 and 4,000 triads, respectively, at the Genomics Core Facility located at the Norwegian University of Science and Technology, Trondheim, Norway. The second set of triads was genotyped using Illumina Global Screening Array v.1.0 for all 6,000 triads at the Erasmus University Medical Center in Rotterdam, Netherlands.

The genotypes were called in Illumina Genome Studio (for the first sample v.2011.1 and for the second v.2.0.3) using only samples with call rate ≥ 0.98 and GenCall score ≥ 0.15 for defining cluster positions. We excluded variants with low call rates, signal intensity, quality scores, heterozygote excess and deviation from Hardy-Weinberg equilibrium based on the following QC parameters: call rate < 98%, cluster separation < 0.4, 10% GC-score < 0.3, AA T Dev > 0.025, HWE P-value < 10-6. Samples were excluded based on call rate < 98% and heterozygosity excess > 4 SD. Study participants with non-Norwegian ancestry were excluded after merging with samples from the HapMap project (ver. 3) to remove ethnic outliers.

Prior to imputation, the genetic dataset was harmonized with the Haplotype Reference Consortium (HRC) v.1.1 imputation panel using the HRC Imputation preparation tool by Will Rayner version 4.2.5. Insertions and deletions were excluded. Allele, marker position, and strand orientation were updated to match the reference panel. A total of 384,855 and 568,275 markers remained eligible for phasing and imputation in the first and second sample, respectively. Pre-phasing was conducted locally using Shapeit v2.790 to allow for phasing utilizing the pedigree data. Imputation was performed on the Sanger Imputation Server using the Positional Burrows-Wheeler Transform and HRC version 1.1 reference panel. After imputation, to avoid inclusion of poorly imputed markers, only markers genotyped in either of the two samples and having a quality score (INFO score) > 0.9 were included in the analysis (i.e. genotyped in one sample and imputed with high quality in the other sample). Additionally, markers with MAF < 0.01 and HWE p-value < 1×10^{-6} were excluded. No markers had missing calls after imputation. After exclusions, 497,187 markers were eligible for analysis.

After preliminary data management 13,934 independent mother and child duo pairs were available. GCTA was used to filter cryptically related individuals > 0.025 by removing one in each pair of samples in mothers and children separately. Additionally, any sample missing birth weight or having birth weight 4 standard deviations away from the mean were excluded. After data management 7,910 mother-offspring pairs were eligible for analysis. Birthweight was z-score transformed and were adjusted for sex, gestational duration and the first four ancestry-informative principal components. M-GCTA performed the REML analysis to resolve the effects of maternal genotype, offspring genotype and their covariance on offspring birth weight.

Meta-analysis

Estimates of the variance components obtained in the ALSPAC and MOBA cohorts were combined using inverse variance weighted meta-analysis of the variance components in the R package meta (DerSimonian and Laird 1986; Schwarzer 2007).

Results

We used the same simulation strategy to generate small sets of data, containing 1000 mother-offspring pairs and 50 singletons. We simulated 250 SNPs in total, apportioning 75 SNPs to have direct offspring genetic effects only on the phenotype, 75 SNPs to have maternal genetic effects only on the phenotype, 50 SNPs that had both offspring and maternal genetic effects and 50 SNPs that had no effect on the phenotype. Table 1 summarizes the results for the simulated mother-offspring pairs across three scenarios (i.e. positive covariance between maternal and fetal genetic effects, negative covariance between maternal and fetal effects, and a mixture of the two). Estimates of model parameters and test statistics obtained from the M-GCTA software package were almost identical to those obtained by analysis using standard GCTA and through analysis in R using the OpenMx package. Likewise, it did not appear to matter whether the model constraint was explicitly or implicitly modelled by the OpenMx software, the parameter estimates were the same. All formulations of the M-GCTA model appeared to correctly recover the underlying model parameters regardless of whether the underlying SNPs influenced the phenotypes in the same direction, different directions, or a mixture of the two. Similar results were obtained using an analysis involving father-offspring pairs (Supplementary Table 1).

We assessed the computational performance of the M-GCTA software by running it on several simulated datasets of varying size (Figure 3 and Supplementary Table 2). Based on the computational costs in standard GCTA-GREML analysis (Yang et al. 2014), it appears as if compute time scales $O(4SN^2 + N^3)$ whereas we estimate that memory required is approximately $N \times S \times 4 \times 2 + N \times N \times 8 \times 7$ bytes (where N is the number of mother-offspring pairs and S is the number of SNP markers).

We applied the M-GCTA software to 4,310 mother-child pairs from the ALSPAC cohort, and found that the child's genotype (σ_O^2 = 23%; s.e. = 10%) made a larger contribution to the variance in birth weight than either the mother's genotype (σ_M^2 = 5%; s.e. = 9%) or twice the covariance between the two (σ_D = 8%; s.e. = 7%) (Table 2). These results closely match estimates from previous studies using the ALSPAC cohort (Horikoshi et al. 2016; Warrington et al. 2018b), which reported that maternal and fetal contributions to birth weight were 4% and 24%, respectively, with a slightly smaller covariance (i.e., 4%) between the two. Similar results were found when we applied M-GCTA to 7,910 mother-offspring pairs from the MoBa cohort. The maternal genome explained 8% (s.e. = 6%) and fetal genome 29% (s.e. = 6%) of the

variation in offspring birth weight (Table 2), with a slightly smaller covariance (i.e., 4% ± 5%) between the two. These results are highly consistent with previous reports using the MoBa cohort (Warrington et al. 2019). A meta-analysis of M-GCTA results from these two cohorts showed that 7% (s.e. = 5%) and 27% (s.e. = 5%) of variance in birth weight were captured by assays of maternal and fetal genetic variation, respectively, and an additional 5% (s.e. = 4%) of variance was attributable to the covariance between the two. No statistical heterogeneity was observed.

Discussion

In this manuscript, we describe M-GCTA, a user-friendly software package that implements G-REML methodology for mother (or alternatively father) child pairs with genome-wide SNP data. The M-GCTA software package accepts genome-wide SNP data from mother (or alternatively father) offspring pairs in PLINK pedigree or binary file format. The software allows the user to construct GRMs indexing genetic similarity across the genome between parents and offspring enabling the estimation of variance due to maternal (or alternatively paternal) and offspring genetic effects. The software also includes several options for data cleaning and quality control, including the ability to detect and automatically remove one mother-offspring pair from each pair of related individuals.

We performed several simulations which show that the M-GCTA software package calculates the same variance component estimates as GCTA, however it is much less time consuming and error prone due to the data cleaning and quality control features included. Our simulations also showed that there was no difference in the variance components estimates according to whether the $\sigma_D = mc$ constraint was explicitly modelled in OpenMx or implicitly modelled in the other formulations.

There are two key computational steps implemented in the M-GCTA software package, building the GRM and estimating the variance components. Accordingly, the compute time and memory usage of the M-GCTA software can be estimated by summing the requirements of both components. Our results show that current desktops/laptops will not have sufficient RAM to run the M-GCTA software on most realistically sized datasets, but that compute servers providing larger RAM will probably be adequate. Extrapolating our results, we expect that M-GCTA could realistically be used to compute GRMs and estimate variance components on N = 50,000 mother-offspring pairs assuming m = 1,000,000 markers genome-wide (i.e. which is our estimate of the number of mother-offspring pairs with genome-wide data that are currently available worldwide as part of the Early Growth Genetics Consortium (Moen et al. 2019)) given the computing resources typically available in many modern scientific institutes.

