Detection of Intergenerational Genetic Effects with Application to *HLA-B* Matching as a Risk Factor for Schizophrenia

Erica J. Childs^a Eric M. Sobel^b Christina G.S. Palmer^{b, c} Janet S. Sinsheimer^{a, b, d}

Departments of ^aBiostatistics, ^bHuman Genetics, ^cPsychiatry and Biobehavioral Sciences, and ^dBiomathematics, University of California, Los Angeles, Calif., USA

Key Words

Family-based association • Maternal-fetal genotype test • Extended maternal-fetal genotype test • Genotype interactions • Complex disease • Olfactory deficits • Gene-by-gene • Gene-by-environment • Major histocompatibility complex

Abstract

Background and Methods: Association studies using unrelated individuals cannot detect intergenerational genetic effects contributing to disease. To detect these effects, we improve the extended maternal-fetal genotype (EMFG) incompatibility test to estimate any combination of maternal effects, offspring effects, and their interactions at polymorphic loci or multiple SNPs, using any size pedigrees. We explore the advantages of using extended pedigrees rather than nuclear families. We apply our methods to schizophrenia pedigrees to investigate whether the previously associated mother-daughter *HLA-B* matching is a genuine risk or the result of bias. **Results:** Simulations demonstrate that using the EMFG test with extended pedigrees increases power and precision, while partitioning extended pedigrees into nuclear families can underestimate intergenerational ef-

fects. Application to actual data demonstrates that mother-daughter *HLA-B* matching remains a schizophrenia risk factor. Furthermore, ascertainment and mate selection biases cannot by themselves explain the observed *HLA-B* matching and schizophrenia association. **Conclusions:** Our results demonstrate the power of the EMFG test to examine intergenerational genetic effects, highlight the importance of pedigree rather than case/control or case-mother/control-mother designs, illustrate that pedigrees provide a means to examine alternative, non-causal mechanisms, and they strongly support the hypothesis that *HLA-B* matching is causally involved in the etiology of schizophrenia in females.

Copyright © 2011 S. Karger AG, Basel

Introduction

Genome-wide association studies of unrelated individuals have been the method of choice for identifying genetic variants associated with common complex traits and diseases. Although many variants have been found, most have small effect sizes and explain only a small portion of heritability [1–4]. These disappointing results suggest that in addition to common genetic variants, other

C.G.S. Palmer and J.S. Sinsheimer contributed equally to this work.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2011 S. Karger AG, Basel
0001-5652/11/0723-0161\$38.00/0

Accessible online at:
www.karger.com/hhe

Christina Palmer, PhD
UCLA Semel Institute
760 Westwood Plaza, Room 47-422, Los Angeles, CA 90095 (USA)
Tel. +1 310 794 4796, E-Mail cpalmer@mednet.ucla.edu

Janet Sinsheimer, PhD
UCLA Gonda Center
695 Charles E. Young Drive South, 5357-C
Los Angeles, CA 90095 (USA)
Tel. +1 310 825 8002, E-Mail janet@mednet.ucla.edu

mechanisms such as rare variants, structural variation, gene-by-gene interactions, gene-by-environment interactions, and intergenerational genetic effects may significantly contribute to complex trait heritability and need to be explored further [3, 5, 6].

Case-control studies, although convenient, limit the questions that can be answered. Distinguishing parent-of-origin effects from maternal-fetal genotype (MFG) incompatibility is impossible without paternal information. In fact, without parental data, it can be impossible to determine whether intergenerational effects such as parent-of-origin effects or MFG incompatibility contribute to disease. Improperly accounting for these effects can diminish power in association studies [3].

MFG incompatibility is a gene-gene interaction that occurs when certain combinations of maternal and fetal genes create an adverse environment for the developing fetus. Methods and study designs for identifying genes involved in MFG incompatibilities are receiving increasing attention [7–23]. The first such test, the MFG test [23], is an affected-only, likelihood-based test for case-parent trios that is adapted from Weinberg's log-linear method for detecting maternal and offspring main genetic effects [24]. The primary strength of the MFG test derives from its ability to jointly model maternal genetic effects, offspring genetic effects, and interactions between maternal and offspring genotypes without confounding. Extensions to the original test allow for: multiple affected offspring in a nuclear family [17], sparse data [19], adjustment for the genetic effects of viability when testing for the effect of MFG incompatibility on disease [14], and handling incomplete parental genotypes [15, 20].

Until recently, the MFG test [14–17, 19, 20, 23] was limited to nuclear families. This forced researchers to either choose one nuclear family per extended pedigree (reducing power) or to analyze related nuclear families as independent (possibly introducing bias). To eliminate this problem, the extended MFG (EMFG) test was developed to include arbitrary pedigree structures in the analysis of MFG incompatibility at a bi-allelic locus [8]. By using pedigrees in their entirety, the EMFG test yields unbiased estimates of the MFG parameter in situations where using nuclear families extracted from extended pedigrees leads to biased estimates [8]. The current article generalizes the EMFG test to allow: (1) the assessment of highly polymorphic loci, such as *HLA* loci or joint analysis of multiple SNPs, and (2) more flexibility in the intergenerational effects that can be tested.

As an example of intergenerational effect analysis, we apply our new EMFG test to provide insight into the role

of *HLA-B* [MIM 142830] maternal-fetal matching in the risk for schizophrenia. There is little doubt that schizophrenia is a highly heritable, genetically complex disorder [25]. However, genome-wide association studies have had only limited success in explaining its genetic underpinnings [26]. Prenatal environmental factors, notably those that result in obstetric complications, increase schizophrenia risk [27–33]. Particularly interesting are prenatal environment risks that are genetic in origin, such as those induced by MFG incompatibility [23]. Palmer et al. [34] use the MFG test [16, 17, 23] to analyze nuclear families extracted from large pedigrees and find that *HLA-B* matching increases schizophrenia risk in female offspring. Although intriguing, two possible artifacts may explain this phenomenon.

First, given that ignoring the affection status of relatives can bias effect sizes [8], the observed association between *HLA-B* matching and schizophrenia in female offspring could be an artifact of analyzing data from selected nuclear families rather than using the actual pedigree structures and affection statuses. Using our new EMFG test for multi-allelic loci, we can determine if the nuclear families extracted from pedigrees [34] introduce a bias against a male MFG effect, leading to a female-specific *HLA-B* artifact.

Second, Palmer's MFG results [34] might be solely an artifact of olfactory deficits [35] that are present in parents of individuals with schizophrenia [36–42]. The relationship between mate choice, olfaction, and *HLA* type has been studied [43–51]. Some, but not all, of these studies find that in the general population disassortative mate selection exists at *HLA* loci due to olfactory preferences [43–45]. Under an olfactory deficit hypothesis, schizophrenics' parents do not avoid *HLA* similar mates, leading to a different distribution of mating types in these parents versus the mating types observed for the parents of unaffected offspring. This lack of olfactory discrimination leads to increased mother-offspring *HLA-B* matching, which could be misinterpreted as a risk factor for schizophrenia. However, if parental olfactory deficits are the sole cause of the observed mother-offspring *HLA-B* matching, we should observe data that are consistent with paternal transmission equilibrium. In contrast, a hypothesis that includes a direct effect of *HLA-B* matching on schizophrenia risk (whether or not olfactory deficits are present) predicts paternal transmission distortion [22]. Thus, the hypothesis that *HLA-B* maternal-fetal matching contributes to schizophrenia susceptibility will be supported by evidence of paternal transmission distortion regardless of the observed parental mating types.

In the current study, we first generalize the EMFG test for use with multi-allelic loci and any form of MFG incompatibility. We then apply this new EMFG test to simulated data to quantify the advantages of using extended pedigrees rather than nuclear families and to evaluate its statistical properties. We also revisit a large Finnish schizophrenia data set that includes previously excluded individuals. The new EMFG test should provide more accurate *HLA-B* matching estimates by better reflecting the true relationships among affected family members. By using extended pedigrees we are also able to better examine whether the study design previously used or the olfactory deficit hypothesis explain the observed association between *HLA-B* matching and schizophrenia in female offspring [34].

