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Abstract:
Maternal and offspring cell contact at the site of placentation presents a plausible setting for maternal-fetal genotype (MFG) interactions affecting fetal growth. We test hypotheses regarding killer cell immunoglobulin-like receptor (KIR) and HLA-C MFG effects on human birth weight by extending the quantitative MFG (QMFG) test.Until recently, association testing for MFG

interactions had limited applications. To improve the ability to test for these interactions, we developed the extended QMFG test, a linear mixed-effect model that can use multi-locus genotype data from families.We demonstrate the extended QMFG test's statistical properties. We also show that if an offspring-only model is fit when MFG effects exist, associations can be missed or misattributed. Furthermore, imprecisely modeling the effects of both KIR and HLA-C could result in a failure to replicate if these loci's allele frequencies differ among populations. To further illustrate the extended QMFG test's advantages, we apply the extended QMFG test to a UK cohort study and the Norwegian Mother and Child Cohort (MoBa) study.We find a significant KIR-HLA-C interaction effect on birth weight. More generally, the QMFG test can detect genetic associations that may be missed by standard genome-wide association studies for quantitative traits.

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Human Birth Weight and Reproductive Immunology: Testing for Interactions between Maternal and Offspring *KIR* and *HLA-C* Genes

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Keywords

Maternal-fetal genotype interaction · KIR · HLA · Gene-gene interaction · Family-based association · Quantitative traits · Variance components · Intergenerational effects · The Norwegian Mother and Child Cohort (MoBa) Study

Abstract

Background/Aims: Maternal and offspring cell contact at the site of placentation presents a plausible setting for maternal-fetal genotype (MFG) interactions affecting fetal growth. We test hypotheses regarding killer cell immunoglobulin-like receptor *(KIR)* and *HLA-C* MFG effects on human birth weight by extending the quantitative MFG (QMFG) test. ***Methods:*** Until recently, association testing for MFG interactions had limited applications. To improve the ability to test for these interactions, we developed the extended QMFG test, a linear mixed-effect model that can use multi-locus genotype data from families. ***Results:*** We demonstrate the extended QMFG test's statistical properties. We also show that if an offspring-only model is fit when MFG effects exist, associations can be missed or misattributed. Furthermore, imprecisely modeling the effects of both *KIR* and *HLA-C* could result in a failure to replicate if these loci's allele frequencies differ among populations. To further illustrate the extended QMFG test's advantages, we apply the extended QMFG test to a UK cohort study and the Norwegian Mother and Child Cohort (MoBa) study. ***Conclusion:*** We find a significant *KIR–HLA-C* interaction effect on birth weight. More generally, the QMFG test can detect genetic associations that may be missed by standard genome-wide association studies for quantitative traits.

Introduction

Complex familial disorders result from interactions between environmental and genetic factors. One such interaction, which can contribute to disease susceptibility and variation in quantitative traits, occurs when the fetal environment is modified by the interaction of proteins expressed from maternal and offspring genes. During preg-

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nancy, the maternal and fetal semiallogenic cells come into direct contact, resulting in an intricate connection between the 2 individuals. Depending on the combination of maternal and offspring genotypes, maternal immune recognition of fetal cells is one possible form of maternal–fetal genotype (MFG) interaction. MFG interactions can alter the conditions in which the fetus develops and have the potential to impact offspring traits. MFG interactions have been shown to be involved in perinatal diseases [1–5] as well as those that occur later in life [6–15].

One example of an MFG interaction involves genes that are thought to regulate human birth weight [16–18]. Besides variation in birth weight being of intrinsic interest in human evolution, extremes in birth weight are strongly associated with obstetric complications and perinatal mortality. For instance, whereas high birth weight causes obstructed labor [19], preeclampsia and fetal growth restriction are 2 consequences of poor placentation in early pregnancy [20]. Trophoblast invasion and spiral artery transformation are important processes that affect the maternal blood supply to the placenta and therefore impact fetal growth. Uterine natural killer (uNK) cells are distinctive maternal lymphocytes, which are only found in a woman's mucosal lining during placentation, that accumulate around the invasive trophoblast cells. Human maternal uNK receptors, which are encoded by the killer cell immunoglobulin-like receptor *(KIR)* gene family, can bind to human leukocyte antigens (HLA) expressed by fetal trophoblast cells thus forming an immune interaction between maternal and fetal cells [21]. Although other genes [22, 23] and environmental factors [24–27] are very likely to influence birth weight, there is evidence that this interaction between uNK KIR and trophoblast HLA influences the balance between restricted and amplified fetal placental cell invasion, transformation of spiral arteries, and, in turn, fetal development.

Trophoblast cells express 3 HLA class I molecules: 2 nonclassical (HLA-G and HLA-E) and 1 classical (HLA-C) [28]. Of these 3, only HLA-C is polymorphic. Although there are 2,902 known *HLA-C* alleles, they can be placed into 2 groups, *C1* and *C2*, which differ in their epitopes, when considering their effect on birth weight [29]. Allotypic recognition of C1 or C2 epitopes varies by *KIR* gene. It is important to note that beyond the influence of the offspring *HLA-C* genotype, the maternal *HLA-C* genotype may also play an important role in placental development. It is hypothesized that, during uNK cell development, the maternal KIR interacts with her own HLA-C molecules, thus "educating" or "licensing" her uNK cells and changing the way they interact with her offspring's HLA-C molecules during placentation [16, 18, 30, 31]. That uNK education occurs in the uterus is supported by evidence that maternal MHC (major histocompatibility complex) class I antigens educate the uNK cells in murine models [32].

Models involving *KIR* genes together with *HLA-C* have been found to be essential in explaining associations with pregnancy disorders including preeclampsia, fetal growth restriction, and recurrent miscarriage [16, 33, 34]. Fifteen *KIR* genes have been identified, mapping to chromosome 19q13.4 within the 1-Mb leukocyte receptor complex [35]. *KIR* genes are denoted by the number of extracellular immunoglobulin domains (2D or 3D) and the length of the cytoplasmic tail (L for long and S for short). This region of the human genome is highly variable. In fact, the number and nature of the genes in the region can differ between individuals. Hiby et al. [17] found that a parsimonious model, which shows the presence or absence of the gene *KIR2DS1* in the mother, was sufficient to present the interaction of *KIR* and *HLA-C* as a predictor of birth weight in normal pregnancies using subjects from the UK and Norway. Thus, for simplicity, in this article, we refer to 2 alleles, – and +, representing the absence and presence of the *KIR2DS1* gene on a chromosome, respectively.