We applied the M-GCTA software package to offspring birth weight data from a large sample of unrelated mother-offspring pairs from the ALSPAC and MoBa cohorts. Birth weight is a complex trait, which is likely to be influenced by both maternal and fetal genetic factors in addition to the environment (Magnus 1984a; Magnus 1984b; Lunde et al. 2007; Horikoshi et al. 2016; Warrington et al. 2019). Using the M-GCTA software we found that the variance in

birth weight explained by tagged maternal, and fetal genetic sources of variation and the covariance between the two was around 4%, 23% and 9% respectively. Our results suggest that fetal genetics are the primary source of individual differences in birth weight that results from genetic factors, and that maternal genetic effects contribute to a much lesser extent, consistent with previous research (Horikoshi et al. 2016; Warrington et al. 2019).

It is worth highlighting that the covariance between maternal and fetal genetic sources of variation estimated by M-GCTA represents a genetic covariance that has been calculated across all SNPs in the GRM. Thus, a positive (or negative) covariance does not necessarily mean that all maternal and fetal loci across the genome affect the offspring's phenotype in the same direction, but rather that in aggregate, the overall effect is in a positive (negative) direction. Focusing on this aggregate measure means that potentially interesting effects at individual loci might be missed. This is illustrated in the simulation results under scenario three where 60% of the SNPs with both maternal and offspring effects operated in the same direction whereas the other 40% operated in opposite directions, and the overall covariance estimated by M-GCTA was negative. Additionally, we report a positive genetic correlation between maternal and offspring genetic effects on birth weight, despite the fact that previous work has identified at least nine individual loci that exert opposing maternal and fetal effects on birth weight putatively through effects on glucose sensing in mother and child (Warrington et al. 2018a; Beaumont et al. 2018). Thus, it is likely that the opposing instances of these individual SNPs are cancelled out by polygenic contributions that exert shared effects on birth weight in the same direction (Warrington et al. 2019). The corollary is that users should be careful not to constrain variance components to be positive in M-GCTA in order to allow for the possibility of negative covariance between maternal and offspring genetic effects.

Whilst we have used offspring birth weight to illustrate the application of the M-GCTA software in this manuscript, our software package will likely be useful for the analysis of many other phenotypes, especially perinatal and early developmental traits such as birth length (Lunde et al. 2007; Eaves et al. 2014; van der Valk et al. 2014), gestational age (Lunde et al. 2007; York et al. 2013), crown-heel length and head circumference (Masuda et al. 2002; Rice and Thapar 2010; Taal et al. 2012), where large scale GWAS have already been performed and maternal and fetal genome-wide SNP data is available in large numbers of participants. As long as genome-wide SNP data is available in a relatively large dataset of mother-offspring (or alternatively father-offspring) pairs, then it is possible to use this software to resolve maternal (or alternatively paternal) and offspring genetic effects. However, the challenge will be in

finding enough genotyped mother (or father) offspring pairs with individual level GWAS data to run sufficiently powered analyses (Evans et al. 2019). Cohorts with large numbers of mother-offspring pairs include the Avon Longitudinal Study of Parents and Children (Fraser et al. 2012; Boyd et al. 2013), the Norwegian MoBa Study (Magnus et al. 2016), the Norwegian HUNT study (Krokstad et al. 2012), and the UK Biobank study (Sudlow et al. 2015).

Although we have focused on estimating maternal effects in this manuscript, as noted, our software can alternatively be used to estimate the contribution of paternal genetic effects on offspring phenotypes. M-GCTA includes explicit routines for the automatic cleaning and analysis of paternal-offspring genotype data, making this process easy for users. Genetic variants in the paternal genome can affect their children's phenotype independently of offspring genotype by contributing to the paternally provided environment, a phenomenon recently referred to as "genetic nurture" (Kong et al. 2018). Whilst extensive studies have been conducted to investigate maternal genetic effects on a variety of offspring phenotypic traits, the number of studies examining paternal genetic effects has been low and has often focused on investigating the evidence for effects mediated via epigenetic mechanisms (Curley et al. 2011; Rando 2012). One interesting idea would be to use the estimation of paternal genetic effects as a negative control in maternal studies of perinatal phenotypes similar to what has been done in traditional observational epidemiological studies (Smith 2008; Smith 2012). Paternal genetic effects are unlikely to exert large intrauterine environmental effects on offspring phenotypes, but are likely to share the same potential confounding factors (e.g. population stratification, assortative mating, etc.) as studies focusing on maternal genetic effects. Therefore, for these phenotypes, we would expect stronger evidence for a maternal genetic effect, whereas estimated maternal and paternal variance components of similar size may indicate the presence of residual confounding in the analysis (Richmond et al. 2014). In the future, we intend to expand the M-GCTA model to jointly estimate paternal and maternal effects in the same G-REML framework and simultaneously account for any effects due to assortative mating.

We note that the M-GCTA model involves a number of assumptions common to the G-REML framework including normality of phenotypes, no G×E interactions or non-additive genetic effects, and random mating (Eaves et al. 2014). Additionally, M-GCTA estimates of maternal and offspring genetic effects might be biased if paternal genetic effects and/or parent-of-origin effects (POEs) affect the phenotype under study and are not modelled correctly in the analysis. For example, paternal genotypes will be correlated 0.5 with offspring genotypes. If

paternal genetic effects affect the offspring phenotype then they may incorrectly be modelled as offspring genetic effects and bias estimates of the variance components. POEs refer to the phenomenon in which the effect of an allele depends on whether it is inherited from the mother or the father, the best studied of these effects being genomic imprinting (Guilmatre and Sharp 2012; Lawson et al. 2013). Thus, estimation of parental genetic effects by incorporating maternal (or alternatively paternal) genotypes into the model without further investigation of the underlying mechanisms, as what we do in M-GCTA, may capture confounding effects from POEs. Although the contribution in phenotypic variation from imprinting has not been fully investigated, evidence suggests that less than one percent of the human genome is imprinted (Morison et al. 2005; Lawson et al. 2013; Cuellar Partida et al. 2018). Indeed, the G-REML methodology can be extended to estimate the proportion of phenotypic variance due to parent of origin effects across the genome (Laurin et al. 2018). Similar to M-GCTA, the method requires genome-wide SNP information on parent(s) and children in order to determine the parental origin of allelic transmissions. Using this method, we have shown that POEs on a range of different traits is likely to be very small (Laurin et al. 2018). In theory, a component indexing POEs could be added to the M-GCTA model so that POEs and maternal sources of variation could be estimated together, but future work is necessary to ensure optimal parameterization and the properties of such a combined model.

Finally, since publication of the M-GCTA method (Eaves et al. 2014), a number of high profile procedures that share some similarities to M-GCTA in their methods and/or aims have been published in the literature (Kong et al. 2018; Young et al. 2018). Kong et al. (2018) created a procedure to detect what the authors term “genetic nurturing effects” of parents on their offspring (i.e. these genetic nurturing effects include, but are not limited to, maternal genetic effects). The Kong et al approach regresses offspring phenotype on genome-wide polygenic scores (Evans et al. 2009) consisting of transmitted and untransmitted alleles. From this regression, estimates of the direct effect of alleles and indirect effects through "genetic nurture" on the offspring’s phenotype can be obtained. In addition, the authors show how it is possible to examine parent of origin effects i.e. whether direct and indirect effects differ between paternally and maternally transmitted alleles and whether the presence of assortative mating is likely to influence the estimates obtained. A key difference between M-GCTA and the Kong et al. (2018) approach, is that the latter utilizes genome-wide polygenic scores (which tend not to do an optimal job of tagging information across the genome), whereas M-GCTA summarizes genome-wide information more elegantly in the form of genetic relationship matrices.

