

ROBUST PARTIAL LIKELIHOOD APPROACH FOR DETECTING IMPRINTING AND MATERNAL EFFECTS USING CASE-CONTROL FAMILIES¹

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Genomic imprinting and maternal effects are two epigenetic factors that have been increasingly explored for their roles in the etiology of complex diseases. This is part of a concerted effort to find the “missing heritability.” Accordingly, statistical methods have been proposed to detect imprinting and maternal effects simultaneously based on either a case-parent triads design or a case-mother/control-mother pairs design. However, existing methods are full-likelihood based and have to make strong assumptions concerning mating type probabilities (nuisance parameters) to avoid overparametrization. In this paper we propose to augment the two popular study designs by combining them and including control-parent triads, so that our sample may contain a mixture of case-parent/control-parent triads and case-mother/control-mother pairs. By matching the case families with control families of the same structure and stratifying according to the familial genotypes, we are able to derive a partial likelihood that is free of the nuisance parameters. This renders unnecessary any unrealistic assumptions and leads to a robust procedure without sacrificing power. Our simulation study demonstrates that our partial likelihood method has correct type I error rate, little bias and reasonable power under a variety of settings.

1. Introduction. Genomic imprinting and maternal effects are two epigenetic factors that have been increasingly explored for their roles in the etiology of complex diseases. This is part of a concerted effort to find the “missing heritability” [Manolio et al. (2009)]. Genomic imprinting (maternal or paternal) is an epigenetic process involving methylation and histone modifications in order to silence the expression of a gene inherited from a particular parent (mother or father) without altering the genetic sequence. This process leads to unequal expression of a heterozygous genotype depending on whether the imprinted variant is inherited from the mother or the father. Maternal effect, on the other hand, refers to a situation where the phenotype of an individual is influenced by the genotype of the mother. Maternal effects usually occur due to the additional mRNAs or proteins passed from mother to fetus during pregnancy, which may result in an individual

Received March 2011; revised February 2012.

¹Supported in part by NSF Grant DMS-12-08968.

Key words and phrases. Genomic imprinting, maternal effect, case-parent/control-parent triads, case-mother/control-mother pairs, mating type probabilities.

showing the phenotype due to the genotype of the mother regardless of one's own genotype.

The first imprinted gene in humans was found almost 20 years ago [Gianoukakis et al. (1993)]. Since then, a number of genetic disorders have been found to be associated with imprinting defects. The most well known include Beckwith–Wiedemann syndrome, Silver–Russell syndrome, Angelman syndrome and Prader–Willi syndrome [Falls et al. (1999)]. Although it has been estimated that about 1% of all mammalian genes are imprinted [Morison, Paton and Cleverley (2001)], only a limited number have been identified thus far. With the availability of the massively parallel sequencing technology, scientists are now able to carry out direct studies of imprinting genomewide in mouse efficiently [Gregg et al. (2010), Wang et al. (2008)]. Nevertheless, the controlled mating setup that was successful in mouse studies is not feasible in humans. Therefore, there is still a pressing need for the development of robust and powerful statistical methods for detecting imprinting effects based on single nucleotide polymorphism data.

A variety of diseases, especially those that are related to pregnancy outcomes, such as childhood diseases and birth defects, have been hypothesized to be influenced by maternal effects. Well-known examples of diseases in which maternal effects play a role include childhood cancer and spina bifida [Haig (1993, 2004), Jensen et al. (2006)]. Maternal effects are also suspected to be involved in other diseases such as schizophrenia [Palmer et al. (2006)] and high blood pressure [Yang and Lin (2009)]. Although imprinting and maternal effects arise from two different biological processes, their effects as expressed in the phenotypes can mask one another [Hager, Cheverud and Wolf (2008)]. Thus, it is important that these two confounding effects be studied jointly to avoid false positives/negatives.

Two popular designs for studying genomic imprinting and/or maternal effects are case-parent triads and case-mother pairs, the latter may be supplemented by control-mother pairs [Weinberg, Wilcox and Lie (1998), Weinberg and Umbach (2005), Shi et al. (2008), Ainsworth et al. (2011)]. The use of triads is attractive, as both imprinting and maternal effects can be studied jointly to handle the issue of confounding. However, it is well known that fathers are usually much harder to recruit than mothers for a genetic study, and thus a study design with case-mother/control-mother pairs may be easier to meeting its target sample size. Nevertheless, the pair design will lead to reduction in the number of familial genotype categories, and thus only maternal effect is usually studied even with the assumption of mating symmetry [Shi et al. (2008)]. The hybrid design of Vermeulen et al. (2009) proposed to use control-mother pairs to enrich the sample of case-parent triads, leading to a case-parent triad/control-mother design. Such a design increases the number of family genotype categories so that mating frequencies can be estimated without the mating symmetry assumption.