Until recently, association testing for MFG interactions with quantitative traits was limited to retrospective likelihood designs [36–38], which model the distribution of genotypes conditional on the phenotypes [39]. These designs are direct extensions of association testing for MFG interactions with qualitative traits [40, 41]. In addition to potential difficulties in parameter interpretation, such approaches have typically been limited to case-parent trios and cannot easily account for the main effects of other covariates [42, 43]. To address these modeling limitations, the quantitative MFG (QMFG) test was developed [43]. This linear mixed-effect approach can quickly and accurately test for various scenarios of joint maternal and offspring effects and can handle pedigrees of any size.

The QMFG test was originally developed to address interactions that occur at a single locus, but this limited its applicability to only a small number of scenarios. Thus, we extend the model to multiple loci in this article. The *KIR–HLA-C* interaction as a predictor of birth weight serves as an interesting and important example where our extension to the QMFG test can provide new insights. We apply the QMFG test to the UK cohort [16, 17, 33, 34] and the Norwegian Mother and Child Study (MoBa) cohort [44] used by Hiby et al. [17] to extend their findings and test the hypothesis of a *KIR–HLA-C* MFG interaction effect on human birth weight.

Methods

The QMFG Model

Consider a pedigree f with N_f offspring who are genotyped, phenotyped for a quantitative trait, and have mothers who are genotyped. Note that the pedigree f can be multigenerational so that an offspring can also be a mother (see Fig. 1 for simple examples). We start with the case in which there are no environmental covariates and the variance can be partitioned into 2 components, the additive genetic variance (σ_a^2) and the environmental variance (σ_e^2). The vector of quantitative trait values y_f has N_f entries and can be expressed as

$$y_f = \mu + X_f\beta + A_f + e_f = \nu_f + A_f + e_f. \quad (1)$$

The parameter μ denotes a vector of N_f grand means. X_f is an $N_f \times m$ matrix where the rows correspond to the offspring in pedigree f. Each row contains a single 1 entry corresponding to the observed maternal-offspring genotypes and the rest of the entries are 0. β is the m-component column vector of regression coefficients for all possible MFG combinations. A_f and e_f are vectors of random effects such that $A_f \sim MVN(0, 2\sigma_a^2\,\Phi_f)$, where Φ_f is an $N_f \times N_f$ matrix of kinship coefficients, and $e_f \sim MVN(0, \sigma_e^2 I)$, where I is an $N_f \times N_f$ identity matrix [45]. This model can be extended to include other variance components such as shared environmental variance or dominance genetic variance or additional covariates [45]. In the case of birth weight, additional covariates may include gestational age, sex, and ethnicity/cohort.

The number of genetic parameters in β depends on the model. Inspired by Hiby et al. [17], we model the MFG effects of a single biallelic locus (i.e. *HLA-C*) so that there are 7 possible mother-offspring genotype combinations. Note that for the birth weight example, we refer to the *HLA-C* alleles as *C1* and *C2*, which more precisely corresponds to the classification of the numerous *HLA-C* alleles into 2 groups of *HLA-C* alleles based on having a C1 or C2 epitope. If we add to the model the interaction of this locus with a maternally acting biallelic locus (i.e. alleles – and + representing the absence or presence of *KIR2DS1*), then we have 21 mother-offspring genotype combinations. The vector of QMFG regression coefficients for these 2 loci therefore consists of parameters of the form β_{ijk} where i denotes the number of maternal *KIR2DS1*+ alleles, j denotes the number of maternal *C2* alleles, and k denotes the number of offspring *C2* alleles (Table 1, column 4). As with the single SNP version of the QMFG test, one of the parameters for the MFG effects is made the reference state (in our case, we make $\beta_{000} = 0$ the reference state, so 0 copies of the variant alleles, + and *C2*) to avoid nonidentifiability.

Model Descriptions

A variety of models (Models 0–9, Tables 1 and 2) are fit to simulated data. All models include parameters for a grand mean (μ), sex effect (β_{sex}), and variance components (σ_a^2 and σ_e^2). To make estimation practical, we impose constraints on the 20 QMFG parameters based on our prior understanding of a possible underlying immune response. The most general *KIR-HLA-C* model that we consider for birth weight, Model 1, imposes restrictions so that there are 3 freely estimated parameters: (1) an effect for the maternal *KIR*, modeled as a + allele dominant effect, (2) β_{KIR}, an effect for those offspring with more *C2* than their mother, denoted by β_{more}, and (3) a *KIR–HLA-C* interaction effect when the mother has at least one + allele and the offspring has more *C2* alleles than their mother, β_{int} (Table 1, column 5). Thus, $\beta_{001} = \beta_{012} = \beta_{more}$, $\beta_{i00} = \beta_{i10} = \beta_{i11} = \beta_{i21} = \beta_{KIR}$ for $i = 1, 2$, and $\beta_{i01} = \beta_{i12} = \beta_{more} + \beta_{KIR} + \beta_{int}$ for $i = 1, 2$. Otherwise, $\beta_{ijk} = 0$. Models 0 and 2–5 include additional constraints on the parameters of Model 1 (Table 2, rows 4–7) depending upon the hypothesis.

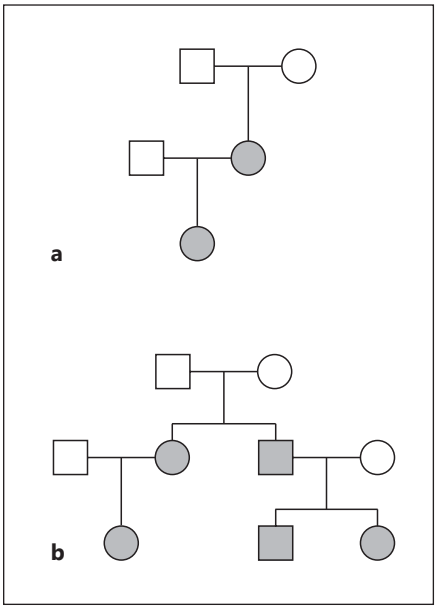

Fig. 1. Family structures. Offspring used in analyses are shaded in gray. **a** Grandparents-parents-offspring family with 2 offspring. **b** Extended 3-generation family with 5 offspring.

Because genome-wide association studies (GWAS) typically include an individual's own genotype but not their mother's genotype (offspring-only), Models 6–9 are used to explore the effects of misspecification when an offspring-only model is incorrectly applied to data generated under an MFG interaction scenario. For Model 6 (Table 1, column 6), offspring *HLA-C* effects are denoted by parameters $\beta_{C1/C2}$ and $\beta_{C2/C2}$. Estimating an additive offspring *HLA-C* effect requires the additional constraint $\beta_{C1/C2} = \beta_{C2/C2}$ (Model 7; Table 2, row 9). For Model 8, we can similarly define offspring + effects by parameters $\beta_{-/+}$ and $\beta_{+/+}$ (Table 2, row 10). Estimating an additive offspring + effect requires the additional constraint $2\beta_{-/+} = \beta_{+/+}$ (Model 9; Table 2, row 11).