The same group of authors also created a procedure called Relatedness Disequilibrium Regression (RDR) which shares many methodological similarities with M-GCTA. RDR attempts to estimate heritability (or alternatively SNP heritability) by exploiting variation in relatedness between pairs of individuals due to random Mendelian segregation (which by definition should be orthogonal to environmental factors). Specifically, RDR first quantifies how much more or less related pairs of individuals are compared to what would be expected from the relatedness of their parents. Using this information, the authors show how it is possible to partition the phenotypic variance into components due to the direct effect of individuals' genotypes on their own traits (i.e. heritability), as well as the variance of the part of the environmental component of the phenotype that is correlated with the parental phenotype (which include maternal effects and other forms of "genetic nurture"), and the covariance between both of these components. However, application of RDR requires thousands of genotyped parents of probands (i.e. genotyped mothers, fathers and their offspring), meaning that the procedure currently has very limited practical utility. In contrast, M-GCTA only requires genotyped mother-offspring pairs (or alternatively father-offspring pairs), meaning that the method can be applied much more widely currently (but contingent on the assumptions discussed above).In conclusion, in this manuscript we have described a user-friendly tool for estimating the proportion of phenotypic variance due to tagged maternal (or alternatively paternal) and offspring genetic effects on offspring phenotypes using large studies where genome-wide genotype data are available on mother- (or father-) offspring pairs. We applied the M-GCTA software package to birth weight using mother-offspring pairs from two large population based birth cohorts, the ALSPAC and MoBa studies, and showed how genetic variation in birth weight was predominantly due to fetal genetic rather than maternal genetic sources of variation. In the future, we expect that the M-GCTA software will be used in the genetic analysis of parent-offspring pairs from large cohorts to further enhance understanding of parental genetic effects on offspring health-related phenotypes.

Acknowledgments

We are extremely grateful to all the families who took part in ALSPAC, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers,

receptionists and nurses. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). The UK Medical Research Council and the Wellcome Trust (Grant ref: 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. The ALSPAC GWAS data were generated by Sample Logistics and Genotyping Facilities at the Wellcome Trust Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. This publication is the work of the authors and DME will serve as guarantor for the contents of this paper.

Compliance with ethical standards

Funding NMW is supported by a National Health and Medical Research Council Early Career Fellowship (grant number 1104818). This work and DME are supported by an NHMRC Senior Research Fellowship (1137714) and NHMRC project grants (GNT1085159, GNT1085130, GNT1125141, GNT1125200, GNT1157714). This work was supported by grants (to PRN) from the European Research Council (AdG #293574), the Bergen Research Foundation ("Utilizing the Mother and Child Cohort and the Medical Birth Registry for Better Health"), Stiftelsen Kristian Gerhard Jebsen (Translational Medical Center), the University of Bergen, the Research Council of Norway (FRIPRO grant #240413), the Western Norway Regional Health Authority (Strategic Fund "Personalized Medicine for Children and Adults"), and the Norwegian Diabetes Foundation; and (to SJ) Helse Vest's Open Research Grant.

Conflict of interest Zhen Qiao, Jie Zheng, Øyvind Helgeland, Marc Vaudel, Stefan Johansson, Pål Njølstad, George Davey Smith, Nicole Warrington and David Evans declare that they have no conflict of interest.

Ethical approval Ethical approval was obtained from the ALSPAC Law and Ethics Committee, MoBa Ethics Board and other relevant ethics committees. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent has been provided by all study participants.

References

Beaumont RN, Warrington NM, Cavadino A, Tyrrell J, Nodzenski M, Horikoshi M, Geller F, Myhre R, Richmond RC, Paternoster L, Bradfield JP, Kreiner-Møller E, Huikari V, Metrustry S, Lunetta KL, Painter JN, Hottenga JJ, Allard C, Barton SJ, Espinosa A, Marsh JA, Potter C, Zhang G, Ang W, Berry DJ, Bouchard L, Das S; Early Growth Genetics (EGG) Consortium, Hakonarson H, Heikkinen J, Helgeland Ø, Hocher B, Hofman A, Inskip HM, Jones SE, Kogevinas M, Lind PA, Marullo L, Medland SE, Murray A, Murray JC, Njølstad PR, Nohr EA, Reichetzeder C, Ring SM, Ruth KS, Santa-Marina L, Scholtens DM, Sebert S, Sengpiel V, Tuke MA, Vaudel M, Weedon MN, Willemsen G, Wood AR, Yaghootkar H, Muglia LJ, Bartels M, Relton CL, Pennell CE, Chatzi L, Estivill X, Holloway JW, Boomsma DI, Montgomery GW, Murabito JM, Spector TD, Power C, Järvelin MR, Bisgaard H, Grant SFA, Sørensen TIA, Jaddoe VW, Jacobsson B, Melbye M, McCarthy MI, Hattersley AT, Hayes MG, Frayling TM, Hivert MF, Felix JF, Hyppönen E, Lowe WL Jr, Evans DM, Lawlor DA, Feenstra B, Freathy RM (2018) Genome-wide association study of offspring birth weight in 86,577 women identifies five novel loci and highlights maternal genetic effects that are independent of fetal genetics. Hum Mol Genet 27:742-756

Bernardo J (1996) Maternal effects in animal ecology. Am Zool 36:83-105

Boker S, Neale M, Maes H, Wilde M, Spiegel M, Brick T, Spies J, Estabrook R, Kenny S, Bates T, Mehta P, Fox J (2011) OpenMx: An Open Source Extended Structural Equation Modeling Framework. Psychometrika 76:306-317

Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G (2013) Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol 42:111-127

Corey L, Nance W (1978) The monozygotic half-sib model: a tool for epidemiologic research. Prog Clin Biol Res 24A:201-9

Cuellar Partida G, Laurin C, Ring SM, Gaunt TR, McRae A, Visscher PM, Montgomery G, Martin NG, Hemani G, Suderman M (2018) Genome-wide survey of parent-of-origin effects on DNA methylation identifies candidate imprinted loci in humans. Hum Mol Genet 27:2927-2939

Curley JP, Mashoodh R, Champagne FA (2011) Epigenetics and the origins of paternal effects. Horm Behav 59:306-314

DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. Control Clin Trials 7:177-188

Eaves LJ, Pourcain BS, Smith GD, York TP, Evans DM (2014) Resolving the effects of maternal and offspring genotype on dyadic outcomes in genome wide complex trait analysis ("M-GCTA"). Behav Genet 44:445-455

Evans DM, Moen G-H, Hwang D, Lawlor DA, Warrington NM (2019) Elucidating the Role of Maternal Environmental Exposures on Offspring Health and Disease Using Two-Sample Mendelian Randomization. Int J Epidemiol 48:861-875

Evans DM, Visscher PM, Wray NR (2009) Harnessing the information contained within genome-wide association studies to improve individual prediction of complex disease risk. Hum Mol Genet 18:3525-3531

Evans DM, Zhu G, Dy V, Heath AC, Madden PA, Kemp JP, McMahon G, St Pourcain B, Timpson NJ, Golding J (2013) Genome-wide association study identifies loci affecting blood copper, selenium and zinc. Hum Mol Genet 22:3998-4006