Both nonparametric and parametric statistical methods have been proposed in the literature for analyzing triads and pairs data. Nonparametric methods are mainly for detecting the imprinting effect under the assumption of no maternal ef-

fect [Weinberg (1999), Zhou et al. (2009)]. Such methods are very attractive, as they are simple, elegant and powerful, but they may suffer from severely inflated type I error rate or power loss if the assumption is violated. On the other hand, existing parametric methods can usually study both imprinting and maternal effects (with triad data), but they usually need to make rather strong assumptions to avoid overparametrization. The typical assumptions made are regarding mating type probabilities, the most extreme of which is random mating, leading to the Hardy–Weinberg equilibrium (HWE). More mild assumptions include parental allelic changeability and mating symmetry. It is well accepted that the assumption of HWE is unlikely to hold, however, even the validity of the least stringent one, mating symmetry, may still be in doubt in reality. Mating selections are usually guided by cultural values and social rules in general, and, further, mating symmetry is easily violated if there is any gender-specific assortative mating in the population.

Many complex diseases, such as cancers and hypertension, are rather common. However, rare diseases are sometimes assumed in existing methods so that the probabilities of child-mother pair genotype combinations can be treated as approximately the same in the control and in the general populations [Shi et al. (2008)]. Although the rationale seems rather intuitive, analytical as well as simulation results indicate that the rare disease assumption is in fact only a necessary, but not a sufficient condition, for the frequencies to be roughly equal. It is the interplay of allele frequency and the underlying genetic model, not the rare disease assumption alone, that determines whether the pair frequencies are roughly equal.

In this paper, we propose a partial Likelihood approach for detecting *Imprinting* and *Maternal Effects* (LIME) simultaneously using a family-based case-control design. Specifically, our sampled data is a mixture of case-parent/control-parent triads and case-mother/control-mother pairs. By including control families in our design, we can match case families with control families of the same structure (i.e., triads vs. triads and pairs vs. pairs) and factor out common terms involving mating type probabilities, the nuisance parameters. This design makes it possible to formulate a novel partial likelihood approach, wherein the likelihood component of interest is free of the nuisance parameters. This circumvents the problem of overparametrization and unrealistic assumptions that plague existing methods. Further, LIME can make use of all available data information (complete triads and those with missing fathers). It does not rely on any assumption about mating type probabilities or rarity of the disease, which makes it robust to departure from the usual assumptions without sacrificing much power.

2. Method. The genotype scores (the number of the variant alleles carried by an individual) of the mother, father and child in a triad are denoted by M , F and C , respectively, which takes values in $\{0, 1, 2\}$. In case-mother or control-mother pairs, the paternal genotype score F is missing. The disease status $D = 1$ indicates that the child is affected and 0 otherwise. We use the following multi-

plicative relative risk model of disease penetrance:

$$(1) \quad P(D = 1|M, F, C) = \delta R_1^{I(C=1)} R_2^{I(C=2)} R_{im}^{I(C=1 \text{ and origin}=M)} S_1^{I(M=1)} S_2^{I(M=2)},$$

where δ is the phenocopy rate of the disease; R_1 and R_2 are the variant allele effect of 1 and 2 copies carried by the child, respectively; R_{im} is the effect when the single copy of the variant allele carried by the child is inherited from the mother; S_1 and S_2 are the maternal effect when the mother carries 1 and 2 copies of the variant allele, respectively; and $I(\cdot)$ is the usual indicator function that is equal to 1 or 0 depending on whether the condition within the parentheses is met or not. Likelihood ratio tests can be conducted to test: (1) for association, $H_0: R_1 = R_2 = R_{im} = S_1 = S_2 = 1$ vs. H_a : at least one of these parameters is not 1; (2) for (maternal/paternal) imprinting, $H_0: R_{im} = 1$ vs. $H_a: R_{im}(< / >) \neq 1$; (3) for maternal effect, $H_0: S_1 = S_2 = 1$ vs. $H_a: S_1$ or S_2 is not 1. Note that this model was used by [Weinberg, Wilcox and Lie \(1998\)](#), which was adopted by [Shi et al. \(2008\)](#) but without the term for the imprinting effect for their pair sampling design. This model is also equivalent to one of the models used recently by [Ainsworth et al. \(2011\)](#).

Denote the numbers of case-parent triads, control-parent triads, case-mother pairs and control-mother pairs in the sample by N_t^1, N_t^0, N_p^1 and N_p^0 , respectively. Two special cases of this general setup are as follows: (1) $N_p^0 = N_p^1 = 0$, if there are no missing fathers and thus all the families are triads; and (2) $N_t^0 = N_t^1 = 0$, if all fathers are missing, and thus all the families are child-mother pairs. Therefore, our method represents a unified approach as it is applicable to triad only data as in [Weinberg, Wilcox and Lie \(1998\)](#), or pairs only data as in [Shi et al. \(2008\)](#), or a mixture of the two.