Inference

The log-likelihood of the data for pedigree f is

$$L_f = -\frac{1}{2}\ln|\Omega_f| - \frac{1}{2}\left(y_f - \nu_f\right)^t \Omega_f^{-1}\left(y_f - \nu_f\right). \quad (2)$$

The covariance matrix is denoted by $\Omega_f = 2\sigma_a^2\Phi_f + \sigma_e^2 I_f$. Because pedigrees are independent, the log-likelihood, L, of the full dataset is $L = \sum_f L_f$. For inference, we use likelihood ratio tests (LRTs) or Akaike Information Criteria (AIC). LRTs can easily handle comparisons between models and their submodels from Table 2. The LRT statistic is equal to twice the difference in log-likelihoods maximized under the null and alternative models. The LRT asymptotically follows a χ^2 distribution with degrees of freedom equal to the difference in the number of parameters under the null

Table 1. Maternal-offspring genotype combinations and model parameters for the *KIR2DS1* and *HLA-C*

Maternal *KIR2DS1*	Maternal *HLA-C*	Offspring *HLA-C*	General QMFG model	Model 1: maternal *KIR*, more offspring *HLA-C2*, and interaction effects	Model 6: *HLA-C* offspring effects
–/–	*C1/C1*	*C1/C1*	0	0	0
–/–	*C1/C1*	*C1/C2*	β_{001}	β_{more}	$\beta_{C1/C2}$
–/–	*C1/C2*	*C1/C1*	β_{010}	0	0
–/–	*C1/C2*	*C1/C2*	β_{011}	0	$\beta_{C1/C2}$
–/–	*C1/C2*	*C2/C2*	β_{012}	β_{more}	$\beta_{C2/C2}$
–/–	*C2/C2*	*C1/C2*	β_{021}	0	$\beta_{C1/C2}$
–/–	*C2/C2*	*C2/C2*	β_{022}	0	$\beta_{C2/C2}$
–/+	*C1/C1*	*C1/C1*	β_{100}	β_{KIR}	0
–/+	*C1/C1*	*C1/C2*	β_{101}	$\beta_{KIR} + \beta_{more} + \beta_{int}$	$\beta_{C1/C2}$
–/+	*C1/C2*	*C1/C1*	β_{110}	β_{KIR}	0
–/+	*C1/C2*	*C1/C2*	β_{111}	β_{KIR}	$\beta_{C1/C2}$
–/+	*C1/C2*	*C2/C2*	β_{112}	$\beta_{KIR} + \beta_{more} + \beta_{int}$	$\beta_{C2/C2}$
–/+	*C2/C2*	*C1/C2*	β_{121}	β_{KIR}	$\beta_{C1/C2}$
–/+	*C2/C2*	*C2/C2*	β_{122}	β_{KIR}	$\beta_{C2/C2}$
+/+	*C1/C1*	*C1/C1*	β_{200}	β_{KIR}	0
+/+	*C1/C1*	*C1/C2*	β_{201}	$\beta_{KIR} + \beta_{more} + \beta_{int}$	$\beta_{C1/C2}$
+/+	*C1/C2*	*C1/C1*	β_{210}	β_{KIR}	0
+/+	*C1/C2*	*C1/C2*	β_{211}	β_{KIR}	$\beta_{C1/C2}$
+/+	*C1/C2*	*C2/C2*	β_{212}	$\beta_{KIR} + \beta_{more} + \beta_{int}$	$\beta_{C2/C2}$
+/+	*C2/C2*	*C1/C2*	β_{221}	β_{KIR}	$\beta_{C1/C2}$
+/+	*C2/C2*	*C2/C2*	β_{222}	β_{KIR}	$\beta_{C2/C2}$

Table 2. Model descriptions

Type	Model	Genetic effects modeled	Additional constraints	Genetic parameters estimated[4]
Null model[1]	0	None	$\beta_{KIR} = \beta_{more} = \beta_{int} = 0$ or $\beta_{C1/C2} = \beta_{C2/C2} = 0$ or $\beta_{-/+} = \beta_{+/+} = 0$	0
QMFG models[2]	1	Maternal *KIR2DS1* More *HLA-C2* Interaction	None	β_{KIR} β_{more} β_{int}
	2	Interaction	$\beta_{KIR} = \beta_{more} = 0$	β_{int}
	3	Maternal *KIR2DS1* More *HLA-C2*	$\beta_{int} = 0$	β_{KIR} β_{more}
	4	Maternal *KIR2DS1*	$\beta_{more} = \beta_{int} = 0$	β_{KIR}
	5	More *HLA-C2*	$\beta_{KIR} = \beta_{int} = 0$	β_{more}
Standard offspring models[3]	6	Genotypic offspring *HLA-C* effect	None	$\beta_{C1/C2}$ $\beta_{C2/C2}$
	7	Additive offspring *HLA-C* effect	$2\beta_{C1/C2} = \beta_{C2/C2}$	$\beta_{C1/C2}$
	8	Genotypic offspring *KIR2DS1* effect	None	$\beta_{-/+}$ $\beta_{+/+}$
	9	Additive offspring *KIR2DS1* effect	$2\beta_{-/+} = \beta_{+/+}$	$\beta_{-/+}$

[1] Model 0 is a submodel of all other models (Models 1–9). [2] Models 2–5 are submodels of 1 (parameterization shown in Table 1). [3] Model 7 is a submodel of Model 6 and Model 9 is a submodel of Model 8 (parameterization of Model 6 is shown in Table 1). [4] All models additionally estimate a grand mean (μ), sex effect (β_{sex}), and variance components (σ^2_a and σ^2_e).

Table 3. Simulation scenarios

Scenario	*KIR* and *HLA-C* genetic effects	Simulated values[1]
I	None	$\beta_{KIR} = \beta_{more} = \beta_{int} = 0$
II	Interaction	$\beta_{KIR} = \beta_{more} = 0$ $\beta_{int} = \{0.05, 0.07, ..., 0.35\}$
III	Maternal *KIR2DS1* More *HLA-C2* Interaction	$\beta_{KIR} = 0.05$ $\beta_{more} = -0.1$ $\beta_{int} = \{0.05, 0.07, ..., 0.35\}$

[1] All models additionally simulate a grand mean μ (3.5 kg), sex effect (β_{sex} = –0.2 kg if female), and variance components (σ_a^2 = 0.2025 kg^2 and and σ_e^2 = 0.0475 kg^2).

and alternative hypotheses. When comparing models that are not nested, we use the AIC. If c is the number of freely estimated parameters,

$$AIC = 2c - 2L. \quad (3)$$

We then select the model that minimizes the AIC as our best model.