Falconer DS, Mackay TFC (1996) Introduction to Quantitative Genetics. Longman

Fatemifar G, Hoggart CJ, Paternoster L, Kemp JP, Prokopenko I, Horikoshi M, Wright VJ, Tobias JH, Richmond S, Zhurov AI (2013) Genome-wide association study of primary tooth eruption identifies pleiotropic loci associated with height and craniofacial distances. Hum Mol Genet 22:3807-3817

Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A (2012) Cohort profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol 42:97-110

Guilmatre A, Sharp A (2012) Parent of origin effects. Clin Genet 81:201-209

Hewitt JK (2015) Announcement of the Fulker Award for a Paper Published in Behavior Genetics, Volume 44, 2014. Behav Genet 45:699

Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, Feenstra B, van Zuydam NR, Gaulton KJ, Grarup N, Bradfield JP, Strachan DP, Li-Gao R, Ahluwalia TS, Kreiner-Møller E, Rueedi R, Lyytikäinen L, Cousminer DL, Wu Y, Thiering E, Wang CA, Have CT, Hottenga JJ, Vilor-Tejedor N, Joshi PK, Tai Hui Boh E, Ntalla I, Pitkänen N, Mahajan A, van Leeuwen EM, Joro R, Lagou V, Nodzenski M, Diver LA, Zondervan KT, Bustamante M, Marques-Vidal P, Mercader JM, Bennett AJ, Rahmioglu N, Nyholt DR, Ma

RCW, Tam CHT, Tam WH, CHARGE Consortium Hematology Working Group, Ganesh SK, van Rooij FJA, Jones SE, Loh PR, Ruth KS, Tuke MA, Tyrrell J, Wood AR, Yaghootkar H, Scholtens DM, Paternoster L, Prokopenko I, Kovacs P, Atalay M, Willems SM, Panoutsopoulou K, Wang X, Carstensen L, Geller F, Schraut KE, Murcia M, van Beijsterveldt CEM, Willemsen G, Appel EVR, Fonvig CE, Trier C, Tiesler CMT, Standl M, Kutalik Z, Bonas-Guarch S, Hougaard DM, Sánchez F, Torrents D, Waage J, Hollegaard MV, de Haan HG, Rosendaal FR, Medina-Gomez C, Ring SM, Hemani G, McMahon G, Robertson NR, Groves CJ, Langenberg C, Luan J, Scott RA, Zhao JH, Mentch FD, MacKenzie SM, Reynolds RM, Lowe Jr WL, Tönjes A, Stumvoll M, Lindi V, Lakka TA, van Duijn CM, Kiess W, Körner A, Sørensen TIA, Niinikoski H, Pahkala K, Raitakari OT, Zeggini E, Dedoussis GV, Teo YY, Saw SM, Melbye M, Campbell H, Wilson JF, Vrijheid M, de Geus EJCN, Boomsma DI, Kadarmideen HN, Holm JC, Hansen T, Sebert S, Hattersley AT, Beilin LJ, Newnham JP, Pennell CE, Heinrich J, Adair LS, Borja JB, Mohlke KL, Eriksson JG, Widén EE, Kähönen M, Viikari JS, Lehtimäki T, Vollenweider P, Bønnelykke K, Bisgaard H, Mook-Kanamori DO, Hofman A, Rivadeneira F, Uitterlinden AG, Pisinger C, Pedersen O, Power C, Hyppönen E, Wareham NJ, Hakonarson H, Davies E, Walker BR, Jaddoe VWV, Jarvelin MR, Grant SFA, Vaag AA, Lawlor DA, Frayling TM, Davey Smith G, Morris AP, Ong KK, Felix JF, Timpson NJ, Perry JRB, Evans DM, McCarthy MI, Freathy RM (2016) Genome-wide associations for birth weight and correlations with adult disease. Nature 538:248-52

Kong A, Thorleifsson G, Frigge ML, Vilhjalmsson BJ, Young AI, Thorgeirsson TE, Benonisdottir S, Oddsson A, Halldorsson BV, Masson G (2018) The nature of nurture: Effects of parental genotypes. Science 359:424-428

Krokstad S, Langhammer A, Hveem K, Holmen T, Midthjell K, Stene T, Bratberg G, Heggland J, Holmen J (2012) Cohort profile: the HUNT study, Norway. Int J Epidemiol 42:968-977

Laurin C, Cuellar-Partida G, Hemani G, Smith GD, Yang J, Evans DM (2018) Partitioning Phenotypic Variance Due to Parent-of-Origin Effects Using Genomic Relatedness Matrices. Behav Genet 48:67-79

Lawson HA, Cheverud JM, Wolf JB (2013) Genomic imprinting and parent-of-origin effects on complex traits. Nat Rev Genet 14:609-617

Lunde A, Melve KK, Gjessing HK, Skjærven R, Irgens LM (2007) Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. Am J Epidemiol 165:734-741

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Sunderland, MA

Magnus P (1984a) Causes of variation in birth weight: a study of offspring of twins. Clin Genet 25:15-24

Magnus P (1984b) Further evidence for a significant effect of fetal genes on variation in birth weight. Clin Genet 26:289-296

Magnus P, Birke C, Vejrup K, Haugan A, Alsaker E, Daltveit AK, Handal M, Haugen M, Høiseth G, Knudsen GP (2016) Cohort profile update: the Norwegian mother and child cohort study (MoBa). Int J Epidemiol 45:382-388

Maher B (2008) Personal genomes: The case of the missing heritability. Nature 456:18-21

Masuda K, Osada H, Iitsuka Y, Seki K, Sekiya S (2002) Positive association of maternal G protein β3 Subunit 825T allele with reduced head circumference at birth. Pediatr Res 52:687-691

Mather K, Jinks JL (1982) Components of variation. In Biometrical Genetics. Springer, pp 135-175

Meyer K (1992) Variance components due to direct and maternal effects for growth traits of Australian beef cattle. Livest Prod Sci 31:179-204

Moen G-H, Hemani G, Warrington NM, Evans DM (2019) Calculating power to detect maternal and offspring genetic effects in genetic association studies. Behav Genet 49:327-339

Morison IM, Ramsay JP, Spencer HG (2005) A census of mammalian imprinting. Trends Genet 21:457-465

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, De Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-575

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rando OJ (2012) Daddy issues: paternal effects on phenotype. Cell 151:702-708

Rice F, Thapar A (2010) Estimating the relative contributions of maternal genetic, paternal genetic and intrauterine factors to offspring birth weight and head circumference. Early Hum Dev 86:425-432

Richmond RC, Al-Amin A, Smith GD, Relton CL (2014) Approaches for drawing causal inferences from epidemiological birth cohorts: a review. Early Hum Dev 90:769-780

Schwarzer G (2007) meta: An R package for meta-analysis. R News 7:40-45

Smith GD (2008) Assessing intrauterine influences on offspring health outcomes: can epidemiological studies yield robust findings? Basic Clin Pharmacol Toxicol 102, 245-256

Smith GD (2012) Negative control exposures in epidemiological studies. Epidemiology 23, 350-351

Speed D, Cai N, Johnson MR, Nejentsev S, Balding DJ, Consortium U (2017) Reevaluation of SNP heritability in complex human traits. Nat Genet 49:986-992

Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M (2015) UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 12(3):e1001779