Subjects and Methods

Subjects

Study subjects are part of a Finnish schizophrenia study, which has been previously described [34, 52–55]. Subjects provided informed consent. The research was approved by the Ministry of Social Affairs and Health (Finland) and the Institutional Review Board at the University of California, Los Angeles (USA). Probands with schizophrenia born between 1940 and 1969 and their relatives were identified through nationwide health and population registers. Two independent DSM-IV [56] best-estimate lifetime diagnoses were made from all available inpatient and outpatient records for probands and their relatives.

Palmer et al. [34] use 274 nuclear families from this study where DNA is available for at least 1 parent and 1–6 offspring affected with schizophrenia, schizoaffective psychosis disorder, or schizophrenia spectrum disorder for a total of 484 affected offspring. We expand the original sample by including relatives who could not be analyzed previously, such as half siblings, first cousins, and affected parents. We also reconnect nuclear families where offspring are second cousins, or more distantly related, and were previously treated as unrelated families. These two steps yield a study sample with a total of 553 genotyped, affected individuals (419 diagnosed with schizophrenia, 80 with schizoaffective disorders, and 54 with schizophrenia spectrum disorders) distributed over 230 pedigrees, an additional 69 affected individuals. The number of affected individuals per pedigree ranges from 1 to 14 with one-third of pedigrees having 3 or more affected members. Of the 230 pedigrees, 69 (30%) are extended pedigrees. Of these 69 extended pedigrees, 48 (21% of the study sample) contain affected individuals in multiple generations (e.g. parent-offspring combinations), including a total of 31 affected mothers and 17 affected fathers with affected offspring. As before [34], 60% of affected individuals are male.

Genotyping Methods

DNA and genotyping details are found elsewhere [34, 57]. In short, an *HLA* clinical diagnostic and reference laboratory with high inter- and intrasite reliability for DNA-based typing of *HLA*

class I and II genes was used for genotyping. Individuals were genotyped blind to their diagnostic status for *HLA-B* antigens. The genotyping failure rate was 5%, mainly due to insufficient quantities of DNA. Individuals were coded as homozygous when only a single allele was observed.

We use the Trim Pedigrees [58] option of Mendel (version 10) [59] to remove any individuals who are not necessary for informing the relationships between affected individuals in the pedigree. We use Mendel's Mistyping option [60] to detect genotyping errors and find no errors over those found previously [34]. We also use Mendel to test for the Hardy-Weinberg equilibrium (HWE) in the pedigrees [61] because HWE is an assumption of the multi-allelic version of the EMFG test and because violation of HWE is evidence against the olfactory deficit model.

Statistical Analyses – The Generalized EMFG Test for Multi-Allelic Loci.

For a pedigree with n members, the EMFG test fits a conditional likelihood of the observed marker phenotypes $\mathbf{G}$ given the trait phenotypes $\mathbf{D}$

$$L(\mathbf{G}|\mathbf{D}) = \quad (1)$$

$$\frac{\sum_{\mathbf{g}_1} \cdots \sum_{\mathbf{g}_n} \prod_j \text{Prior}(\mathbf{g}_j) \prod_i \text{Pr}(\mathbf{G}_i | \mathbf{g}_i) \prod_{[c,r,s]} \text{Pr}(\mathbf{D}_c | \mathbf{g}_c, \mathbf{g}_r) \text{Trans}(\mathbf{g}_c | \mathbf{g}_r, \mathbf{g}_s)}{\sum_{\mathbf{g}_1} \cdots \sum_{\mathbf{g}_n} \prod_j \text{Prior}(\mathbf{g}_j) \prod_{[c,r,s]} \text{Pr}(\mathbf{D}_c | \mathbf{g}_c, \mathbf{g}_r) \text{Trans}(\mathbf{g}_c | \mathbf{g}_r, \mathbf{g}_s)}.$$

Here $\text{Prior}(\mathbf{g}_j)$ represents founder j 's genotype frequency at the proposed genotype $\mathbf{g}_j$. We assume random mating at the locus of interest to avoid overparameterization [8]. $\text{Pr}(\mathbf{G}_i | \mathbf{g}_i)$ is an indicator variable equaling 1 if, for individual i , $\mathbf{g}_i$ is consistent with the observed marker phenotype $\mathbf{G}_i$, and 0 if it is inconsistent. When $\mathbf{G}_i$ is missing, $\text{Pr}(\mathbf{G}_i | \mathbf{g}_i) = 1$. Offspring and maternal genetic effects, and MFG interactions are parameterized through $\text{Pr}(\mathbf{D}_c | \mathbf{g}_c, \mathbf{g}_r)$, the penetrance, which is combined with $\text{Trans}(\mathbf{g}_c | \mathbf{g}_r, \mathbf{g}_s)$ (the transmission probability for the offspring, mother, and father triple (c, r, s)). The denominator sums over all possible ordered (phased) genotypes for the n pedigree members, and is similar to standard ascertainment correction [62]. The likelihood in equation 1 is summed over all ordered genotypes $\mathbf{g}$ that are consistent with the observed genotypes $\mathbf{G}$ in the pedigree. As genotype phases are usually unknown, the number of terms in this summation can be large if genotypes are missing for many individuals. To include affected and genotyped founders in the EMFG test, each must be provided with two parents who have no genotypes or phenotypes.

As presented here, the EMFG test is an affected-only analysis. All unaffected individuals are considered to be phenotype unknown. This low prevalence disease assumption [15] is reasonable for schizophrenia, which has less than 2% prevalence. For diseases with a prevalence less than ~10%, unaffected offspring contribute little to the likelihood, and therefore the affected-only approach is a reasonable approximation [14, 63]. For more common diseases, the test can be modified to estimate the penetrance for unaffected offspring, requiring the baseline disease prevalence to be estimated [14].

We modify the EMFG test [8] for use with highly polymorphic loci like *HLA* by making two adjustments to equation 1: (a) we

estimate founder allele frequencies rather than genotype frequencies to reduce nuisance parameters, and (b) we generalize the penetrance function $\Pr(D_c | g_c, g_r)$ to handle the wide range of MFG incompatibilities that are possible with multi-allelic loci. We make the first adjustment by assuming HWE at the locus of interest. The second adjustment is straightforward but requires careful consideration. There are $q(2q^2 - q + 1)/2$ distinct MFG combinations for a locus with q alleles. Each combination could be assigned its own MFG parameter, but this would result in too many parameters. In practice, we reduce the parameter number by drawing on prior biological knowledge. With maternal-fetal matching, we are not interested in the specific alleles present in the mother-offspring genotype pair, but rather how many alleles they share identical by state (IBS). There are 4 possible mother-offspring genotype IBS combinations: (combination A) identical mother-offspring genotypes ($(g_r = i/i, g_c = i/i)$ or $(g_r = i/j, g_c = i/j)$) with relative risk μ_A ; (combination B) the mother is homozygous and the offspring is heterozygous ($(g_r = i/i, g_c = i/j)$) with relative risk μ_B ; (combination C) the mother is heterozygous and the offspring is homozygous ($(g_r = i/j, g_c = i/i)$) with relative risk μ_C , and (combination D) the mother and offspring genotypes are both heterozygous but not identical ($(g_r = i/j, g_c = i/k)$) with relative risk μ_D . These relative risks are modeled in the penetrance function, $\Pr(D_c | g_c, g_r)$. Because these are relative risks, one of them is constrained to 1.0 and serves as the reference.

If we have strong prior knowledge that certain forms of matching do not modify disease risk, we can further reduce the number of relative risk parameters. In our example, the only combinations that lead to an adverse prenatal environment are those in which the offspring's alleles are identical to (combination A), or a subset of (combination C), the maternal alleles [34, 64]. In this case, we constrain both μ_B and μ_D to equal 1.0. We also force combinations A and C to have the same relative risk of disease by including the constraint $\mu_A = \mu_C = \mu$.