There are 15 possible combinations of (M, F, C) for triads; their enumeration and labeling (type) are listed in the top segment of [Table 1](#). The joint probability of the disease status of the child (D) and the triad genotype combination (M, F, C) can be decomposed as

$$P(D, M, F, C) = P(M, F)P(C|M, F)P(D|M, F, C),$$

where $P(M, F)$ is the population probability of a particular parental pair (also called mating type), which is denoted by μ_{mf} for $M = m$ and $F = f$. Note that we do not make any assumption about the mating type probabilities such as HWE or even mating symmetry, and thus μ_{mf} is not necessarily equal to μ_{fm} . On the other hand, $P(C|M, F)$ is the transmission probability, which follows the Mendelian law of segregation. For the penetrance probability, $P(D|M, F, C)$, we use the multiplicative relative risk model as given in equation (1). For all types other than type 8 ([Table 1](#)), if a child has one copy of the variant allele, the parental origin can be unambiguously ascertained, and hence the relevant factors can be

TABLE 1
Joint probabilities of disease status and triad genotypes^a

Type	M	F	C	$P(D = 1, M, F, C)$	$P(D = 0, M, F, C)$
1	0	0	0	$\mu_{00} \cdot 1 \cdot \delta$	$\mu_{00} \cdot 1 \cdot [1 - \delta]$
2	0	1	0	$\mu_{01} \cdot \frac{1}{2} \cdot \delta$	$\mu_{01} \cdot \frac{1}{2} \cdot [1 - \delta]$
3	0	1	1	$\mu_{01} \cdot \frac{1}{2} \cdot \delta R_1$	$\mu_{01} \cdot \frac{1}{2} \cdot [1 - \delta R_1]$
4	0	2	1	$\mu_{02} \cdot 1 \cdot \delta R_1$	$\mu_{02} \cdot 1 \cdot [1 - \delta R_1]$
5	1	0	0	$\mu_{10} \cdot \frac{1}{2} \cdot \delta S_1$	$\mu_{10} \cdot \frac{1}{2} \cdot [1 - \delta S_1]$
6	1	0	1	$\mu_{10} \cdot \frac{1}{2} \cdot \delta S_1 R_1 R_{im}$	$\mu_{10} \cdot \frac{1}{2} \cdot [1 - \delta S_1 R_1 R_{im}]$
7	1	1	0	$\mu_{11} \cdot \frac{1}{4} \cdot \delta S_1$	$\mu_{11} \cdot \frac{1}{4} \cdot [1 - \delta S_1]$
8	1	1	1	$\mu_{11} \cdot \frac{1}{4} \cdot \delta S_1 R_1 (1 + R_{im})$	$\mu_{11} \cdot \frac{1}{4} \cdot [2 - \delta S_1 R_1 (1 + R_{im})]$
9	1	1	2	$\mu_{11} \cdot \frac{1}{4} \cdot \delta S_1 R_2$	$\mu_{11} \cdot \frac{1}{4} \cdot [1 - \delta S_1 R_2]$
10	1	2	1	$\mu_{12} \cdot \frac{1}{2} \cdot \delta S_1 R_1$	$\mu_{12} \cdot \frac{1}{2} \cdot [1 - \delta S_1 R_1]$
11	1	2	2	$\mu_{12} \cdot \frac{1}{2} \cdot \delta S_1 R_2$	$\mu_{12} \cdot \frac{1}{2} \cdot [1 - \delta S_1 R_2]$
12	2	0	1	$\mu_{20} \cdot 1 \cdot \delta S_2 R_1 R_{im}$	$\mu_{20} \cdot 1 \cdot [1 - \delta S_2 R_1 R_{im}]$
13	2	1	1	$\mu_{21} \cdot \frac{1}{2} \cdot \delta S_2 R_1 R_{im}$	$\mu_{21} \cdot \frac{1}{2} \cdot [1 - \delta S_2 R_1 R_{im}]$
14	2	1	2	$\mu_{21} \cdot \frac{1}{2} \cdot \delta S_2 R_2$	$\mu_{21} \cdot \frac{1}{2} \cdot [1 - \delta S_2 R_2]$
15	2	2	2	$\mu_{22} \cdot 1 \cdot \delta S_2 R_2$	$\mu_{22} \cdot 1 \cdot [1 - \delta S_2 R_2]$
				$P(D = 1, M, C)$	$P(D = 0, M, C)$
1, 2	0	-	0	$(\mu_{00} + \frac{1}{2}\mu_{01}) \cdot \delta$	$(\mu_{00} + \frac{1}{2}\mu_{01}) \cdot [1 - \delta]$
3, 4	0	-	1	$(\frac{1}{2}\mu_{01} + \mu_{02}) \cdot \delta R_1$	$(\frac{1}{2}\mu_{01} + \mu_{02}) \cdot [1 - \delta R_1]$
5, 7	1	-	0	$(\frac{1}{2}\mu_{10} + \frac{1}{4}\mu_{11}) \cdot \delta S_1$	$(\frac{1}{2}\mu_{10} + \frac{1}{4}\mu_{11}) \cdot [1 - \delta S_1]$
6, 8, 10	1	-	1	$\frac{1}{2}\mu_{10} \cdot \delta S_1 R_1 R_{im}$ $+ \frac{1}{4}\mu_{11} \cdot \delta S_1 R_1 (1 + R_{im})$ $+ \frac{1}{2}\mu_{12} \cdot \delta S_1 R_1$	$\frac{1}{2}\mu_{10} \cdot [1 - \delta S_1 R_1 R_{im}]$ $+ \frac{1}{4}\mu_{11} \cdot [2 - \delta S_1 R_1 (1 + R_{im})]$ $+ \frac{1}{2}\mu_{12} \cdot [1 - \delta S_1 R_1]$
9, 11	1	-	2	$(\frac{1}{4}\mu_{11} + \frac{1}{2}\mu_{12}) \cdot \delta S_1 R_2$	$(\frac{1}{4}\mu_{11} + \frac{1}{2}\mu_{12}) \cdot [1 - \delta S_1 R_2]$
12, 13	2	-	1	$(\mu_{20} + \frac{1}{2}\mu_{21}) \cdot \delta S_2 R_1 R_{im}$	$(\mu_{20} + \frac{1}{2}\mu_{21}) \cdot [1 - \delta S_2 R_1 R_{im}]$
14, 15	2	-	2	$(\frac{1}{2}\mu_{21} + \mu_{22}) \cdot \delta S_2 R_2$	$(\frac{1}{2}\mu_{21} + \mu_{22}) \cdot [1 - \delta S_2 R_2]$