Type I Error, Power, and Model Misspecification

Because our models are linear mixed models, it is straightforward to calculate power using noncentrality parameters (NCPs) [46, 47] when the study sample is composed of mother-offspring pairs or small families (e.g., 2-generation nuclear families). However, as the families get large or we wish to examine the effects of model misspecification, using an NCP is more difficult and so we rely on simulations. We first verify that our simulations are accurate by comparing them to power estimated from the NCP with mother-offspring pairs, nuclear families with 2 siblings, and grandparents-parents-offspring families (for an example of a grandparents-parents-offspring family, see Fig. 1a; for a more detailed explanation of NCP, see online suppl. materials; for all online suppl. material, see www.karger.com/doi/10.1159/000456033). Then, we conduct simulation studies with 300 three-generation 5-offspring families, each having the structure shown in Figure 1b in order to examine the type I error, power, and parameter estimate accuracy for the QMFG test with multiple loci and extended families.

For the purpose of examining the type I error, Scenario I data are simulated under the null hypothesis of no genetic effects at the studied loci. Scenario II data are simulated under conditions so that birth weight is affected only when an offspring has more *C2* than his or her mother and the mother has at least 1 copy of the *KIR2DS1* gene (a dominant-acting maternal + allele). Scenario III involves the same interaction effect in Scenario II as well as separate effects for the dominant-acting maternal + allele that is independent of *HLA-C* and for more *C2* in the offspring than in the mother that is independent of *KIR2DS1*. These simulation scenarios are summarized in Table 3. Unless otherwise specified, the + allele frequency is 20% and the *C2* allele frequency is 30%; these values are chosen based on frequencies observed in white British populations [17].

Each simulation run consists of 2,000 repetitions in which birth weight is simulated with a grand mean μ (3.5 kg) and a sex effect to reduce the average weight if the offspring is female (β_{sex} = –0.2 kg). For the most part, variance components are simulated to allow for a high heritability of birth weight as found by Demerath et al. [48] (σ_a^2 = 0.2025 kg^2 and σ_e^2 = 0.0475 kg^2; residual heritability h^2 = 0.81), although we also examine the effects of lower heritability with and without shared environmental variation.

Genomic control values (λ) are reported as an assessment of type I error accuracy [49]. The significance level used to estimate power is 0.001. If r is the proportion of rejected tests and N is the number of simulation repetitions, the standard errors for the power estimates are calculated as

$$SE = \sqrt{\frac{r(1-r)}{N}}.$$

Proportion of variation explained is estimated empirically by dividing the difference in residual variance under the null and alternative models by the residual variance under the null hypothesis. All simulations and analyses are conducted using the statistical genetics software package Mendel [50].

UK Cohort and the MoBa Study

Details of the participants from the UK cohort study [16, 17, 33, 34] and MoBa [44] (a Norway-wide prospective population-based study of over 110,000 pregnancies conducted by the Norwegian Institutes of Public Health) were previously published. Hiby et al. [17] used a subset of these mother-offspring pairs from the UK and Norway and found a significant association between the maternal *KIR2DS1* and increased offspring birth weight, especially in the presence of more *C2* in offspring than their mothers. However, they did not explicitly test for an interaction between these loci or determine if there were additional independent effects of the loci. In this article, we use the same mother-offspring pairs, *KIR2DS1* genotypes, and *HLA-C* genotypes as Hiby et al. [17] in an effort to allow direct comparison to their analyses and to refine the characterization of the effects. Ethical approval was obtained from the Cambridge Research Ethics Committee (reference No. 01/197 and 05/Q0108/367; Cambridgeshire, UK) for the UK study and from the Regional Committee for Medical Research Ethics and the Data Inspectorate for the MoBa study (reference No. HBREC.2016.12). All adult subjects provided informed written consent. A detailed description of the genotyping is described elsewhere [16, 33, 51]. Both mothers and offspring were genotyped for *HLA-C* and mothers' *KIR* genotypes were determined.

Pregnancies with preeclampsia or fetal growth restriction as well as normal pregnancies were included in these cohorts. In both studies, small babies (<5th centile) were heavily oversampled and large babies (≥90th centile) were slightly oversampled. As in Hiby et al. [17], we run our analyses only on the offspring that had birth weight data >5th centile, were firstborn singletons from full-term births (38–42 weeks), and whose mothers were over the age of 18 and had no medical conditions including preexisting and gestational diabetes, hypertension, renal disease, and autoimmune disease. Of the 1,316 pregnancies that fit these criteria, 7 pregnancies were dropped due to a missing offspring *HLA-C* genotype. Our analyses, therefore, include 1,309 pregnancies. Of these, 403 came from the UK cohort and an additional 906 came from the MoBa cohort. These birth weights were approximately normally distributed within their respective cohorts.

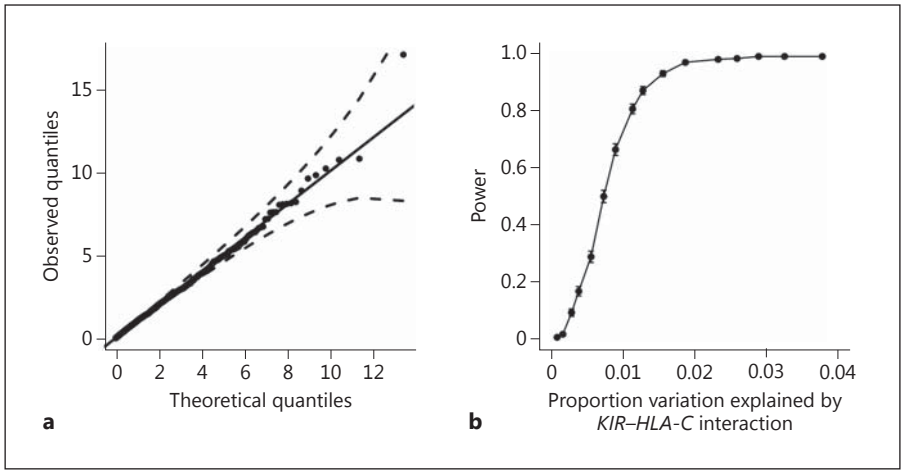

Fig. 2. Statistical properties of the extended QMFG when testing for a *KIR–HLA-C* interaction (df = 1). Power calculated by simulation (2,000 replicates). **a** Q-Q plot. Genotypes and birth weight phenotypes were simulated for 300 pedigrees under the null hypothesis of no genetic effects of *HLA-C* or *KIR* (Scenario I; genomic control value, $\lambda = 1.055$). **b** The power to detect a *KIR–HLA-C* interaction effect when data are simulated under Scenario II (*KIR–HLA-C* interaction only). Error bars represent approximate 95% confidence intervals.