Introducing additional relative risks into the penetrance function accommodates the main effects of offspring risk alleles. These new parameters represent the relative risk of disease for an offspring who carries one or two copies of a risk allele compared to an offspring who carries no copies. Similarly, maternal genetic effects can be modeled [23] that represent relative risks of disease for offspring whose mother carries one or two copies of the risk allele compared to an offspring whose mother carries no copies. Although each possible maternal and offspring genotype could have its own associated risk, for highly polymorphic loci this model would result in too many parameters for accurate estimation. Thus we suggest constraining the offspring effects to $\rho_{2,s} = \rho_{1,s}^2$ and the maternal effects to $\eta_{2,s} = \eta_{1,s}^2$ for each allele s to reduce the number of parameters to $2(q - 1)$, where q is the number of alleles and one allele is the reference.

Simulation Studies

Through simulation studies, we examine type I error, power, parameter estimation, and other properties of the EMFG test for multi-allelic loci. Each analysis described below simulates a locus with 10 alleles and frequencies to mimic *HLA-B* frequencies in our Finnish schizophrenia data (0.0575, 0.0593, 0.0625, 0.0847, 0.0860, 0.0921, 0.1020, 0.1185, 0.1687, 0.1688). We simulate 1,000 samples, each containing 50 three-generation pedigrees with affected individuals in 2 generations (see fig. 1). We use sex-neutral effect sizes of 1.0 and 1.5. We calculate the average parameter estimates

Fig. 1. Pedigree structure used for simulations. Dark shading represents individuals who are always affected in simulations. Hatching represents individuals who are sometimes affected (see text). Individual 7 is male and individual 8 is female in half of the pedigrees; their sexes are reversed in the other half.

of the male and female MFG effects, standard errors, coverage, and rejection rates using the EMFG test. The rejection rate is the proportion of samples where the likelihood ratio test rejects the null hypothesis of no MFG effect at the 0.05 significance level. Coverage is the proportion of 95% confidence intervals (CI) that contain the MFG relative risk's true value.

The EMFG test for binary loci can have an inflated type I error when HWE does not hold [8]. To test the effects of violating HWE in the multi-allelic setting, we simulate an example of population stratification with 1,000 samples from two populations. Each sample consists of 50 families with affected individuals in 2 generations (fig. 1) for a total of 200 founders. One half of the families are simulated using the Finnish *HLA-B* frequencies and the other half are simulated using allele frequencies of 0.1197, 0.0709, 0.1072, 0.1847, 0.0505, 0.1644, 0.1635, 0.0396, 0.0526, and 0.0469. Samples are simulated with effect sizes of 1.0 and 1.5. We estimate the MFG parameter, rejection rates, and coverages using the EMFG test. As measures of the degree of population stratification, we use the Allele Frequencies option of Mendel to calculate Wright's F_{st} statistic and to test for equivalent allele frequencies between the two populations [65].

To determine the usefulness of extended pedigrees over nuclear families, we use a large MFG effect size of 2.5 along with a large number of pedigrees to ensure detection of any potential biases. We simulate these samples using the Finnish *HLA-B* allele frequencies. In the first set of simulations, we generate 300 three-generation pedigrees with only the last generation affected (fig. 1, individuals 7 and 8). We analyze the data as extended pedigrees (scenario 1) and use two different nuclear family approaches: (i) a conservative approach of randomly selecting one grandchild and his/her parents from each pedigree, resulting in the analysis of 300 case-parent trios per sample (scenario 2), and (ii) a maximum sample size approach of including both grandchildren and their parents in the analysis for a total of 600 case-parent trios per sample (scenario 3). In a second set of simulations, we classify all offspring in the second and third generations as affected (fig. 1, individuals 5–8) and repeat the entire simulation analysis (scenario

Table 1. Statistical properties of the EMFG test

μ	Constraints	$\hat{\mu}_m$ (SE)	Coverage (SE)	$\hat{\mu}_f$ (SE)	Coverage (SE)	Rejection rate (SE)
1.0	$\mu_m = \mu_f$	0.970 (0.155)	0.945 (0.007)	$= \hat{\mu}_m$	$= \text{Cov}(\hat{\mu}_m)$	0.060 (0.008)
1.5	$\mu_m = \mu_f$	1.475 (0.207)	0.950 (0.007)	$= \hat{\mu}_m$	$= \text{Cov}(\hat{\mu}_m)$	0.775 (0.013)
1.5	none	1.461 (0.335)	0.953 (0.007)	1.480 (0.342)	0.970 (0.005)	0.673 (0.015)

μ = Simulation conditions used to create the samples. Constraints = Constraints enforced on parameters to fit the model of interest. $\hat{\mu}_m$ = Relative risk for males who match with their mother at *HLA-B*. SE = Standard error. $\hat{\mu}_f$ = Relative risk for females who match with their mother at *HLA-B*.

os 4–6). For the conservative approach we choose individuals 1, 2, 5, and 6 (the most informative nuclear family per pedigree, scenario 5). For the maximum sample size approach we include each nuclear family, resulting in the analysis of 900 nuclear families (scenario 6).

Analysis of Schizophrenia Pedigrees

Because the EMFG test can now be applied to a multi-allelic locus, we can estimate a sex-specific *HLA-B* matching effect in the schizophrenia sample. We use the World Health Organization criteria [66] to classify *HLA-B* alleles. However, the 20 observed alleles are too many for our modest sample size because this would result in 19 estimates of allele frequencies, many of which are quite rare. We use the Combining Alleles option of Mendel [67] to bin alleles, and thus limit the number of alleles to ten. One potential objection to combining alleles is that it may result in binning low-risk mother-offspring combinations with high-risk ones. However, for MFG matching, our binning scheme biases results towards the null hypothesis of no MFG incompatibility and thus is conservative. For example, if allele *k* is rare and is combined with frequent allele *j*, we misclassify a (*i/j*, *i/k*) mother-offspring low-risk combination as a (*i/j*, *i/j*) mother-offspring high-risk combination, which reduces the estimate of the MFG matching effect. As a sensitivity test, we also make genotypes with very rare alleles missing and find no difference in our results (data not shown).

We test for a sex-specific *HLA-B* matching effect on schizophrenia by fitting three models: no MFG effects (model 0); separate MFG effects for males and females (model 1), and a female MFG effect while the male MFG effect is constrained to the null (model 2). We define offspring as being MFG incompatible (i.e. matching) with their mother if the offspring's alleles are equivalent to, or a subset of, the mother's alleles (combinations A and C). Constraints are placed on parameters to fit models of interest. Likelihood ratio tests are used to compare null and alternative hypotheses. Although not observed by Palmer et al. [34], findings by Nunes et al. [68] suggest that *HLA-B*15* and *HLA-B*35* are associated with schizophrenia, so we also test whether offspring or maternal genetic effects for these two alleles are significant in the presence of the MFG matching effect.

Because of the absence of control families in our study design, we cannot directly test for olfactory deficits. However, we can determine if an olfactory deficit model is the sole explanation for the observed association between *HLA-B* matching and schizophrenia, as opposed to MFG incompatibility alone or in combination

with olfactory deficits, by focusing on the parental mating pairs that are informative for matching and then testing for evidence of transmission distortion. In these informative pairs, mothers and fathers are IBS = 1, and we determine which of the father's alleles is transmitted. For homozygous mother-heterozygous father pairs (*i/i***i/j*), offspring can be matched (*i/i*) or mismatched (*i/j*) with their mother. For mating type pairs *i/j***i/k*, offspring can be matched (*i/i* or *i/j*) or mismatched (*i/k* or *j/k*). If matching is a risk factor for schizophrenia, we will see significantly more matched affected offspring than mismatched affected offspring in these two mating type pairs. In other words, we will see apparent over-transmission of the paternal allele shared by the mating pair. To test this hypothesis we determine the number of *HLA-B* matched offspring in these two mating type groups and compare the observed proportions to what is expected under the null hypothesis of no transmission distortion (50%) using a Wald test.