^aM, F and C are the number of variant allele(s) carried by the mother, the father and the child in a family, which are equal to 0, 1 or 2; F = - indicates paternal genotype is missing in case-mother and control-mother pairs; μ_{mf} denotes the probability of parental pairs in which the mothers carry m copies and the fathers carry f copies of the variant allele, that is, mating type probability of (M, F) = (m, f); δ is the phenocopy rate of the disease in the population; R_1 and R_2 are relative risks due to 1 and 2 two copies of the variant allele carried by the offspring, respectively; R_{im} is the relative risk due to the single copy of the variant allele being inherited from the mother; S_1 and S_2 are the maternal effect of 1 and 2 copies of the variant allele carried by the mother, respectively.

easily extracted from (1) and multiplied to the joint probability. For type 8, in which $(M, F, C) = (1, 1, 1)$, the variant allele carried by the child can be inherited either from the mother or the father with equal probabilities and, as such, both possibilities need to be considered, leading to the summation of two probabilities weighted equally. For all 15 types, the specific joint probabilities for the case-parent and control-parent triads are given in the last two columns of the top segment of Table 1. We can see from the table that the parameters concerning the mating type probabilities (μ_{mf}) are factored out nicely from the parameters of the disease model, both for the case and the control families.

For pairs, because the father’s genotype is missing, the 15 (M, F, C) combinations for triads collapse into 7 (M, C) types, as indicated in the bottom segment of Table 1. In other words, each (M, C) type is the combination of potential triad types had the paternal genotype been observed for those child-mother pairs. For example, for $(M, C) = (0, 0)$, the father’s genotype can be either 0 or 1 and, therefore, the first type for (M, C) is the combinations of types 1 and 2 for triads. Accordingly, the joint distribution of disease status and genotype is the sum of the probabilities of the collapsing triads types. That is,

$$P(D, M, C) = \sum_{F=0,1,2} P(D, M, F, C) = \sum_{F=0,1,2} P(M, F, C)P(D|M, F, C),$$

where $P(M, F, C)$ may be 0 if F is not compatible with the observed genotypes. For example, for $(M, C) = (0, 0)$, $F = 2$ is incompatible and, therefore, the summation is only over 0 and 1. For most (M, C) combinations, the penetrance $P(D|M, F, C)$ can be factorized out of the summation over $F = 0, 1, 2$, since the penetrance is the same for the potential types of triad regardless of the paternal genotype under our model. The only exception is $(M, C) = (1, 1)$, in which case the penetrance term is different for the three potential types of triad unless the imprinting effect is absent (i.e., $R_{im} = 1$). The specific joint probabilities for the case-mother and control-mother pairs are given in the last two columns of the bottom segment of Table 1. We can see from the table that, other than when $(M, C) = (1, 1)$, the parameters concerning the mating type probabilities (μ_{mf}) are factored out nicely from the parameters of the disease model, both for the case and the control families.