We fit several models for *KIR2DS1*, *HLA-C*, and birth weight encompassing single-locus and multi-locus effects separately and jointly to the 1,309 mother-offspring pairs from the UK and MoBa cohorts. Models 0–5 are compared using the AIC to determine whether a *KIR–HLA-C* interaction is appropriate for these data. Because Hiby et al. [17] provide results with and without gestational age as a covariate, a sensitivity analysis is performed to ascertain the effect of excluding gestational age from the best model on the estimated parameters. We also consider another model that aims to capture a possible paternal parent-of-origin effect (see online suppl. materials).

Results

Modeling a Two-Locus Interaction Effect

To examine type I error rates of the extended QMFG test, we simulate data under the null hypothesis of no genetic effects of *HLA-C* or *KIR* (Scenario I). Based on the work of Hiby et al. [17], we examine the statistical properties of the interaction-only model that requires that the mother have at least + allele and the offspring have more *C2* alleles than his/her mother to affect a difference in birth weight (Model 2). The results comparing the null model of no genetic effects (Model 0) to the alternative model estimating a *KIR–HLA-C* interaction (Model 2) are displayed as a Q-Q plot in Figure 2a. All the points fall between the confidence bounds, showing that there is no bias in the type I error for the QMFG test in this scenario ($\lambda = 1.055$).

Under Scenario II, data are simulated with *KIR–HLA-C* interaction effects (β_{int}) ranging from 0.05 to 0.35 kg in increments of 0.02 kg. Fitting the same null (Model 0) and alternative (Model 2) models, we calculate the power analytically using NCPs at a significance level of 0.001 and each of the following study designs: 1,500 mother-offspring pairs, 750 two-sibling nuclear families, or 750 grandparents-parents-offspring families (equivalent numbers of mother-offspring pairs in each study). We compare the NCP results to simulation results for each of these study designs. In each case, the analytical power is slightly higher than the power using simulations, suggesting that the simulations may be slightly conservative (see online suppl. materials and online suppl. Fig. S1 for details).

Continuing with Scenario II and using Model 0 as the null model and Model 2 as the alternative model, the power to detect a *KIR–HLA-C* interaction effect for 300 extended families (Fig. 1b) is shown in Figure 2b. When the significance level is 0.001, 80% power is reached when the *KIR–HLA-C* interaction effect is approximately 0.19 kg, that is, when the proportion of variation explained by the *KIR–HLA-C* interaction effect is approximately 0.011. Figure 3a shows that the parameter estimates are unbiased when Model 2 is fit to data simulated with a *KIR–HLA-C* interaction effect of 0.19 kg. Together the type I error rate, power, and bias estimates demonstrate that the QMFG test has good statistical properties.

We also used this scenario and these models to compare the power between study designs. Each design has an equivalent number of mother-offspring pairs. Interestingly, we found that under these conditions 750 grandparents-parents-offspring families tend to have more power than 300 extended families, which have more power than 1,500 mother-offspring pairs, which have more power than 750 two-sibling nuclear families (Fig. 4).

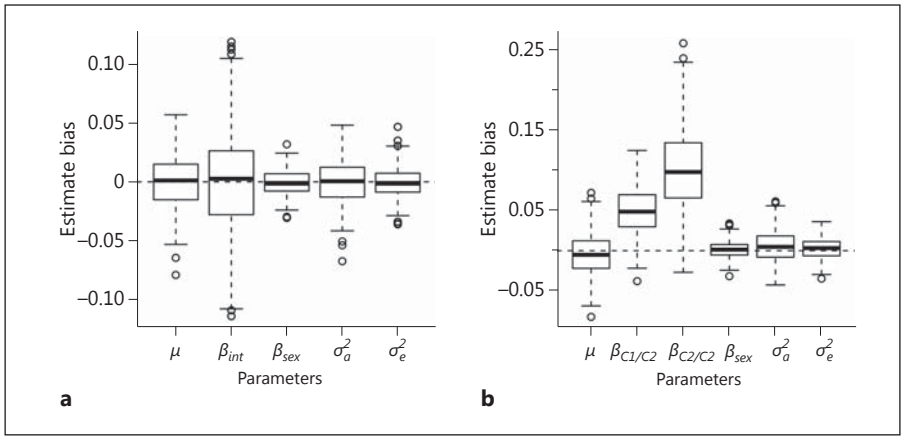

Fig. 3. Parameter estimate bias. Birth weight phenotypes simulated given $\mu = 3.5$ kg, $\beta_{sex} = -0.2$ kg, $\sigma^2_a = 0.2025$ kg^2, and $\sigma^2_e = 0.0475$ kg^2 as well as additional *KIR* and *HLA-C* effects. **a** A *KIR–HLA-C* interaction-only model is fit to Scenario II (*KIR–HLA-C* interaction only) data with a *KIR–HLA-C* interaction effect of 0.19 kg ($\beta_{int} = 0.19$ kg). **b** Model is misspecified as a genotypic offspring *HLA-C* model and is fit to Scenario II data with a *KIR–HLA-C* interaction ($\beta_{int} = 0.35$ kg) leading to substantial bias in the effects of the offspring alleles.

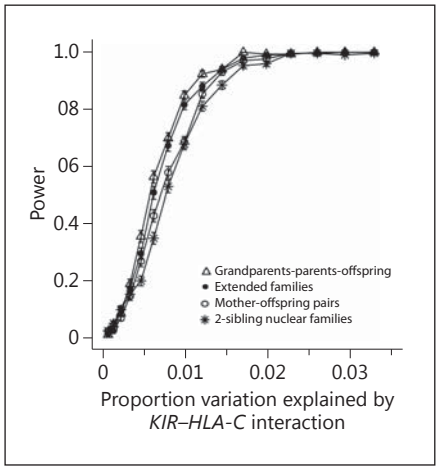

Fig. 4. Effect of study design on power. Error bars represent approximate 95% confidence intervals. Power calculated by simulation (2,000 replicates) for 1,500 mother-offspring pairs, 750 two-sibling nuclear families, 750 grandparents-parents-offspring families, and 300 extended families. Power to detect a *KIR–HLA-C* interaction effect when data are simulated under Scenario II (*KIR–HLA-C* interaction only).