Results

Statistical Properties of the EMFG Test for Multi-Allelic Matching

Simulations under the null hypothesis of no MFG incompatibility effect (table 1, $\mu = 1.0$) demonstrate that the parameter estimation ($\hat{\mu} = 0.970$, SE = 0.155), coverage (0.945), and type I error rate (0.060) are all appropriate. With 50 extended pedigrees, there is 77.5% power to detect an effect size of 1.5. Parameter estimation ($\hat{\mu} = 1.475$, SE = 0.207) and coverage (0.950) are appropriate when a model constraining gender effects is used (table 1, $\mu = 1.5$, $\mu_m = \mu_f$). Under these same conditions, there is a slight reduction of power (67.3%) when estimating separate relative risks for males and females, but parameter estimates and coverage do not change significantly (table 1). These results suggest that the extension of the EMFG test to a multi-allelic locus is statistically sound and our analysis of the schizophrenia data set with 230 pedigrees is well powered to detect moderate effect sizes using a relatively homogeneous population like the Finns.

Table 2. Comparison of analysis approaches when only grandchildren are affected

Scenario	Number of families	$\hat{\mu}_m$ (SE)	Coverage (SE)	$\hat{\mu}_f$ (SE)	Coverage (SE)
1	300 extended pedigrees ¹	2.486 (0.295)	0.958 (0.006)	2.493 (0.296)	0.961 (0.006)
2	300 nuclear families ²	2.478 (0.418)	0.951 (0.007)	2.491 (0.420)	0.958 (0.006)
3	600 nuclear families ³	2.485 (0.295)	0.957 (0.006)	2.492 (0.296)	0.959 (0.006)

$\hat{\mu}_m$ = Relative risk for males who match with their mother at *HLA-B*. SE = Standard error. $\hat{\mu}_f$ = Relative risk for females who match with their mother at *HLA-B*.

¹ 300 simulated extended pedigrees are analyzed where individuals 7 and 8 are affected (see fig. 1).

² One of the affected individuals and his/her parents is randomly selected from the extended pedigrees simulated in scenario 1, resulting in the analysis of 300 nuclear families.

³ Both affected individuals and their parents from the extended pedigrees simulated in scenario 1 are analyzed, resulting in the inclusion of 600 nuclear families.

Because users may be interested in applying the EMFG test to data from more structured populations, we simulate data where the random mating assumption of HWE is violated by population stratification. Our simulated data have an average F_{ST} of 0.021, comparable to F_{ST} values between southern Italians and the Kuusamo subisolate of Finland ($F_{ST} = 0.023$ [69]) or between Russians and Palestinians ($F_{ST} = 0.020$ [70]). This degree of population stratification is severe enough to cause the test of population allelic homogeneity to be rejected in every one of our samples. When samples are simulated with an MFG effect of 1.0, the type I error rate is inflated (10.5%), the MFG matching effect is overestimated and coverage is reduced ($\hat{\mu} = 1.115$, SE = 0.173, coverage = 88.5%). When samples are simulated with an effect size of 1.5, the MFG effect is overestimated and coverage is reduced ($\hat{\mu} = 1.698$, SE = 0.233, coverage = 83.8%, power = 95.6%). When the degree of population stratification is reduced (when $0.00 < F_{ST} < 0.02$), the type 1 error rate approaches 0.05 and the coverage is increased (data not shown). So, for our actual data analysis using schizophrenia Finnish families from Helsinki and Kuusamo, where the F_{ST} has been estimated to be 0.005 [69], the inference should be accurate.

Investigating the Usefulness of Extended Pedigrees

There is no evidence of bias when simulating pedigrees where only the last generation contains affected individuals and data are analyzed from (1) a single nuclear family per pedigree (table 2, scenario 2) or (2) all nuclear families with an affected offspring (table 2, scenario 3). These nuclear family results (table 2, scenario 2: $\hat{\mu}_m = 2.478$, coverage = 0.951; $\hat{\mu}_f = 2.491$, coverage = 0.958; and scenario 3: $\hat{\mu}_m = 2.485$, coverage = 0.957; $\hat{\mu}_f = 2.492$, cov-

erage = 0.959) match almost exactly the extended pedigree analysis of these same pedigrees (table 2, scenario 1: $\hat{\mu}_m = 2.486$, coverage = 0.958; $\hat{\mu}_f = 2.493$, coverage = 0.961).

Childs et al. [8] show that there is biased estimation of the *RHD* incompatibility effect when pedigrees containing affected parents and affected offspring are partitioned into nuclear families and then analyzed. For this reason we also simulated data using pedigrees where affected individuals are in two consecutive generations and there is a sex-neutral MFG matching effect of 2.5 (table 3, scenarios 4–6). No bias is observed in the analysis of the extended pedigrees for either female or male offspring (table 3, scenario 4: $\hat{\mu}_f = 2.504$, coverage = 0.955; $\hat{\mu}_m = 2.495$, coverage = 0.950). Partitioning pedigrees into nuclear families produces underestimates in the female *HLA-B* maternal-fetal matching effect, but it produces no bias in the male *HLA-B* maternal-fetal matching effect if the nuclear families consisting of the grandparents (fig. 1: individuals 1 and 2), their daughter (individual 5) and son (individual 6) are analyzed (table 3, scenario 5: $\hat{\mu}_f = 2.346$, coverage = 0.926; $\hat{\mu}_m = 2.499$, coverage = 0.943). When all nuclear families are treated as independent, underestimation occurs for the MFG matching effect of both genders but it is more pronounced for females (table 3, scenario 6: $\hat{\mu}_f = 2.356$, coverage = 0.912; $\hat{\mu}_m = 2.404$, coverage = 0.936).

The results for the analysis of a nuclear family consisting of individuals 1, 2, 5, and 6 (table 3, scenario 5) show that the effect in female offspring is underestimated but the effect in male offspring is not. This female-specific parameter underestimation is due to conflicts between the possible *HLA-B* maternal-fetal matching combinations, which are not taken under consideration when the data are treated as nuclear families. When affected indi-

Table 3. Comparison of analysis approaches when parents and grandchildren are affected

Scenario	Number of families	$\hat{\mu}_m$ (SE)	Coverage (SE)	$\hat{\mu}_f$ (SE)	Coverage (SE)
4	300 extended pedigrees ¹	2.495 (0.218)	0.950 (0.007)	2.504 (0.228)	0.955 (0.007)
5	300 nuclear families ²	2.499 (0.355)	0.943 (0.007)	2.346 (0.333)	0.926 (0.008)
6	900 nuclear families ³	2.404 (0.209)	0.936 (0.008)	2.356 (0.205)	0.912 (0.009)

$\hat{\mu}_m$ = Relative risk for males who match with their mother at *HLA-B*. SE = Standard error. $\hat{\mu}_f$ = Relative risk for females who match with their mother at *HLA-B*.

¹ 300 simulated extended pedigrees are analyzed where individuals 5–8 are affected (see fig. 1).

² The nuclear family that includes affected offspring 5 and 6 is selected from the extended pedigrees simulated in scenario 4, resulting in the analysis of 300 nuclear families.

³ Every nuclear family with at least one affected offspring from the extended pedigrees simulated in scenario 4 is selected, resulting in the analysis of 900 nuclear families.

Table 4. log likelihoods and relative risk estimates of schizophrenia due to *HLA-B* MFG matching under three models

Model	$\hat{\mu}_m$ (95% CI)	$\hat{\mu}_f$ (95% CI)	log likelihood
0	= 1	= 1	–2,868.179
1	0.890 (0.687, 1.153)	1.449 (1.109, 1.892)	–2,864.638
2	= 1	1.417 (1.089, 1.843)	–2,865.042

$\hat{\mu}_m$ = Relative risk for males who match with their mother at *HLA-B*. CI = Confidence interval. $\hat{\mu}_f$ = Relative risk for females who match with their mother at *HLA-B*.

viduals are present in consecutive generations, there are conflicting optimal genotypes for individual 5, who is the offspring of individual 2 and also the mother of individual 8. MFG matching favors mothers who are heterozygous and offspring who are homozygous. Thus, as offspring, individuals 5 and 6 are more likely to be homozygous than is expected for a random individual in the population. However, as the mother of affected offspring 8 in the nuclear family (with individuals 4, 5, 8), we expect individual 5's genotype to favor heterozygosity. We find that individual 6 is homozygous in 29% of the simulated families, while individual 5 is homozygous in 26% of the families, reflecting the conflicting role for individual 5. When analyzed as part of the extended family, individual 5's two roles are captured correctly. In the analysis of the nuclear family with individuals 1, 2, 5, and 6, the information that individual 5 is an affected mother of an affected offspring is lost. Overall this information loss leads to an

underestimation of the *HLA-B* matching effect in females but not in males when we analyze affected individuals without including their affected offspring. The information regarding the parents' affection statuses is also lost when we analyze the nuclear families with individuals 3, 6, 7 and with individuals 4, 5, 8. This information loss leads to an underestimation of both the male and female MFG effect. Based on these results, we conclude that the analysis of selected nuclear families could lead to an underestimation of MFG matching effects but not to an overestimation of a female-specific MFG matching effect.