Taal HR, St Pourcain B, Thiering E, Das S, Mook-Kanamori DO, Warrington NM, Kaakinen M, Kreiner-Møller E, Bradfield JP, Freathy RM, Geller F, Guxens M, Cousminer DL, Kerkhof M, Timpson NJ, Ikram MA, Beilin LJ, Bønnelykke K, Buxton JL, Charoen P, Chawes BLK, Eriksson J, Evans DM, Hofman A, Kemp JP, Kim CE, Klopp N, Lahti J, Lye SJ, McMahon G, Mentch FD, Müller M, O'Reilly PF, Prokopenko I, Rivadeneira F, Steegers EAP, Sunyer J, Tiesler C, Yaghootkar H; Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Consortium, Breteler MMB, Debette S, Fornage M, Gudnason V, Launer LJ, van der Lugt A, Mosley TH, Seshadri S, Smith AV, Vernooij MW; Early Genetics & Lifecourse Epidemiology (EAGLE) consortium, Blakemore AI, Chiavacci RM, Feenstra B, Fernandez-Benet J, Grant SFA, Hartikainen AL, van der Heijden AJ, Iñiguez C, Lathrop M, McArdle WL, Mølgaard A, Newnham JP, Palmer LJ, Palotie A, Pouta A, Ring SM, Sovio U, Standl M, Uitterlinden AG, Wichmann HE, Vissing NH, DeCarli C, van Duijn CM, McCarthy MI, Koppelman GH, Estivill X, Hattersley AT, Melbye M, Bisgaard H, Pennell CE, Widen E, Hakonarson H, Smith GD, Heinrich J, Jarvelin MR. Early Growth Genetics (EGG) Consortium, Jaddoe VWV (2012) Common variants at 12q15 and 12q24 are associated with infant head circumference. Nat Genet 44:532-538Timpson NJ, Greenwood CM, Soranzo N,

Lawson DJ, Richards JB (2018) Heritable contributions versus genetic architecture. Nat Rev Genet 19:185

van der Valk RJ, Kreiner-Møller E, Kooijman MN, Guxens M, Stergiakouli E, Sääf A, Bradfield JP, Geller F, Hayes MG, Cousminer DL (2014) A novel common variant in DCST2 is associated with length in early life and height in adulthood. Hum Mol Genet 24:1155-1168

Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, Bacelis J, Peng S, Hao K, Feenstra B, Wood AR, Mahajan A, Tyrrell J, Robertson NR, Rayner W, Qiao Z, Moen GH, Vaudel M, Marsit CJ, Chen J, Nodzenski M, Schnurr TM, Zafarmand MH, Bradfield JP, Grarup N, Kooijman MN, Li-Gao R, Geller F, Ahluwalia TS, Paternoster L, Rueedi R, Huikari V, Hottenga JJ, Lyytikäinen LP, Cavadino A, Metrustry S, Cousminer DL, Wu Y, Thiering E, Wang CA, Have CT, Vilor-Tejedor N, Joshi PK, Painter JN, Ntalla I, Myhre R, Pitkänen N, van Leeuwen EM, Joro R, Lagou V, Richmond RC, Espinosa A, Barton SJ, Inskip HM, Holloway JW, Santa-Marina L, Estivill X, Ang W, Marsh JA, Reichetzeder C, Marullo L, Hocher B, Lunetta KL, Murabito JM, Relton CL, Kogevinas M, Chatzi L, Allard C, Bouchard L, Hivert MF, Zhang G, Muglia LJ, Heikkinen J, Early Growth Genetics (EGG) Consortium, Morgen CS, van Kampen AHC, van Schaik BDC, Mentch FD, Langenberg C, Luan J, Scott RA, Hua Zhao JH, Hemani G, Ring SM, Bennett AJ, Gaulton KJ, Fernandez-Tajes J, van Zuydam NR, Medina-Gomez C, de Haan HG, Rosendaal FR, Kutalik Z, Marques-Vidal P, Das S, Willemsen G, Mbarek H, Müller-Nurasyid M, Standl M, Appel EVR, Fonvig CE, Trier C, van Beijsterveldt CEM, Murcia M, Bustamante M, Bonas-Guarch S, Hougaard DM, Mercader JM, Linneberg A, Schraut KE, Lind PA, Medland SE, Shields BM, Knight BA, Chai JF, Panoutsopoulou K, Bartels M, Sánchez F, Stokholm J, Torrents D, Vinding RK, Willems SM, Atalay M, Chawes BL, Kovacs P, Prokopenko I, Tuke MA, Yaghootkar H, Ruth KS, Jones SE, Loh PR, Murray A, Weedon MN, Tönjes A, Stumvoll M, Michaelsen KF, Eloranta AM, Lakka TA, van Duijn CM, Kiess W, Körner A, Niinikoski H, Pahkala K, Raitakari OT, Jacobsson B, Zeggini E, Dedoussis GV, Teo YY, Saw SM, Montgomery GW, Campbell H, Wilson JF, Vrijkotte TGM, Vrijheid M, de Geus EJCN, Geoffrey Hayes M, Kadarmideen HN, Holm JC, Beilin LJ, Pennell CE, Heinrich J, Adair LS, Borja JB, Mohlke KL, Eriksson JG, Widén EE, Hattersley AT, Spector TD, Kähönen M, Viikari JS, Lehtimäki T, Boomsma DI, Sebert S, Vollenweider P, Sørensen TIA, Bisgaard H, Bønnelykke K, Murray JC, Melbye M, Nohr EA, Mook-Kanamori DO, Rivadeneira F, Hofman A, Felix JF, Jaddoe, VWV, Hansen T, Pisinger C, Vaag AA, Pedersen O, Uitterlinden AG, Järvelin MR, Power C, Hyppönen E, Scholtens DM, Lowe Jr WL, Smith GD, Timpson NJ, Morris AP, Wareham NJ,

Hakonarson H, Grant SFA, Frayling TM, Lawlor DA, Njølstad PR, Johansson S, Ong KK, McCarthy MI, Perry JRB, Evans DM, Freathy RM (2019) Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. Nat Genet 51:804-814.

Warrington NM, Freathy RM, Neale MC, Evans DM (2018a) Using structural equation modelling to jointly estimate maternal and fetal effects on birthweight in the UK Biobank. Int J Epidemiol 47:1229-1241

Warrington NM, Richmond R, Fenstra B, Myhre R, Gaillard R, Paternoster L, Wang CA, Beaumont RN, Das S, Murcia M (2018b) Maternal and fetal genetic contribution to gestational weight gain. Int J Obes (Lond) 42:775-784

Wolf JB, Wade MJ (2009) What are maternal effects (and what are they not)? Philos Trans R Soc Lond B Biol Sci 364:1107-1115

Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, Madden PA, Heath AC, Martin NG, Montgomery GW (2010) Common SNPs explain a large proportion of the heritability for human height. Nat Genet 42:565-569

Yang J, Lee SH, Goddard ME, Visscher PM (2011) GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet 88:76-82

Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, de Andrade M, Feenstra B, Feingold E, Hayes MG, Hill WG, Landi MT, Alonso A, Lettre G, Lin P, Ling H, Lowe W, Mathias RA, Melbye M, Pugh E, Cornelis MC, Weir BS, Goddard ME, Visscher PM (2011) Genome partitioning of genetic variation for complex traits using common SNPs. Nat Genet 43:519-525

Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL (2014) Advantages and pitfalls in the application of mixed-model association methods. Nat Genet 46:100–106

York TP, Eaves LJ, Lichtenstein P, Neale MC, Svensson A, Latendresse S, Långström N, Strauss III JF (2013) Fetal and maternal genes' influence on gestational age in a quantitative genetic analysis of 244,000 Swedish births. Am J Epidemiol 178:543-550