Denote the counts of case-parent and control-parent triads with $M = m$, $F = f$ and $C = c$ by n_{mfc}^1 and n_{mfc}^0 , respectively. Similarly, the counts of case-mother and control-mother pairs with $M = m$ and $C = c$ are denoted by n_{mc}^1 and n_{mc}^0 . With the fixed totals N_t^1 , N_t^0 , N_p^1 and N_p^0 , the distributions of the 15 observed triad counts for the cases ($\{n_{mfc}^1\}$) and the controls ($\{n_{mfc}^0\}$), and those of the 7 observed pair counts for the cases ($\{n_{mc}^1\}$) and the controls ($\{n_{mc}^0\}$), are as follows:

$$n_{mfc}^1 \sim \text{Multinomial}(N_t^1, P(M = m, F = f, C = c | D = 1)),$$

$$n_{mfc}^0 \sim \text{Multinomial}(N_t^0, P(M = m, F = f, C = c | D = 0)),$$

$$n_{mc}^1 \sim \text{Multinomial}(N_p^1, P(M = m, C = c | D = 1)),$$

$$n_{mc}^0 \sim \text{Multinomial}(N_p^0, P(M = m, C = c | D = 0)).$$

The cell probabilities $P(M, F, C | D) = P(D, M, F, C) / P(D)$ and $P(M, C | D) = P(D, M, C) / P(D)$, where the probabilities in the numerators are as given in Table 1 and we assume the disease prevalence, $P(D = 1)$, in the source population is known. Such information for most known diseases can be retrieved from the Incidence and Prevalence Database (IPD) (http://www.tdrdata.com/IPD/ipd_init.aspx) or other sources.

The likelihood function of our observed data is as follows:

$$\begin{aligned} & P(n_{mfc}^1, n_{mfc}^0, n_{mc}^1, n_{mc}^0 | \mu_{mf}, \delta, R_1, R_2, R_{im}, S_1, S_2) \\ & \propto \prod_{(m,f,c)} P(M = m, F = f, C = c | D = 1)^{n_{mfc}^1} \\ & \quad \times P(M = m, F = f, C = c | D = 0)^{n_{mfc}^0} \\ & \quad \times \prod_{(m,c) \neq (1,1)} P(M = m, C = c | D = 1)^{n_{mc}^1} P(M = m, C = c | D = 0)^{n_{mc}^0} \\ & \propto \left\{ \prod_{(m,f,c)} (p_{mfc})^{n_{mfc}^1} (1 - p_{mfc})^{n_{mfc}^0 - n_{mfc}^1} \right. \\ & \quad \times \left. \prod_{(m,c) \neq (1,1)} (p_{mc})^{n_{mc}^1} (1 - p_{mc})^{n_{mc}^0 - n_{mc}^1} \right\} \\ & \quad \times \left\{ \prod_{(m,f,c)} [s_{mfc} P(M = m, F = f, C = c)]^{n_{mfc}^1} \right. \\ & \quad \times \left. \prod_{(m,c) \neq (1,1)} [s_{mc} P(M = m, C = c)]^{n_{mc}^1} \right\}, \end{aligned}$$

where $n_{mfc} = n_{mfc}^0 + n_{mfc}^1$ and $n_{mc} = n_{mc}^0 + n_{mc}^1$. Further,

$$s_{mfc} = N_t^1 P(D = 1 | m, f, c) / P(D = 1) + N_t^0 P(D = 0 | m, f, c) / P(D = 0),$$

$$s_{mc} = N_p^1 P(D = 1 | m, c) / P(D = 1) + N_p^0 P(D = 0 | m, c) / P(D = 0),$$

$$p_{mfc} = \frac{N_t^1 P(D = 1 | m, f, c) / P(D = 1)}{s_{mfc}},$$

$$p_{mc} = \frac{N_p^1 P(D = 1 | m, c) / P(D = 1)}{s_{mc}}.$$

Note that the above expressions are independent of the nuisance parameters (matching type probabilities μ_{mf}), as we will see more clearly, from the derivation of the

formula below, the nuisance parameters in the numerator and denominator cancel out.