Application of the EMFG Test to Schizophrenia Data

Based on our simulation results it is possible that the previous failure to detect an *HLA-B* matching effect in males [34] is an artifact of breaking the extended pedigrees into nuclear families. Because the new EMFG can now handle extended pedigrees, we retested the hypotheses using the full pedigrees as ascertained. We analyzed the primary hypothesis of a sex-specific *HLA-B* matching effect by first comparing the model in which the male- and female-specific MFG effects are estimated (table 4, model 1) to a model in which both are constrained to the null value (table 4, model 0). This comparison yields evidence to support an *HLA-B* matching effect on schizophrenia ($\chi^2 = 7.082$, d.f. = 2, $p = 0.029$), with a male relative risk estimate ($\hat{\mu}_m$) of 0.890 (95% CI = 0.687, 1.153) and a female relative risk estimate ($\hat{\mu}_f$) of 1.449 (95% CI = 1.109, 1.892). We then fit a third model where we constrain the male MFG effect to 1.0 and only estimate the female MFG matching effect (table 4, model 2). Comparison of the null hypothesis model of no MFG incompatibility (model 0) to a female-specific MFG matching ef-

Table 5. Frequency of *HLA-B* matched and mismatched female offspring among informative mating pairs

Mother	Father	Offspring	Number of mother-father-offspring trios ^a
i/i ^b	i/j	i/i	1
i/i	i/j	i/j	0
i/j	i/k	i/i or i/j	19
i/j	i/k	i/k or j/k	4

^a Number of affected female offspring randomly selected per informative mating pairs where mothers and fathers share one allele IBS.

^b i, j, and k do not represent specific *HLA-B* alleles.

fect model (model 2) shows that the female relative risk estimate ($\hat{\mu}_f$) of 1.417 is significantly different from its null value of 1.0 ($\chi^2 = 6.274$, d.f. = 1, $p = 0.012$, 95% CI = 1.089, 1.843). When the model estimating both male and female relative risks (model 1) is compared to the model estimating only the female relative risk (model 2), the likelihood ratio test is not significant, providing support for the model where the *HLA-B* matching effect is exclusive to female offspring ($\chi^2 = 0.808$, d.f. = 1, $p = 0.369$). Due to a previous finding that *HLA-B*15* and *HLA-B*35* are associated with schizophrenia [68], we also tested for offspring and maternal genetic effects in the presence of the MFG matching effect. In each of these analyses, no significant genetic effects are apparent ($p > 0.13$) and the MFG matching effect remains significant (p values < 0.014). In summary, the *HLA-B* matching effect on schizophrenia is exclusive to female offspring and is not due to maternal or offspring genetic effects.

Examination of the Mate Choice – Olfactory Deficit Model

Before addressing the hypothesis that the observed MFG is due solely to olfactory deficits, we first note that in our study sample HWE at *HLA-B* holds ($p = 0.42$). This result supports the hypothesis of olfactory deficits as well as the use of the EMFG test in our analyses. We examine the ability of olfactory deficits alone to explain the *HLA-B* matching results by randomly selecting one affected female offspring from the 24 mating pairs informative for matching in which there is at least one affected female offspring. We find 20 (83%) who are *HLA-B* MFG matched with their mothers and 4 (17%) who are *HLA-B* MFG mismatched (table 5). These proportions are significantly different from the null hypothesis of no paternal trans-

mission distortion (50%) ($p = 0.001$) and support the alternative hypothesis that paternal transmission distortion is present. We observe similar results supporting the presence of paternal transmission distortion when we use both male and female affected offspring and randomly select one offspring from each informative mating pair (29 *HLA-B* matched (66%) and 15 *HLA-B* mismatched (34%) offspring in 44 mating pairs, $p = 0.035$). Because paternal transmission equilibrium would be expected if the olfactory deficit were the sole cause of the observed association between *HLA-B* maternal-fetal matching and schizophrenia, this result supports a causal role of *HLA-B* matching between mother and female offspring in the etiology of schizophrenia. However, this result does not rule out a scenario where there are olfactory deficits contributing to the observed effects in addition to a causal role for *HLA-B* matching.

Discussion

In this article, we provide a general model and announce software for testing interactions between maternal and offspring genotypes by further extending the flexibility of the EMFG test. The EMFG test is an analysis option within the free, statistical genetics software package Mendel (version 11 or later). This new EMFG test allows for the use of a multi-allelic locus with arbitrary pedigree structures. The test examines whether risk alleles that act through the offspring (offspring genetic effects), mother (maternal genetic effects), or an interaction of maternal and offspring genotypes (MFG incompatibility) modulate disease risk in the offspring. Our new EMFG test is flexible in defining MFG incompatibility specific to the scientific hypotheses in question. As an example, multi-locus SNP genotypes can be used by letting haplotypes serve as the underlying unobserved states and the unordered multi-locus genotypes serve as the observed marker phenotypes [67]. This construct is consistent with our theory development because the likelihood equation 1 sums over all possible genotypes, and alleles need not be co-dominant [20].

Our simulations show that chopping up extended pedigrees into nuclear families can lead to biases in parameter estimation when affected individuals are present in multiple generations. This problem is a result of losing information provided by relatives. With regards to MFG matching, the affection status of grandchildren provides information in the analysis of affected mothers that is lost when analyzing nuclear families or case-

mother/control-mother designs. Thus, whenever possible, we recommend that families be analyzed as ascertained. Although this finding relates directly to analyses of MFG incompatibility, it is likely that conclusions drawn from these simulations will apply to the testing of other intergenerational mechanisms. This advantage must be weighed against the disadvantage of having to assume HWE holds. Our simulations show that when there is population stratification as extreme as Northern Europeans and Middle Eastern Arabs [70], there can be slight upwardly biased estimates of the MFG effect and biased type I errors. Thus, if population stratification or another violation of HWE is suspected, we suggest conducting a test of HWE in a control sample from the same study population before using the EMFG test. If significant, we recommend using the nuclear family MFG test for multi-allelic loci [14, 23, 34].

Family studies are optimal for examining intergenerational genetic effects because they allow for the detection of various types of genotypic variation, which would be confounded without parental genotype data. Schizophrenia provides an excellent example of a disease where addressing more complicated genetic mechanisms than the simple offspring risk allele models tested in genome-wide association studies may provide more insights. We apply the EMFG test to a data set containing previously analyzed individuals [34] and additional family members not previously used. Our results support the previous finding that *HLA-B* MFG matching increases schizophrenia risk in female offspring. Moreover, the 95% CIs in the current study are roughly two-thirds the size previously found [34]. This indicates that reconnecting nuclear families can yield significantly more precise estimates for risk factors. Our analysis bolsters the argument that *HLA-B* maternal-fetal matching is a much larger risk factor for females than males.

Before embarking on studies to clarify the causal role of *HLA-B* matching in schizophrenia etiology, it is important to examine other explanations for the observed association. By using the EMFG test we rule out one possible explanation, namely that the MFG parameter estimates are upwardly biased as a result of choosing nuclear families from extended pedigrees. Simulation studies reveal that under an *HLA-B* matching MFG model, selecting nuclear families from extended pedigrees can lead to underestimation of the female MFG effect when affected females have affected offspring, but it does not lead to overestimation. The demonstration of a statistically significant female *HLA-B* MFG matching effect and the absence of a male effect in the Finnish extended pedigree

data effectively lay to rest the hypothesis that the original result [34] is an artifact of its study design.