Young AI, Frigge ML, Gudbjartsson DF, Thorleifsson G, Bjornsdottir G, Sulem P, Masson G, Thorsteinsdottir U, Stefansson K, Kong A (2018) Relatedness disequilibrium regression estimates heritability without environmental bias. Nat Genet 50:1304-1310

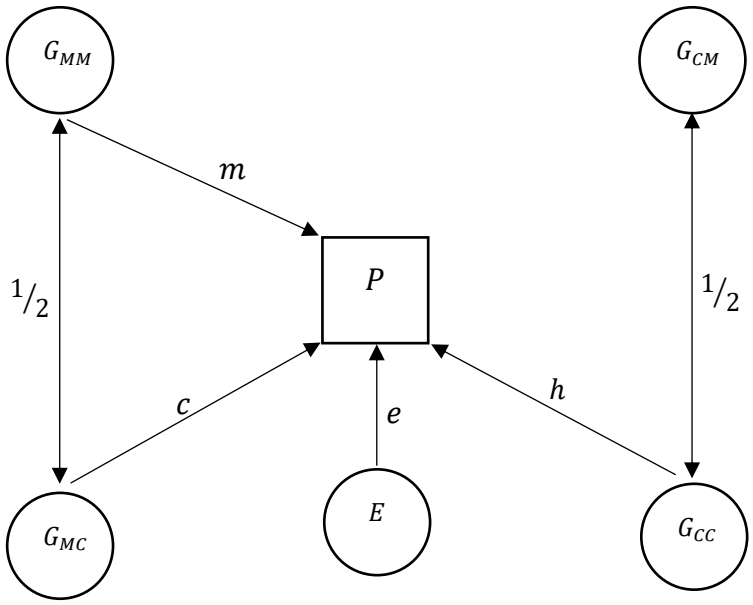

Figure 1. Path model illustrating the relationship between latent maternal and offspring genetic factors and the observed offspring phenotype for a single mother-offspring pair. G_{MM} represents maternal genotypes at loci that exert an effect on the offspring phenotype P via path m (i.e. maternal genetic effects). G_{MC} represents these same loci in offspring and the degree to which these loci affect the offspring phenotype is quantified by the path coefficient c. G_{CC} represents offspring genotypes at loci that induce no maternal effects when present in the mother's genome but contribute directly to the offspring phenotype P via path h. Finally, G_{CM} represents the maternal loci that do not affect the offspring phenotype P. The same latent genetic factors in mothers and offspring are expected to correlate 0.5 with each other. Finally, E represents the effect of residual environmental and untagged genetic factors that contribute to phenotype P through the causal path e.

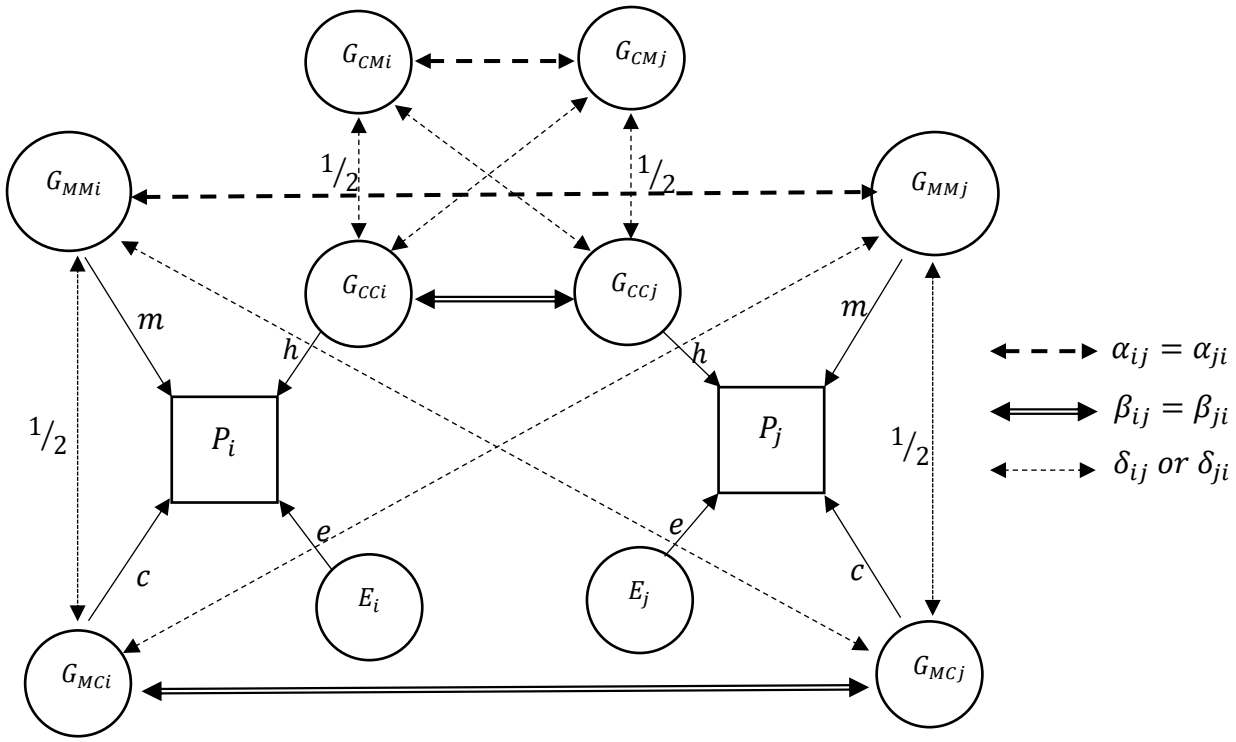

Figure 2. M-GCTA path model showing the relationship between two unrelated mother-offspring pairs (e.g. pair *i* and pair *j*). The correlations between the latent genetic factors are defined as follows: α_{ij} is the genomic relatedness coefficients between mothers of pair *i* and *j*, β_{ij} is the relatedness coefficient between the offspring of pair *i* and *j*, and δ_{ij} is the relatedness coefficients between the mother of pair *i* and the child of pair *j*.