In our likelihood formulation, note that the pair type (1, 1) is not included since the nuisance parameters and the risk parameters cannot be nicely separated as discussed above. The potential effects of excluding this data type will be addressed in the discussion section. With this exclusion, one can see from the above factorization that only the second component of the likelihood (factors within the second set of curly brackets in the last formula) contains the nuisance parameters μ_{mf} 's. Therefore, the first component is our partial likelihood, which can be maximized instead of the full likelihood to avoid estimating the nuisance parameters [Cox (1975)]. In fact, the partial likelihood component can be regarded as the likelihood of the reorganized data conditional on each possible triad (M, F, C) or pair (M, C) type. Within each type, counts of the cases and controls follow a “renormalized” binomial distribution with the appropriate probabilities. For example, for a triad type (m, f, c), the probabilities of observing a case are

$$\begin{aligned}
 p_{mfc} &= \frac{E(n_{mfc}^1)}{E(n_{mfc}^1 + n_{mfc}^0)} = \frac{N_t^1 P(m, f, c|D = 1)}{N_t^1 P(m, f, c|D = 1) + N_t^0 P(m, f, c|D = 0)} \\
 &= \frac{N_t^1 P(D = 1|m, f, c)/P(D = 1)}{N_t^1 P(D = 1|m, f, c)/P(D = 1) + N_t^0 P(D = 0|m, f, c)/P(D = 0)}.
 \end{aligned}$$

This manipulation turns data from a retrospective design into a “prospective” likelihood stratified according to each type. That is, the partial likelihood is the kernel of Binomial(n_{mfc} , p_{mfc}) and Binomial(n_{mc} , p_{mc}), and n_{mfc} and n_{mc} may be viewed as “fixed” as in a binomial distribution. Thus, although the second component of the likelihood contains the parameters of interest, it does not depend on the data. It is also worth reemphasizing that, by modeling the counts using partial likelihood instead of the full likelihood, the dimensionality of the parameter space is greatly reduced. Only phenocopy rate and genotype relative risks are estimated from the partial likelihood. Consequently, no assumption is needed regarding the underlying mating type probabilities in the population.

3. Two existing methods for comparison.

3.1. *Constrained log-linear model.* A special case of our family case-control design, in which $N_t^1 = N_t^0 = 0$, was considered in a recent study [Shi et al. (2008)] to detect maternal effects. They used the log-linear relative risk model for the case-mother and control-mother pairs, but assuming the absence of imprinting. Thus, their model is a special case of our model as specified in (1) by setting $R_{im} = 1$. However, since their full likelihood approach requires the estimation of mating type probabilities, they propose to explore three levels of assumptions regarding these probabilities, namely, Mendelian inheritance, mating symmetry and parental

allelic exchangeability, leading to what they call a constrained log-linear model (CLL).

In addition to the assumption about mating type probabilities, the expected frequencies of control-mother pairs were assumed to be the same as the expected frequencies of any child-mother pairs for each type regardless of whether the child is affected or not. Considering the retrospective nature of the case-mother/control-mother design, the fact that the child in a control-mother pair is unaffected should be taken into account by multiplying the unaffected probability by the expected frequency of each (M, C) combination. Unless the conditional probability of being unaffected given every (M, C) combination is close to 1, approximating the frequencies of control-mother pairs using the frequencies of child-mother pairs in the general population would be inaccurate. Shi et al. (2008) justified the use of this approximation under the rare disease assumption. This assumption only implies that the marginal unaffected probability in the whole population is close to 1, but the conditional unaffected probability given some of the (M, C) type may not be close to 1 at all. In particular, when $(M, C) = (2, 2)$, the conditional probability that the child is unaffected, $1 - \delta S_2 R_2$, might be much smaller than 1 for reasonably large relative risks S_2 and R_2 . For example, suppose the phenocopy rate $\delta = 0.05$, and the relative risks are $S_2 = 2$ and $R_2 = 3$ as in one of the settings in Shi et al. (2008), then $1 - \delta S_2 R_2$ is only 0.7. Thus, although the rare disease assumption is necessary, it is certainly not sufficient in justifying the frequency approximation used in CLL.

A careful dissection of CLL reveals that the control-mother expected frequencies approximation was also needed for the mating type probability assumption under Mendelian inheritance. It is indeed true that, under Mendelian inheritance, the sum of the expected frequencies of child-mother pairs with $(M, C) = (1, 0)$ and with $(M, C) = (1, 2)$ equals the expected frequency with $(M, C) = (1, 1)$ if the pair is randomly sampled from the population. However, this does not hold for control-mother pairs due to the involvement of unaffected probabilities as discussed above, which complicates the simple relationship in the constraint.

3.2. Log-linear likelihood ratio test. The log-linear likelihood ratio test (LL-LRT) was the first method proposed for detecting imprinting and maternal effects simultaneously using case-parent triad data [Weinberg, Wilcox and Lie (1998)]. It assumes multiplicative relative risks and models the counts of the 15 case-parent triads using a log-linear model as in (1). It is necessary to make the assumption of mating symmetry when using LL-LRT; otherwise, the number of parameters in the model would exceed the degrees of freedom of the count data.