We also show that families provide the ability to distinguish between (1) MFG matching that is a consequence of increased genotype similarity of the parental pairs of the affected offspring (such as the olfactory deficit model), and (2) MFG matching that leads to apparent paternal transmission distortion. Our results allow us to refute the hypothesis that the *HLA-B* MFG matching between mother and offspring is solely a byproduct of olfactory deficits in the parents of schizophrenics. We need to reiterate that it is still possible that olfactory deficits are present in the parents of schizophrenics, and that these olfactory deficits set the stage for the MFG matching as a schizophrenia risk factor. However, in order to examine whether olfactory deficits contribute, we would need a different study design that includes families unaffected by schizophrenia.

Discovering factors associated with schizophrenia is only the first step to understanding the etiology of this complicated disorder. The biological basis for the female-specific effect of *HLA-B* matching on schizophrenia needs to be elucidated [35]. One possible mechanism is that *HLA-B* matching results in increased prenatal/obstetric complications and that female fetuses are more likely to survive these complications [71], therefore resulting in the observation of a stronger female *HLA-B* maternal-fetal matching effect in schizophrenia studies. Many studies examine the association of *HLA* maternal-fetal or sibling matching on fetal reproductive outcome [64, 72–88] and many, but not all, of these studies find that *HLA* matching is associated. To our knowledge, only one study [80] specifically examines whether male viability is differentially affected by *HLA-B* maternal-fetal matching. This study finds that surviving males are less likely to be *HLA-B* matched with their mothers than surviving females [80], suggesting that male fetuses are more susceptible to the adverse effects of matching.

Other hypotheses address the mechanisms through which putative effects of *HLA-B* matching, such as low birth weight or preeclampsia, increase schizophrenia risk. One hypothesis posits that preeclampsia leads to abnormal fetal blood flow that results in further complications such as chronic fetal hypoxia or malnutrition [89], both of which have previously been associated with schizophrenia [29, 30, 33, 90–94]. In addition, preeclampsia results in an oxidatively stressed or hypoxic placenta that engages a maternal inflammatory response [95]. These processes may damage the microvascular system of the fetal brain [96, 97] and increase susceptibility to schizophrenia [96].

In summary, our analyses provide additional evidence that *HLA-B* matching is part of the etiology of schizophrenia, and thus should be further explored. It is important to recognize that neither explanation for the observed increase in *HLA-B* matching could have been examined if we had used a case-mother/control-mother design. We also show that it is important to collect data on extended pedigrees rather than mother-offspring pairs because intergenerational effects may easily be missed when ignoring the affection status of a proband's relatives. *HLA-B* matching is not only an example of the broader phenomenon of MFG incompatibility, but is also an important example of an intergenerational mechanism. Our results demonstrate the need for statistical

methods and study designs that can test hypotheses regarding intergenerational effects that may underlie a number of complex diseases. The EMFG test addresses this need.

Acknowledgments

This paper is dedicated to Dr. Leena Peltonen for her consistent support of our MFG research. The authors thank Dr. Kenneth Lange for his assistance in modifying the Mendel software. Mendel software is available through the Human Genetics Department of the University of California, Los Angeles, at <http://www.genetics.ucla.edu/software/>. The research was supported in part by USPHS grants MH59490, GM53275, T32-HG002536, and F31-HG005056.

References

- Hindorf LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA: Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci USA* 2009;106:9362–9367.
- Visscher PM, Hill WG, Wray NR: Heritability in the genomics era – concepts and misconceptions. *Nat Rev Genet* 2008;9:255–266.
- Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM: Finding the missing heritability of complex diseases. *Nature* 2009;461:747–753.
- Maher B: Personal genomes: the case of the missing heritability. *Nature* 2008;456:18–21.
- Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, Nadeau JH: Missing heritability and strategies for finding the underlying causes of complex disease. *Nat Rev Genet* 2010;11:446–450.
- Zhou J, Pelka S, Lange K, Palmer CGS, Sinsheimer JS: Dissecting prenatal, postnatal, and inherited effects: ART and design. *Genet Epidemiol* 2011;35:437–446.
- Chen JB, Zheng HT, Wilson ML: Likelihood ratio tests for maternal and fetal genetic effects on obstetric complications. *Genet Epidemiol* 2009;33:526–538.
- Childs EJ, Palmer CGS, Lange K, Sinsheimer JS: Modeling maternal-offspring gene-gene interactions: the extended-MFG test. *Genet Epidemiol* 2010;34:512–521.
- Cordell HJ: Properties of case/pseudocontrol analysis for genetic association studies: effects of recombination/ascentertainment, and multiple affected offspring. *Genet Epidemiol* 2004;26:186–205.
- Cordell HJ: Estimation and testing of gene-environment interactions in family-based association studies. *Genomics* 2009;93:5–9.
- Cordell HJ, Barratt BJ, Clayton DG: Case/pseudocontrol analysis in genetic association studies: a unified framework for detection of genotype and haplotype associations, gene-gene and gene-environment interactions, and parent-of-origin effects. *Genet Epidemiol* 2004;26:167–185.
- Cordell HJ, Clayton DG: Genetic epidemiology 3 – genetic association studies. *Lancet* 2005;366:1121–1131.
- Gjessing HK, Lie RT: Case-parent triads: estimating single- and double-dose effects of fetal and maternal disease gene haplotypes. *Ann Hum Genet* 2006;70:382–396.
- Hsieh HJ, Palmer CGS, Harney S, Newton JL, Wordsworth P, Brown MA, Sinsheimer JS: The v-MFG test: investigating maternal, offspring and maternal-fetal genetic incompatibility effects on disease and viability. *Genet Epidemiol* 2006;30:333–347.
- Hsieh HJ, Palmer CGS, Sinsheimer JS: Allowing for missing data at highly polymorphic genes when testing for maternal, offspring and maternal-fetal genotype incompatibility effects. *Hum Hered* 2006;62:165–174.
- Kraft P, Hsieh HJ, Cordell HJ, Sinsheimer J: A conditional-on-exchangeable-parental-genotypes likelihood that remains unbiased at the causal locus under multiple-affected-sibling ascertainment. *Genet Epidemiol* 2005;29:87–90.
- Kraft P, Palmer CGS, Woodward AJ, Turunen JA, Minassian S, Paunio T, Lonnqvist J, Peltonen L, Sinsheimer JS: RHD maternal-fetal genotype incompatibility and schizophrenia: extending the MFG test to include multiple siblings and birth order. *Eur J Hum Genet* 2004;12:192–198.
- Li S, Lu Q, Fu W, Romero R, Cui Y: A regularized regression approach for dissecting genetic conflicts that increase disease risk in pregnancy. *Stat Appl Genet Mol Biol* 2009;8:Article 45.
- Minassian SL, Palmer CGS, Sinsheimer JS: An exact maternal-fetal genotype incompatibility (MFG) test. *Genet Epidemiol* 2005;28:83–95.
- Minassian SL, Palmer CGS, Turunen JA, Paunio T, Lonnqvist J, Peltonen L, Woodward JA, Sinsheimer JS: Incorporating serotypes into family based association studies using the MFG test. *Ann Hum Genet* 2006;70:541–553.
- Parimi N, Tromp G, Kuivaniemi H, Nien JK, Gomez R, Romero R, Goddard KA: Analytical approaches to detect maternal/fetal genotype incompatibilities that increase risk of pre-eclampsia. *BMC Med Genet* 2008;9:60.
- Sinsheimer JS, Elston RC, Fu WJ: Gene-gene interaction in maternal and perinatal research. *J Biomed Biotechnol* 2010;2010:853612.
- Sinsheimer JS, Palmer CGS, Woodward JA: Detecting genotype combinations that increase risk for disease: the maternal-fetal genotype incompatibility test. *Genet Epidemiol* 2003;24:1–13.
- Weinberg CR, Wilcox AJ, Lie RT: A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. *Am J Hum Genet* 1998;62:969–978.
- McGuffin P, Gottesman II: Risk factors for schizophrenia. *N Engl J Med* 1999;341:370–371.
- Wray NR, Visscher PM: Narrowing the boundaries of the genetic architecture of schizophrenia. *Schizophr Bull* 2010;36:14–23.