Modeling Two-Locus Main and Interaction Effects

We check for bias in type I error rates for our most complex 2-locus model (Model 1, with parameters for an effect of the dominant maternal + allele [β_{KIR}], an effect of an offspring having more *C2* [β_{more}], and an effect of their interaction [β_{int}]). We simulate data with no genetic effects (Scenario I) and under Scenario III that involves a *KIR–HLA-C* interaction and main effects of a dominant-acting maternal + allele and more *C2* in the offspring. We find similar type I error rates, power, and lack of bias in the estimates when comparing Model 1 to Model 0 as we found for the interaction effect alone (online suppl. Fig. S2–S4). We also compare Model 1 to a model with only independent effects of *HLA-C* and *KIR2DS1* (no interaction, Model 3). We find accurate type I error rates and lack of bias in the estimates (results not shown) and increased power over the 3-degree-of-freedom test for the same effect size (see online suppl. materials for details; online suppl. Fig. S2–S3).

Model Misspecification

In this section, we examine the effect of using a model that is more general than the true model and the effects of using overly restrictive or incorrect models. Unsurprisingly, using a more general model leads to a loss of power but parameter estimates are unbiased (see online suppl. materials and online suppl. Fig. S5 and S6 for details).

For simulations in which an interaction between the maternal *KIR* and maternal-offspring *HLA-C* provides the only genetic effect on birth weight (Scenario II), we investigate whether a standard offspring-only analysis typically used in a GWAS would detect an association at *HLA-C*. When data simulated under Scenario II (Table 3) are tested using an offspring *HLA-C* genotype model (Model 6), with a + frequency of 0.2, power is drastically reduced (Fig. 5a) compared to the correct QMFG model that includes an interaction effect (Model 2). The parameter estimate bias and boxplots for Model 6 analyses are displayed in Figure 3b for the case in which the data are simulated with an interaction effect size of 0.35 kg. Over the 2,000 simulations, the grand mean is slightly underestimated and the variance components are overestimated. Online supplementary Figure S7 shows the power when testing Scenario II-generated data using an additive

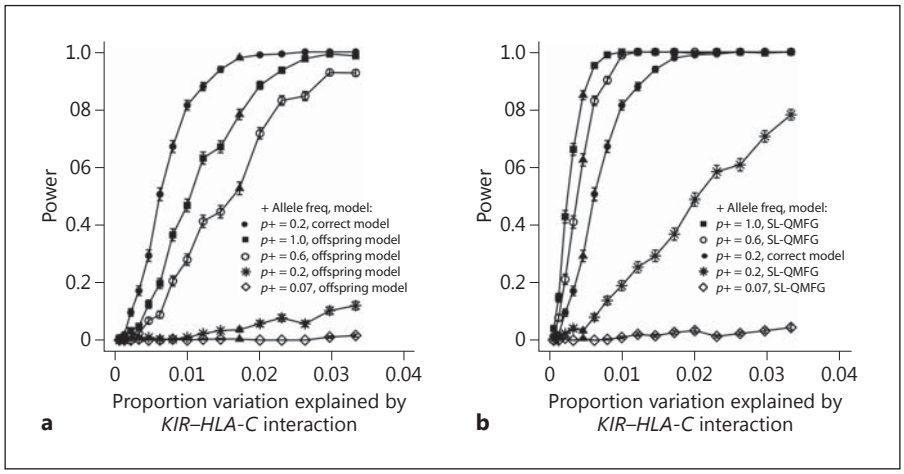

Fig. 5. Effect of model misspecification on power. Error bars represent approximate 95% confidence intervals. **a** Power to detect genotypic *HLA-C* offspring effects. Data simulated under Scenario II (*KIR–HLA-C* interaction only) with several *KIR2DS1* frequencies (*p*+). The correct model (Model 2) is fit when data are simulated with + frequency equal to 0.2. Additionally, the model is misspecified such that it tests for genotypic offspring *HLA-C* effects (df = 2). Simulations in which the *KIR–HLA-C* interaction effect is 0.25 kg (the effect size when power is close to 80% for the genotypic model when *p*+ = 1.0) are represented with triangles. **b** Effect of *KIR2DS1* frequency on the single-locus QMFG (SL-QMFG) test. Data simulated under Scenario II (*KIR–HLA-C* interaction only) and the model is misspecified such that it tests for an effect of a single-locus MFG effect of more offspring *HLA-C2* (df = 1). Simulations in which the *KIR–HLA-C* interaction effect is 0.13 kg (the effect size when power is 85% when *p*+ = 1.0) are represented with triangles.

offspring *C2* model (Model 7 vs. Model 0). Power is slightly higher than for the offspring genotypic test (Model 6), likely due to the reduced degrees of freedom, but remains severely attenuated compared to an analysis using Model 2 when the + frequency is equal.

Analogously, the offspring model might be used to test for an association of *KIR2DS1* with the trait. When using the data simulated under Scenario II and an offspring genotypic (Model 8) or additive (Model 9) model to detect the effect of the presence of the + allele, power is again drastically reduced (online suppl. Fig. S8A and S8B, respectively). Online supplementary Figure S9 shows the grand mean and genotypic parameters estimates are biased upwards for the genotypic model. Taken together, these results lead us to conclude that MFG multi-locus interactions would often be missed in standard GWAS analyses.

Effect of Population Frequency on the Standard Offspring Model

In the previous sections, we assumed the + allele frequency is 0.2 (*p*+ = 0.2) based on the frequency of the *KIR2DS1* gene in the chromosomes of the white British population. In African populations, the *KIR2DS1* gene is found less frequently [52]. Given that frequencies differ between populations, we evaluate how this frequency would change power when using standard offspring/effect-only models. We simulate samples with + frequencies equal to 0.07, 0.2, 0.6, and 1.0 and again use *KIR–HLA-C* interaction effect sizes ranging from 0.05 to 0.35 kg (Scenario II). As the + frequency increases, power also increases for both the offspring *HLA-C* genotypic and additive models. For the specific case where the *KIR–HLA-C* interaction effect size is 0.25 kg (Fig. 5a, denoted by triangles), the estimated power to detect a genotypic offspring *HLA-C* effect ranges from 0.0035 (SE = 0.0013) when *p*+ = 0.07 to 0.78 (SE = 0.009) when *p*+ = 1.0. Similar results are observed when testing these data for an additive offspring *C2* effect (online suppl. Fig. S7, triangles). Thus, when using a standard offspring-only analysis, conclusions about the importance of *HLA-C* on the birth weight depend on the + frequency in the mothers when the true underlying effect stems from a *KIR–HLA-C* interaction.

Effect of Population Frequency on the Single-Locus QMFG Test

We next consider the effect of *KIR2DS1* frequency when the *HLA-C* maternal-offspring effect is modeled but the actual effect is due to a *KIR–HLA-C* interaction.