4. Simulation. To evaluate the performance of the proposed method and to compare it with the existing methods, we create 8 simulation settings of the relative risks due to variant allele, imprinting and maternal effects (Table 2). The first 4 settings with the imprinting relative risk being equal to 1 (i.e., no imprinting effect)

TABLE 2
Simulation settings of the relative risks

Parameter	Settings							
	1	2	3	4	5	6	7	8
R_1	1	2	1	1	1	3	1	3
R_2	1	3	3	3	3	3	3	3
R_{im}	1	1	1	1	3	1/3	3	1/3
S_1	1	1	1	2	1	1	2	2
S_2	1	1	1	2	1	1	2	2

are the same as the simulation settings in Shi et al. (2008). Settings 5 and 6 have paternal and maternal imprinting effects, respectively, but no maternal effect. Settings 7 and 8 have paternal and maternal imprinting effects, respectively, and also maternal effect. Prevalence of the disease is set to be 0.05 (rare) or 0.15 (common). Note that the summation over the 15 joint probabilities $P(D = 1, M, F, C)$ equals the disease prevalence $P(D = 1)$, and thus the phenocopy rate can be solved from the equation since relative risks and prevalence are set in our simulation and the phenocopy rate is the only unknown quantity.

We consider three variant allele frequencies (VAF), 0.1, 0.3 and 0.7, and simulate the parental genotypes under two scenarios. Under the first scenario, the population is in HWE and the probabilities of a paternal or maternal genotype score being 0, 1 and 2 are $(1 - p)^2$, $2(1 - p)p$ and p^2 , respectively, where p is the variant allele frequency, $p \in \{0.1, 0.3, 0.7\}$. Since the population is in HWE, allelic exchangeability (AE) and mating symmetry (MS) are implied. In the other scenario, the probabilities of a genotype score being 0, 1 and 2 are $(1 - p)^2(1 - \zeta) + (1 - p)\zeta$, $2p(1 - p)(1 - \zeta)$ and $p^2(1 - \zeta) + p\zeta$, respectively, where ζ is the inbreeding parameter [Weir (1996)], which is set to be 0.1 and 0.3 for males and females, respectively. As such, neither AE nor MS holds in the population under the second scenario.

For each triad, the genotype of the child is sampled according to the transmission probability given the previously simulated parental genotypes, and then the disease status is generated as a Bernoulli trial with the success probability being equal to the phenocopy rate multiplied by the relevant relative risks. This process is repeated until 150 case-parent triads and 150 control-parent triads are obtained. This sample size was chosen to be the same as in Shi et al. (2008) to facilitate comparison. We then count the 15 types of child-parent triads among case families and control families. Ignoring all the fathers, we also count the 7 types of child-mother pairs among case families and control families. Finally, we also create a mixture sample of triads and pairs by randomly setting the father’s genotype to be missing with probability 0.5 in each triad. This last setting allowing for miss-

ing paternal genotype is the most realistic in genetic epidemiology studies. The number of replication for each simulation setting is set to be 1000.

LIME is applied to the pair/triad mixture counts (LIME-mix). CLL is applied to the pairs (150 case-mother pairs and 150 control-mother pairs) with the constraint arising from AE. Since CLL models pair counts and does not consider the imprinting effect, LIME is also applied to pair counts without the imprinting effect (LIME-pair) purely for the purpose of comparison with CLL. On the other hand, LL-LRT is applied to the case-parent triads (150 complete case-parent triads). The simulation results are summarized in the following three subsections.

4.1. *Bias.* Relative bias of a parameter estimate is defined as $(\hat{\theta} - \theta)/\theta$, where $\hat{\theta}$ is the estimate of θ and $\theta \in \{R_1, R_2, R_{im}, S_1, S_2\}$ as given in the multiplicative relative risk model. The scatterplots shown in Figure 1(a) are relative biases of LIME-pair versus relative biases of CLL. Since neither CLL nor LIME-pair models imprinting effect, it is only appropriate to compare their biases under the settings in which the imprinting effect is absent. Hence, each panel in Figure 1(a) plots the biases of $\hat{R}_1, \hat{R}_2, \hat{S}_1$ and \hat{S}_2 under the first 4 simulation settings of the relative risks. The columns of panels in Figure 1(a) correspond to the three variant allele frequencies, while the rows correspond to the four combinations of the two prevalence settings (0.05 and 0.15) and whether AE holds. Recall that AE is the constraint imposed in CLL.

The scatterplots shown in Figure 1(b) are relative biases of LIME-mix versus relative biases of LL-LRT. Since both LIME-mix and LL-LRT model variant allele, imprinting and maternal effects simultaneously, each panel plots the biases of $\hat{R}_1, \hat{R}_2, \hat{R}_{im}, \hat{S}_1$ and \hat{S}_2 under all 8 simulation settings. Thus, there are more points in Figure 1(b) than in Figure 1(a). The rows of Figure 1(b) correspond to the four combinations of the prevalence settings and whether AE holds. Note that AE implies MS, an assumption made in LL-LRT.