- 27 Cannon M, Jones PB, Murray RM: Obstetric complications and schizophrenia: historical and meta-analytic review. *Am J Psychiatry* 2002;159:1080–1092.
- 28 Cannon TD, Rosso IM, Hollister JM, Bearden CE, Sanchez LE, Hadley T: A prospective cohort study of genetic and perinatal influences in the etiology of schizophrenia. *Schizophr Bull* 2000;26:351–366.
- 29 Dalman C, Allebeck P, Cullberg J, Grunewald C, Koster M: Obstetric complications and the risk of schizophrenia – a longitudinal study of a national birth cohort. *Arch Gen Psychiatry* 1999;56:234–240.
- 30 Geddes JR, Verdoux H, Takei N, Lawrie SM, Bovet P, Eagles JM, Heun R, McCreadie RG, McNeil TF, O'Callaghan E, Stober G, Willinger U, Murray RM: Schizophrenia and complications of pregnancy and labor: an individual patient data meta-analysis. *Schizophr Bull* 1999;25:413–423.
- 31 Kendell RE, Juszcak E, Cole SK: Obstetric complications and schizophrenia: a case control study based on standardised obstetric records. *Br J Psychiatry* 1996;168:556–561.
- 32 Nicodemus KK, Marenco S, Batten AJ, Vakalanka R, Egan MF, Straub RE, Weinberger DR: Serious obstetric complications interact with hypoxia-regulated/vascular-expression genes to influence schizophrenia risk. *Mol Psychiatry* 2008;13:873–877.
- 33 Rifkin L, Lewis S, Jones P, Toone B, Murray R: Low birth weight and schizophrenia. *Br J Psychiatry* 1994;165:357–362.
- 34 Palmer CGS, Hsieh HJ, Reed EF, Lonnqvist J, Peltonen L, Woodward JA, Sinsheimer JS: HLA-B maternal-fetal genotype matching increases risk of schizophrenia. *Am J Hum Genet* 2006;79:710–715.
- 35 Palmer CG: Evidence for maternal-fetal genotype incompatibility as a risk factor for schizophrenia. *J Biomed Biotechnol* 2010;2010:576318.
- 36 Brewer WJ, Wood SJ, Pantelis C, Berger GE, Copolov DL, McGorry PD: Olfactory sensitivity through the course of psychosis: relationships to olfactory identification, symptomatology and the schizophrenia odour. *Psychiatry Res* 2007;149:97–104.
- 37 Cumming AG, Matthews NL, Park S: Olfactory identification and preference in bipolar disorder and schizophrenia. *Eur Arch Psychiatry Clin Neurosci* 2011;261:251–259.
- 38 Ishizuka K, Tajinda K, Colantuoni C, Morita M, Winicki J, Le C, Lin S, Schretlen D, Sawa A, Cascella NG: Negative symptoms of schizophrenia correlate with impairment on the University of Pennsylvania smell identification test. *Neurosci Res* 2010;66:106–110.
- 39 Kopala LC, Good KP, Morrison K, Bassett AS, Alda M, Honer WG: Impaired olfactory identification in relatives of patients with familial schizophrenia. *Am J Psychiatry* 2001;158:1286–1290.
- 40 Moberg PJ, Agrin R, Gur RE, Gur RC, Turetsky BI, Doty RL: Olfactory dysfunction in schizophrenia: a qualitative and quantitative review. *Neuropsychopharmacology* 1999;21:325–340.
- 41 Strauss GP, Allen DN, Ross SA, Duke LA, Schwartz J: Olfactory hedonic judgment in patients with deficit syndrome schizophrenia. *Schizophr Bull* 2010;36:860–868.
- 42 Turetsky BI, Kohler CG, Gur RE, Moberg PJ: Olfactory physiological impairment in first-degree relatives of schizophrenia patients. *Schizophr Res* 2008;102:220–229.
- 43 Santos PSC, Schinemann JA, Gabardo J, Bicalho MD: New evidence that the MHC influences odor perception in humans: a study with 58 Southern Brazilian students. *Horm Behav* 2005;47:384–388.
- 44 Wedekind C, Furi S: Body odour preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? *Proc Biol Sci* 1997;264:1471–1479.
- 45 Wedekind C, Seebeck T, Bettens F, Paepke AJ: MHC-dependent mate preferences in humans. *Proc Biol Sci* 1995;260:245–249.
- 46 Havlicek J, Roberts SC: MHC-correlated mate choice in humans: a review. *Psychoneuroendocrinology* 2009;34:497–512.
- 47 Jacob S, McClintock MK, Zelano B, Ober C: Paternally inherited HLA alleles are associated with women's choice of male odor. *Nat Genet* 2002;30:175–179.
- 48 Lie HC, Simmons LW, Rhodes G: Genetic dissimilarity, genetic diversity, and mate preferences in humans. *Evol Hum Behav* 2010;31:48–58.
- 49 Roberts SC, Gosling LM, Carter V, Petrie M: MHC-correlated odour preferences in humans and the use of oral contraceptives. *Proc Biol Sci* 2008;275:2715–2722.
- 50 Thornhill R, Gangestad SW, Miller R, Scheyd G, McCollough JK, Franklin M: Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behav Ecol* 2003;14:668–678.
- 51 Derti A, Cenik C, Kraft P, Roth FP: Absence of evidence for MHC-dependent mate selection within hapmap populations. *PLoS Genet* 2010;6:e1000925.
- 52 Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R, Suhonen J, Ellonen P, Chan GY, Sinsheimer JS, Sobel E, Juvonen H, Arajärvi R, Partonen T, Suvisaari J, Lonnqvist J, Meyer J, Peltonen L: Chromosome 1 loci in Finnish schizophrenia families. *Hum Mol Genet* 2001;10:1611–1617.
- 53 Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD, Juvonen H, Varilo T, Arajärvi R, Kokko-Sahin ML, Lonnqvist J, Peltonen L: Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. *Hum Mol Genet* 2000;9:1049–1057.
- 54 Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajärvi R, Juvonen H, Kokko-Sahin ML, Vaisanen L, Mannila H, Lonnqvist J, Peltonen L: A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. *Am J Hum Genet* 1999;65:1114–1124.
- 55 Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA, Rinard K, Foti A, Terwilliger JD, Juvonen H, Suvisaari J, Arajärvi R, Suokas J, Partonen T, Lonnqvist J, Meyer J, Peltonen L: Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 2001;10:3037–3048.
- 56 American Psychiatric Association Task Force on DSM-IV: Diagnostic and Statistical Manual of Mental Disorders: DSM-IV, ed 4. Washington, DC, American Psychiatric Association, 1994.
- 57 Blin N, Stafford DW: A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res* 1976;3:2303–2308.
- 58 Lange K, Sinsheimer JS: The pedigree trimming problem. *Hum Hered* 2004;58:108–111.
- 59 Lange K, Cantor R, Horvath S, Perola M, Sabbati C, et al: MENDEL version 4.0: a complete package for the exact genetic analysis of discrete traits in pedigree and population data sets. *Am J Hum Genet* 2001;69 (suppl):504.
- 60 Sobel E, Papp JC, Lange K: Detection and integration of genotyping errors in statistical genetics. *Am J Hum Genet* 2002;70:496–508.
- 61 Zhou JJ, Lange K, Papp JC, Sinsheimer JS: A heterozygote-homozygote test of Hardy-Weinberg equilibrium. *Eur J Hum Genet* 2009;17:1495–1500.
- 62 Lange K: Mathematical and Statistical Methods for Genetic Analysis, ed 2. New York, Springer, 2002.
- 63 Clayton D: A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. *Am J Hum Genet* 1999;65:1170–1177.
- 64 Ober C, Hyslop T, Elias S, Weitkamp LR, Hauck WW: Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. *Hum Reprod* 1998;13:33–38.
- 65 Lange K, Sinsheimer JS, Sobel E: Association testing with Mendel. *Genet Epidemiol* 2005;29:36–50.
- 66 Schreuder GMT, Hurley CK, Marsh SGE, Lau M, Maier M, Kollman C, Noreen HJ: The HLA dictionary 2001: a summary of HLA-A, -B, -C, -DRB1/3/4/5 and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR and -DQ antigens. *Eur J Immunogenet* 2001;28:565–596.