Table 4. Comparison of *KIR–HLA-C* models for the UK and MoBa cohort data (n = 1,309)

Model	Genetic effects	Genetic parameters[1]	log-likelihood	Proportion of variation explained[2]	AIC	ΔAIC from best model
0	None	0	264.88	N/A	–523.75	6.86
1	Maternal *KIR2DS1* More *C2* Interaction	3	271.31	0.0098	–530.61	0
2	Interaction	1	267.00	0.0033	–526.00	4.61
3	Maternal *KIR2DS1* More *C2*	2	269.21	0.0065	–528.41	2.2
4	Maternal *KIR2DS1*	1	268.87	0.0061	–529.73	0.88
5	More *C2*	1	265.08	<0.0001	–522.16	8.45

[1] All models additionally adjusted for sex, gestational age, and cohort (UK and MoBa). [2] Proportion of residual variation explained by *KIR* and *HLA-C* effects compared to Model 0.

Setting + frequencies equal to 0.07, 0.2, 0.6, and 1.0, we simulate data with a *KIR–HLA-C* interaction effect (Scenario II) and test for an effect of more offspring *C2*. Here Model 5 corresponds to our alternative hypothesis and Model 0 corresponds to the null hypothesis. As shown in Figure 5b, power to detect the *HLA-C* effect increases greatly as the + frequency increases. When the *KIR–HLA-C* interaction effect size is 0.13 kg (proportion of variation explained ~0.006), power to detect a more *C2* effect increases from 0.0065 (SE = 0.0018) when $p+ = 0.07$ to 0.85 (SE = 0.008) when $p+ = 1.0$ (Fig. 5b, triangles). Thus, when using a single-locus MFG test that explicitly models the *HLA-C* effects, the ability to find a significant effect of *HLA-C* on the trait depends on the population frequency of the *KIR2DS1* gene when the true underlying effect stems from a *KIR–HLA-C* interaction.

Effect of Reduced Heritability and Shared Environment

Previously published estimates of birth weight heritability are highly variable [48, 53–55]. Although Demerath et al. [48] estimate the heritability to be 81%, other groups find that the heritability is substantially lower. As an example, Lunde et al. [54] estimate that fetal genetic factors explain ~30% of the normal variation in birth weight so we alter our simulated variance components such that the residual heritability is reduced to 30%. Keeping the total variation in birth weight fixed at 0.25 kg^2, we simulate data given Scenario II effect sizes with $\sigma_a^2 = 0.075$ kg^2 and $\sigma_e^2 = 0.175$ kg^2 (residual narrow-sense heritability $h^2 = 0.30$). Online supplementary Figure S10 shows the effects of reduced heritability on power. Compared to the 82% power to detect an effect size of 0.19 kg (variance explained 0.011) when heritability was simulated to be 81%, power is 71%.

Demerath et al. [48] do not address the possibility of shared environmental effects, which could be due to smoking by the mother during pregnancy or her exposure to secondhand smoke [25]. Lunde et al. [54] report that the effect of shared environment between full siblings accounts for ~15% of the total variation of birth weight. To account for the effects of shared environment, we add an additional variance component σ_{sh}^2 such that $\Omega = 2\,\sigma_a^2\,\Phi + \sigma_{sh}^2\,\mathrm{H} + \sigma_e^2\,\mathrm{I}$ [45]. The household indicator matrix, $\mathrm{H} = (h_{ij})$, has entries 0 or 1 depending on whether subjects i and j are siblings. Keeping the residual narrow-sense heritability at 30%, we simulate data such that 15% of the total variability is due to a shared environment effect for full siblings with the variance parameters $\sigma_a^2 = 0.075$ kg^2, $\sigma_{sh}^2 = 0.0375$ kg^2, and $\sigma_e^2 = 0.1375$ kg^2. The variance due to the shared environment has little effect on the power for a given heritability (online suppl. Fig. S10).

Application of the QMFG Test to Birth Weight Data

Using the same mother-offspring pairs as Hiby et al. [17], we fit various models (Models 1–5) that include single-locus and 2-locus interaction effects between the *KIR2DS1* and *HLA-C* genotypes for mothers and their offspring as well as the null model (Model 0). To compare how well the models fit these data, we use AIC (Table 4).

Table 5. Effect estimates and 95% confidence intervals (CI) for Model 1 and the UK and MoBa cohort data with and without adjusting for gestational age

Covariates	Cohort and sex of fetus	Cohort, sex of fetus, and gestational age
Number of subjects	1,309	1,309
Mean effect of maternal *KIR2DS1* (CI)	0.0445 kg (–0.0302, 0.1192)	0.0481 kg (–0.0146, 0.1108)
Mean effect of more *C2* (CI)	–0.1180 kg (–0.2201, –0.0159)	–0.0867 kg (–0.1724, -0.001)
Mean effect of interaction (CI)	0.2027 kg (0.0504, 0.3550)	0.1337 kg (0.0059, 0.2615)
Residual variance (SE)	0.3468 kg^2 (0.0136)	0.2427 kg^2 (0.0095)
Proportion of variation explained	0.0111	0.0098

The minimum AIC is provided by Model 1, which includes effects for the maternal *KIR2DS1* and for more offspring *C2* than maternal *C2*, in addition to the *KIR–HLA-C* interaction.

The effect estimates for Model 1 adjusting for cohort, sex, and gestational age are shown in Table 5 (column 3). For this model, the reference group consists of offspring that have less or equal *C2* alleles than their mother and whose mothers have 0 copies of *KIR2DS1*. According to our results, offspring with more *C2* than their mother and whose mother has 0 copies of *KIR2DS1* are on average 0.0867 kg lighter at birth compared to the reference group adjusting for sex, gestational age, and cohort ($p = 0.047$). Offspring with less or equal *C2* than their mother and whose mother has at least 1 copy of the *KIR2DS1* are estimated to be 0.0481 kg heavier at birth compared to the reference group adjusting for sex, gestational age, and cohort ($p = 0.133$). The interaction effect estimate (0.1337 kg, $p = 0.040$) indicates that on average an offspring who has both more *C2* than their mother and whose mother has at least 1 copy of the *KIR2DS1* is 0.0951 kg heavier at birth compared to the reference group adjusting for sex, gestational age, and cohort. Note that the interaction effect remains significant when the single-locus effects of *KIR* and *HLA-C* are not included in the model (Model 2 vs. Model 0; $p = 0.039$). As a sensitivity analysis, we also fit Model 1 without adjusting for gestational age and discover the effect estimates do not differ greatly (Table 5, column 2).