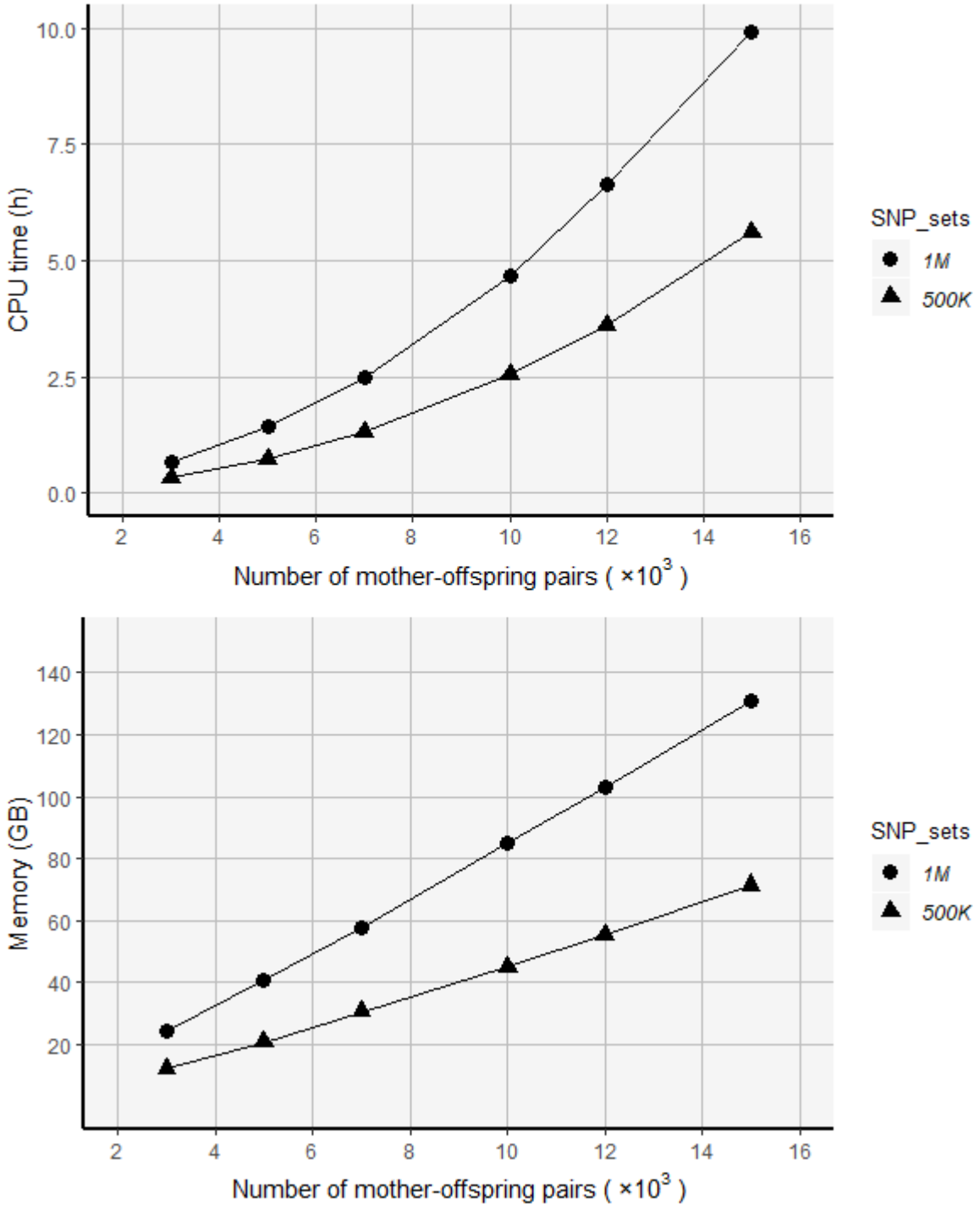

Figure 3. CPU times and Memory Requirements for the M-GCTA software. CPU times (upper panel) and memory use (lower panel) are plotted for runs on subsets of simulated data sets with increasing sample size and number of markers (*m*=500,000 and 1,000,000, respectively). Note, the x axis refers to *N*, the number of mother-offspring pairs, the total number of individuals is 2N. Reported CPU times are the medians of five identical runs using 20 cores of a 3.2 GHz Intel Xeon E5-2667 v3 processor. Reported CPU times are the total time required to compute the genetic relationship matrix (GRM) between mothers and offspring, and perform REML

analysis. Note that the CPU time and memory use may vary by a small factor as a function of the computing environment. Software version: M-GCTA, beta 0.1.1. Numerical data with more details are provided in Supplementary Table 2.

Table 1. Comparison of variance components estimates from mother-offspring simulated data using the M-GCTA software package, ordinary GCTA and the OpenMx package. Parameter estimates are presented with standard errors in parentheses (1000 simulations). Abbreviations: σ_M^2 variance due to tagged maternal genetic effects on child's phenotype, σ_O^2 variance due to tagged direct offspring genetic effects, σ_D (twice the) covariance between maternal and offspring genetic effects, σ_e^2 variance due to residual effects. Note that σ_D can be positive or negative, and represents twice the covariance between maternal and offspring genetic effects. lnL refers to the log likelihood. LRT= $-2 \times (L\text{-}L_0)$, where LRT is the likelihood ratio test, L is the likelihood of the current model and L_0 refers to full/saturated model. *df* is the degrees of freedom and equals the difference in the number of parameters between the two models. *m*, *h*, *c*, *e* are the estimated path coefficients in Figure 1. Under this formulation $\sigma_M^2 = m^2$, $\sigma_O^2 = h^2 + c^2$, $\sigma_D = mc$, and $\sigma_E^2 = e^2$.

	σ_M^2	σ_O^2	σ_D	σ_E^2	-2lnL	LRT (χ^2)	*df*	*p*-value
Scenario 1								
Simulated values	15.46 (0.71)	15.47 (0.71)	6.15 (1.00)	4.00 (0.18)	–	–	–	–
M-GCTA software								
Full model (default)	15.49 (0.81)	15.48 (0.82)	6.16 (0.51)	4.01 (0.25)	3588.435	–	–	–
$\sigma_D = 0$	16.27 (0.81)	16.25 (0.83)	–	3.93 (0.24)	3619.216	30.781	1	1.44×10^{-8}
$\sigma_M^2 = \sigma_D = 0$	–	25.45 (1.51)	–	15.65 (0.84)	4231.646	643.211	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	25.49 (1.52)	–	–	15.65 (0.81)	4231.817	643.382	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	41.12 (1.88)	4717.640	1129.205	3	$<2x10^{-16}$
Standard GCTA software								
Full Model	15.49 (0.81)	15.48 (0.82)	6.16 (0.51)	4.01 (0.25)	3588.435	–	–	–
$\sigma_D = 0$	16.27 (0.81)	16.25 (0.83)	–	3.93 (0.24)	3619.216	30.781	1	1.44×10^{-8}
$\sigma_M^2 = \sigma_D = 0$	–	25.45 (1.51)	–	15.65 (0.84)	4231.646	643.211	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	25.49 (1.52)	–	–	15.65 (0.81)	4231.817	643.382	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	41.12 (1.88)	4717.640	1129.205	3	$<2x10^{-16}$

OpenMx package								
Full Model	15.49 (0.81)	15.48 (0.82)	6.16 (0.51)	4.00 (0.25)	5421.333	–	–	–
$\sigma_D = 0$	16.27 (0.81)	16.25 (0.83)	–	3.93 (0.24)	5452.130	30.797	1	1.43×10^{-8}
$\sigma_M^2 = \sigma_D = 0$	–	25.45 (1.51)	–	15.64 (0.84)	6065.599	644.266	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	25.49 (1.52)	–	–	15.63 (0.81)	6065.769	644.436	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	41.08 (1.88)	6552.324	1130.991	3	$<2x10^{-16}$
Alternative model specification with the constraint $\sigma_D = mc$	*m*	*h*	*c*	*e*				
Full Model	3.93 (0.10)	3.60 (0.13)	1.57 (0.14)	2.00 (0.06)	5421.333	–	–	–
$c = 0$	4.03 (0.10)	4.03 (0.10)	–	1.98 (0.06)	5452.130	30.797	1	1.43×10^{-8}
$m = c = 0$	–	5.03 (0.35)	–	3.95 (0.11)	6065.599	644.266	2	$<2x10^{-16}$
$h = c = 0$	5.00 (0.72)	–	–	3.95 (0.10)	6065.769	644.436	2	$<2x10^{-16}$
$m = h = c = 0$	–	–	–	6.41 (0.15)	6552.324	1130.991	3	$<2x10^{-16}$
Scenario 2								
	σ_M^2	σ_O^2	σ_D	σ_E^2	-2lnL	LRT (χ^2)	*df*	*p*-value
Simulated values	15.50 (0.72)	15.47 (0.71)	-6.20 (1.00)	4.00 (0.18)	–	–	–	–
M-GCTA software								
Full model (default)	15.47 (0.83)	15.47 (0.83)	-6.16 (0.65)	4.00 (0.25)	3562.294	–	–	–
$\sigma_D = 0$	14.60 (0.80)	14.60 (0.80)	–	4.10 (0.26)	3595.715	33.421	1	3.71×10^{-9}
$\sigma_M^2 = \sigma_D = 0$	–	13.16 (1.09)	–	15.62 (0.83)	4099.941	537.647	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	13.13 (1.08)	–	–	15.65 (0.81)	4100.851	538.557	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	28.79 (1.28)	4361.639	799.344	3	$<2x10^{-16}$
Standard GCTA software								
Full Model	15.47 (0.83)	15.47 (0.83)	-6.16 (0.65)	4.00 (0.25)	3562.294	–	–	–
$\sigma_D = 0$	14.60 (0.80)	14.60 (0.80)	–	4.10 (0.26)	3595.715	33.421	1	3.71×10^{-9}
$\sigma_M^2 = \sigma_D = 0$	–	13.16 (1.09)	–	15.62 (0.83)	4099.941	537.647	2	$<2x10^{-16}$