- 67 Sinsheimer JS, McKenzie CA, Keavney B, Lange K: SNPs and snails and puppy dogs' tails: analysis of SNP haplotype data using the gamete competition model. *Ann Hum Genet* 2001;65:483–490.
- 68 Nunes SOV, Borelli SD, Matsuo T, Watanabe MAE, Itano EN: The association of the HLA in patients with schizophrenia, schizoaffective disorder, and in their biological relatives. *Schizophr Res* 2005;76:195–198.
- 69 Nelis M ET, Magi R, Zimprich F, Zimprich A, Toncheva D, Karachanak S, Piskackova T, Balascek I, Peltonen L, Jakkula E, Rehnstrom K, Lathrop M, Heath S, Galan P, Schreiber S, Meitinger T, Pfeufer A, Wichmann HE, Meleghe B, Polgar N, Toniolo D, Gasparini P, D'Adamo P, Klovins J, Nikitina-Zake L, Kucinskas V, Kasnauskiene J, Lubinski J, Debnick T, Limborska S, Khrunin A, Estivill X, Rabionet R, Marsal S, Julia A, Antonarakis SE, Deutsch S, Borel C, Attar H, Gagnebin M, Macek M, Krawczak M, Remm M, Metspalu A: Genetic structure of Europeans: a view from the North-East. *PLoS One* 2009;4:e5472.
- 70 Tian C KR, Nassir R, Lee A, Villoslada P, Klareskog L, Hammarstrom L, Garchon HJ, Pulver AE, Ransom M, Gregersen PK, Seldin MF: European population genetic substructure: further definition of ancestry informative markers for distinguishing among diverse European ethnic groups. *Mol Med* 2009;15:371–383.
- 71 Vatten LJ, Skjaerven R: Offspring sex and pregnancy outcome by length of gestation. *Early Hum Dev* 2004;76:47–54.
- 72 Beydoun H, Saftlas AF: Association of human leucocyte antigen sharing with recurrent spontaneous abortions. *Tissue Antigens* 2005;65:123–135.
- 73 Bolis PF, Bianchi MM, Lafianza A, Franchi M, Belvedere MC: Immunogenetic aspects of preeclampsia. *Biol Res Pregnancy Perinatol* 1987;8:42–45.
- 74 Fujisawa S: HLA antigens-antibodies system and its association with severe toxemia of pregnancy. *Nippon Sanka Fujinka Gakkai Zasshi* 1985;37:124–130.
- 75 Moghraby JS, Tamim H, Anacan V, Al Khalaf H, Moghraby SA: HLA sharing among couples appears unrelated to idiopathic recurrent fetal loss in Saudi Arabia. *Hum Reprod* 2010;25:1900–1905.
- 76 Ober C: HLA and pregnancy: the paradox of the fetal allograft. *Am J Hum Genet* 1998;62:1–5.
- 77 Ober CL, Martin AO, Simpson JL, Hauck WW, Amos DB, Kostyu DD, Fotino M, Allen FH: Shared HLA antigens and reproductive performance among Hutterites. *Am J Hum Genet* 1983;35:994–1004.
- 78 Schneider K, Knutson F, Tamsen L, Sjöberg O: HLA antigen sharing in preeclampsia. *Gynecol Obstet Invest* 1994;37:87–90.
- 79 Unander AM, Olding LB: Habitual abortion: parental sharing of HLA antigens, absence of maternal blocking antibody, and suppression of maternal lymphocytes. *Am J Reprod Immunol* 1983;4:171–178.
- 80 Astolfi P, Cuccia M, Caruso C, Favoino B, Fazzari M, Mantovani V, Lulli P, Illeni MT, Martinetti M: Sharing at the major histocompatibility complex affects the secondary sex ratio in differing ways. *Hum Hered* 1996;46:155–165.
- 81 Brunori ID, Battini L, Simonelli M, Clemente F, Brunori E, Mariotti ML, Genazzani AR: Increased HLA-DR homozygosity associated with pre-eclampsia. *Hum Reprod* 2000;15:1807–1812.
- 82 Cowan LD, Hudson L, Bobele G, Chancellor I, Baker J: Maternal-fetal HLA sharing and risk of newborn encephalopathy and seizures: a pilot study. *J Child Neurol* 1994;9:173–177.
- 83 Larizza D, Martinetti M, Dugoujon JM, Tinelli C, Calcaterra V, Cuccia M, Salvaneschi L, Severi F: Parental GM and HLA genotypes and reduced birth weight in patients with Turner's syndrome. *J Pediatr Endocrinol Metab* 2002;15:1183–1190.
- 84 Montgomery GW, Zhu G, Hottenga JJ, Duffy DL, Heath AC, Boomsma DI, Martin NG, Visscher PM: HLA and genome-wide allele sharing in dizygotic twins. *Am J Hum Genet* 2006;79:1052–1058.
- 85 Ober C, Simpson JL, Ward M, Radvany RM, Andersen R, Elias S, Sabbagha R: Prenatal effects of maternal-fetal HLA compatibility. *Am J Reprod Immunol Microbiol* 1987;15:141–149.
- 86 Reznikoff-Etievant MF, Bonneau JC, Alcalay D, Cavelier B, Toure C, Lobet R, Netter A: HLA antigen-sharing in couples with repeated spontaneous abortions and the birth-weight of babies in successful pregnancies. *Am J Reprod Immunol* 1991;25:25–27.
- 87 Verp MS, Sibul M, Billstrand C, Belen G, Hsu M, Ober C: Maternal-fetal histocompatibility in intrauterine growth retarded and normal weight babies. *Am J Reprod Immunol* 1993;29:195–198.
- 88 Zollner S, Wen XQ, Hanchard NA, Herbert MA, Ober C, Pritchard JK: Evidence for extensive transmission distortion in the human genome. *Am J Hum Genet* 2004;74:62–72.
- 89 Buka SL, Tsuang MT, Lipsitt LP: Pregnancy/delivery complications and psychiatric diagnosis. A prospective study. *Arch Gen Psychiatry* 1993;50:151–156.
- 90 Brown AS, Susser ES, Butler PD, Andrews RR, Kaufmann CA, Gorman JM: Neurobiological plausibility of prenatal nutritional deprivation as a risk factor for schizophrenia. *J Nerv Ment Dis* 1996;184:71–85.
- 91 Hulshoff HE, Hoek HW, Susser E, Brown AS, Dingemans A, Schnack HG, van Haren NEM, Ramos LMP, Gispens-de Wied CC, Kahn RS: Prenatal exposure to famine and brain morphology in schizophrenia. *Am J Psychiatry* 2000;157:1170–1172.
- 92 St Clair D, Xu MQ, Wang P, Yu YQ, Fang YR, Zhang F, Zheng XY, Gu NF, Feng GY, Sham P, He L: Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959–1961. *JAMA* 2005;294:557–562.
- 93 Susser ES, Lin SP: Schizophrenia after prenatal exposure to the Dutch Hunger Winter of 1944–1945. *Arch Gen Psychiatry* 1992;49:983–988.
- 94 Xu MQ, Sun WS, Liu BX, Feng GY, Yu L, Yang L, He G, Sham P, Susser E, St Clair D, He L: Prenatal malnutrition and adult schizophrenia: further evidence from the 1959–1961 Chinese famine. *Schizophr Bull* 2009;35:568–576.
- 95 Cudihy D, Lee RV: The pathophysiology of pre-eclampsia: current clinical concepts. *J Obstet Gynaecol* 2009;29:576–582.
- 96 Hanson DR, Gottesman II: Theories of schizophrenia: a genetic-inflammatory-vascular synthesis. *BMC Med Genet* 2005;6:7.
- 97 Huleihel M, Golan H, Hallak M: Intrauterine infection/inflammation during pregnancy and offspring brain damages: possible mechanisms involved. *Reprod Biol Endocrinol* 2004;2:17.