We note that the presence of more *C2* in the offspring than their mothers implies allogenic *C2* inherited from the father. Without either paternal *HLA-C* genotypes or *HLA-C* phasing of the offspring genotypes, testing for a paternal parent-of-origin effect is not possible. However, we can test alternatives to the more *C2* hypothesis. In particular, if the effect of the *C2* is due to paternal *C2* then *C2/C2* offspring with *C2/C2* mothers should have similar weights to *C1/C2* offspring with *C1/C1* mothers or *C2/C2* offspring with *C1/C2* mothers (online suppl. Table S1, Model b). We find that this model fits slightly better than the Model 1 (online suppl. Table S1), further supporting the paternal parent-of-origin hypothesis.

Discussion

The link between maternal uNK cells and offspring trophoblast cells within the placenta presents a plausible setting for MFG interactions that affect fetal development. Motivated by a previous study that found a significant effect of the maternal *KIR2DS1* on human birth weight for offspring with more *HLA-C* alleles bearing *C2* epitope than their mother [17], we extend the QMFG test to multiple loci and test the hypothesis of a *KIR–HLA-C* interaction effect on birth weight.

Our simulation studies and analytical power determinations show the statistical validity of the QMFG test when extended to interactions between the maternal *KIR*, maternal *HLA-C*, and offspring *HLA-C*. In situations in which the model assumptions are consistent with the simulation scenario, the QMFG test has valid type I error rates, parameter estimate bias centered around 0, and high power (both by simulation or using NCPs) even when the proportion of variation explained is low. To verify that these features are not altered by family structure, we examine equivalent numbers of mother-offspring pairs as independent mother-offspring pairs, 2-sibling nuclear families, and grandparents-parents-offspring families. Power depends on the study design; with equivalent numbers of mother-offspring pairs, grandparents-parents-offspring families have slightly more power than extended families, which have more power than indepen-

dent mother-offspring pairs. Interestingly 2-sibling nuclear families have less power than the independent mother-offspring pairs, all of which has practical ramifications when planning a study. We also find that reduced heritability leads to a reduction in power but shared environment has little effect on the power.

We investigate scenarios in which the true underlying MFG interaction is partially or entirely misspecified. As expected, power is reduced but parameter estimates are unbiased when a more general model is used in a case where a restricted model is sufficient. The ability to detect an association at a locus involved in an MFG interaction using only offspring genotypes depends on the underlying nature of the MFG interaction, but in general power to detect the locus is greatly diminished. We specifically show that if a standard model, which considers only offspring genotypes, is fit to data generated under an underlying *KIR–HLA-C* interaction scenario, associations can be missed or identified incorrectly. These simulation results provide an explanation for why neither *HLA-C* nor *KIR* loci have been found to be significantly associated with birth weight in standard GWAS [22, 23], which fail to account for the effects of maternal genotypes. Similarly, if a single-locus QMFG test is used when 2 or more polymorphic loci are involved in the MFG interaction, power will likely be reduced. In particular, when using the offspring-only or single-locus QMFG analyses, if a researcher is unaware of the existence of the second polymorphic locus (*KIR*), they might find an *HLA-C* effect in a population where the *KIR2DS1* gene is frequent, but fail to replicate the effect in a second population where the *KIR2DS1* gene is infrequent even when *C2* allele frequency is the same in these 2 populations. The researcher would then be inclined to dismiss the first result as a false positive. Thus, like the case of offspring gene-gene interactions, models that incompletely capture the MFG interlocus effects can easily lead to incorrect conclusions.

Due to the practical issues of running simulation studies under all possible models involving multiple loci, we limited our modeling to 2 loci each with 2 alleles under genetic mechanisms pertinent to our real data application. In that way, we were able to reduce the number of genetic parameters from 20 to 3. Naturally, power estimates provided in this article will be higher than those studies where the models cannot be similarly constrained. However, given our results here and in Clark et al. [43], we are confident that with appropriate sample sizes the QMFG test will be statistically sound regardless of the specific MFG interaction being studied.

To date, no other study has looked for an association between *KIR–HLA-C* interactions and birth weight after accounting for single-locus effects. Using subgroup analyses, Hiby et al. [17] found that the effect of maternal *KIR* on birth weight was significant in offspring with more *C2* than their mothers and that this maternal effect was not significant in offspring with less or equal *C2*. From their analyses, it is difficult to determine whether the effect is exclusively a *KIR–HLA-C* interaction effect or whether there are also main effects of maternal *KIR* and more *C2* in the offspring's genotype than the mother's genotype. Using the QMFG test, we extend these previous analyses by building a linear mixed model of *KIR*, *HLA-C*, and birth weight in order to reanalyze data from the UK and MoBa cohorts. Thus, we can determine if the effect of a maternal *KIR2DS1* gene on offspring's birth weight varies depending on whether the offspring has more *C2* by testing the statistical significance of the *KIR–HLA-C* interaction parameter. Using AIC to compare models, the model with effects for maternal *KIR2DS1* and more *C2* alleles in the offspring's genotype than the mother's, and their interaction, is determined to provide a better fit than main effects or interaction alone. For this model, there is a significant interaction effect on offspring birth weight that explains ~1% of the phenotypic variation in human birth weight.

Another advantage of using the QMFG test instead of conducting subgroup analyses is the ability to determine parameter estimates for the maternal *KIR2DS1*, more offspring *C2*, and *KIR–HLA-C* interaction effects. Our findings suggest that there are both significant *KIR–HLA-C* interactions and a main effect of more offspring *C2*. Less certain is a *KIR2DS1* effect independent of *HLA-C*. These results may have implications for prenatal genetic screening to identify pregnant mothers who are at risk for pregnancy complications.

We chose to model the effect of having more *C2* epitopes present in the offspring's *HLA-C* genotype than in the mother's and a dominant-acting maternal *KIR2DS1* effect to allow a direct comparison to the analyses of Hiby et al. [17]. As discussed by these researchers [16], this model is a surrogate for a model that captures the effects of the maternal immune response to the fetus having a non-self HLA-C antigen derived from the father. Hiby's study design did not include paternal *HLA-C* genotypes and therefore comparing the fit of the models presented in this article to a paternal parent-of-origin effect, a very plausible alternative model, is not possible. However, we examined an additional model that is consistent with paternal parent-of-origin effects and found that this model is also plausible.

When the data consist of only mother-offspring pairs, general statistical software packages that include linear mixed model options can be used and, with reparameterization, these packages can also accommodate commonly observed, simple study designs such as nuclear families [56]. However, for studies that collect data from families of varying sizes and complexity, additional software or tools are needed to extract the maternal-offspring genotype combinations, impute missing genotype data, and construct the design and kinship matrices. To perform our analyses, we extended the QMFG test to handle multiple loci in the statistical genetics software package Mendel. These extensions will be available in the next version of the freely available Mendel package. The power of our method to detect significant MFG interactions and our flexible software make the QMFG test an effective tool to consider when studying genetic factors associated with complex traits.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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