$\sigma_O^2 = \sigma_D = 0$	13.13 (1.08)	–	–	15.65 (0.81)	4100.851	538.557	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	28.79 (1.28)	4361.639	799.344	3	$<2x10^{-16}$
OpenMx package								
Full Model	15.47 (0.83)	15.47 (0.83)	-6.16 (0.65)	4.00 (0.25)	5395.222	–	–	–
$\sigma_D = 0$	14.61 (0.80)	14.61 (0.80)	–	4.09 (0.26)	5428.657	33.435	1	3.68×10^{-9}
$\sigma_M^2 = \sigma_D = 0$	–	13.16 (1.09)	–	15.60 (0.83)	5933.860	538.638	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	13.13 (1.08)	–	–	15.63 (0.81)	5934.771	539.549	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	28.76 (1.28)	6195.967	800.745	3	$<2x10^{-16}$
Alternative model specification	*m*	*h*	*c*	*e*				
Full Model	3.93 (0.11)	3.60 (0.09)	-1.57 (0.14)	2.00 (0.06)	5395.222	–	–	–
$c = 0$	3.82 (0.10)	3.82 (0.10)	–	2.02 (0.06)	5428.657	33.435	1	3.68×10^{-9}
$m = c = 0$	–	3.61 (0.35)	–	3.95 (0.10)	5933.860	538.638	2	$<2x10^{-16}$
$h = c = 0$	3.62 (0.15)	–	–	3.95 (0.10)	5934.771	539.549	2	$<2x10^{-16}$
$m = h = c = 0$	–	–	–	5.36 (0.12)	6195.967	800.745	3	$<2x10^{-16}$
Scenario 3								
	σ_M^2	σ_O^2	σ_D	σ_E^2	-2lnL	LRT (χ^2)	*df*	*p*-value
Simulated values	15.48 (0.72)	15.47 (0.73)	-1.21 (1.03)	4.00 (0.18)	–	–	–	–
M-GCTA software								
Full model (default)	15.48 (0.81)	15.47 (0.81)	-1.26 (0.60)	4.00 (0.25)	3602.236	–	–	–
$\sigma_D = 0$	15.31 (0.78)	15.30 (0.78)	–	4.01 (0.25)	3603.750	1.514	1	0.11
$\sigma_M^2 = \sigma_D = 0$	–	18.07 (1.24)	–	15.61 (0.78)	4159.793	557.557	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	18.07 (1.29)	–	–	15.59 (0.82)	4158.808	556.573	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	33.69 (1.51)	4518.627	916.391	3	$<2x10^{-16}$
Standard GCTA software								
Full Model	15.48 (0.81)	15.47 (0.81)	-1.26 (0.60)	4.00 (0.25)	3602.236	–	–	–

$\sigma_D = 0$	15.31 (0.78)	15.30 (0.78)	–	4.01 (0.25)	3603.750	1.514	1	0.11
$\sigma_M^2 = \sigma_D = 0$	–	18.07 (1.24)	–	15.61 (0.78)	4159.793	557.557	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	18.07 (1.29)	–	–	15.59 (0.82)	4158.808	556.573	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	33.69 (1.51)	4518.627	916.391	3	$<2x10^{-16}$
OpenMx package								
Full Model	15.48 (0.81)	15.47 (0.81)	-1.26 (0.60)	3.99 (0.25)	5435.161	–	–	–
$\sigma_D = 0$	15.31 (0.78)	15.30 (0.78)	–	4.00 (0.25)	5436.676	1.515	1	0.11
$\sigma_M^2 = \sigma_D = 0$	–	18.07 (1.24)	–	15.59 (0.78)	5993.729	558.567	2	$<2x10^{-16}$
$\sigma_O^2 = \sigma_D = 0$	18.07 (1.29)	–	–	15.57 (0.82)	5992.741	557.580	2	$<2x10^{-16}$
$\sigma_M^2 = \sigma_O^2 = \sigma_D = 0$	–	–	–	33.66 (1.51)	6353.112	917.951	3	$<2x10^{-16}$
Alternative model specification	*m*	*h*	*c*	*e*				
Full Model	3.93 (0.10)	3.92 (0.10)	-0.32 (0.15)	2.00 (0.06)	5435.161	–	–	–
$c = 0$	3.91 (0.10)	3.91 (0.10)	–	2.00 (0.06)	5436.676	1.515	1	0.11
$m = c = 0$	–	4.19 (0.72)	–	3.95 (0.10)	5993.729	558.567	2	$<2x10^{-16}$
$h = c = 0$	4.21 (0.62)	–	–	3.95 (0.10)	5992.741	557.580	2	$<2x10^{-16}$
$m = h = c = 0$	–	–	–	5.80 (0.13)	6353.112	917.951	3	$<2x10^{-16}$

1

Table 2. Results of applying M-GCTA software on offspring birth weight in the ALSPAC and MoBa cohorts and the meta-analysis of the two. Results are presented as standardized variance components.

	ALSPAC (N = 4310 pairs)		MoBa (N = 7910 pairs)		Meta-analysis				
Variance component	Estimate	s.e	Estimate	s.e	Estimate	s.e	I^2	Q	$P_{\text{heterogeneity}}$
σ_M^2	0.05	0.09	0.08	0.06	0.07	0.05	0.0%	0.08	0.78
σ_O^2	0.23	0.10	0.29	0.06	0.27	0.05	0.0%	0.26	0.61
σ_D	0.08	0.07	0.04	0.05	0.05	0.04	0.0%	0.22	0.64
σ_G^2	0.37	0.10	0.40	0.07	0.39	0.05	0.0%	0.06	0.81

Abbreviations: σ_G^2 variance due to total tagged genetic effects on child's phenotype; σ_M^2 variance due to tagged maternal genetic effects; σ_O^2 variance due to tagged offspring genetic effects; σ_D twice the covariance between tagged maternal and fetal genetic sources of variation; σ_E^2 variance due to residual effects; s.e. standard error; I^2, the I square statistic, measures the proportion of the variability in estimates that is due to heterogeneity; Q, the Cochran Q test (standard chi-squared test) statistic for heterogeneity; $P_{\text{heterogeneity}}$ is the corresponding heterogeneity *p*-value for the Q test statistic.