The intersecting dotted diagonal lines in each panel divide the square into 4 triangular regions. Scattering points in the left and right triangles correspond to the relative biases on the y -axis (LIME-pair/LIME-mix) being smaller than the relative biases on the x -axis (CLL/LL-LRT) in absolute magnitude. In both Figures 1(a) and (b), almost all the points are scattering in the left/right triangle in each panel, which indicates the smaller relative biases of LIME-pair and LIME-mix under all circumstances simulated. When the AE/MS does not hold [3rd and 4th rows of both Figures 1(a) and (b)], the relative biases of CLL/LL-LRT become larger, a phenomenon also noted in Sinsheimer, Palmer and Woodward (2003). In contrast, the relative biases of LIME-pair/Lime-mix remain at around the same level, which is close to zero.

When AE actually holds, the relative biases of CLL become a bit larger when the prevalence changes from 0.05 to 0.15, which reflects the rare disease assumption being necessary (though not sufficient) for CLL to be valid. When both the

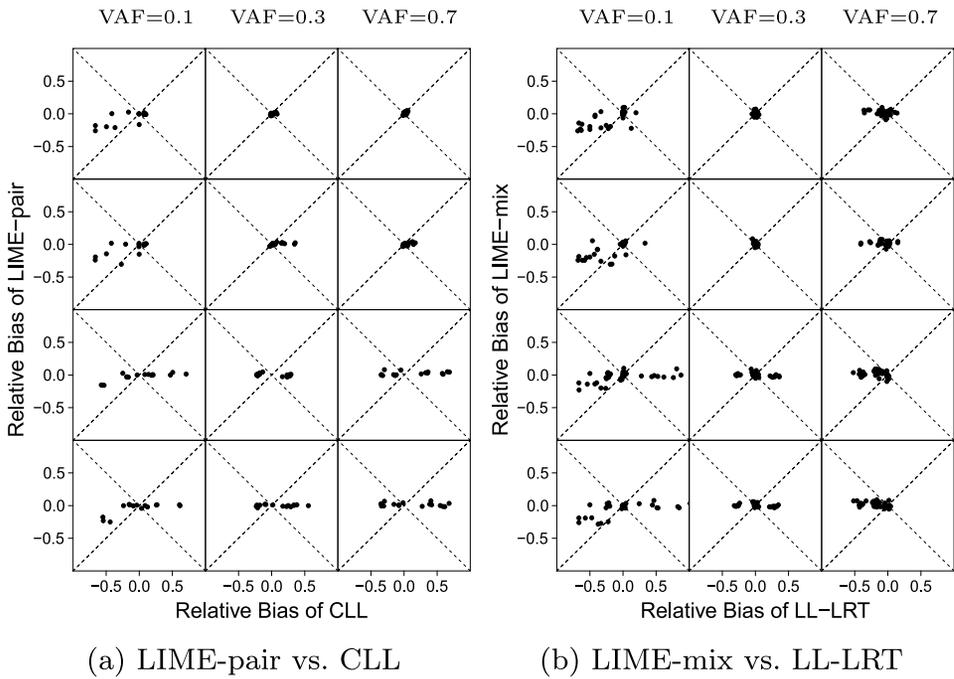


FIG. 1. Relative estimation biases of (a) LIME-pair versus CLL and (b) LIME-mix versus LL-LRT. In both figures (a) and (b), three variant allele frequencies (VAF) are considered and plotted in the three columns. Further, population 1 is in HWE, such that both AE and MS hold, whereas neither AE nor MS holds in population 2. The four rows in the figures correspond to the four combinations of population (1 or 2) and disease prevalence (rare: 0.05 or common: 0.15): row 1: population = 1, prevalence = 0.05; row 2: population = 1, prevalence = 0.15; row 3: population = 2, prevalence = 0.05; row 4: population = 2, prevalence = 0.15.

rare disease (prevalence = 0.05) and AE assumptions hold but the variant allele frequency is small (VAF = 0.1), CLL still has some large relative biases, which mainly correspond to the estimates of R_2 and S_2 . When the VAF is small, it is likely that there is no child-mother pairs with $(M, C) = (2, 2)$ in the sample. The zero cell count due to small VAF leads to large estimation variability for these two parameters. This can also be observed from the large relative biases of LL-LRT in estimating R_2 and S_2 when VAF is small, which are due to the zero counts for $(M, F, C) = (2, 1, 2)$ and $(2, 2, 2)$.

4.2. Type I error rate and power. Figure 2 presents the type I error rates and power of CLL, LIME-pair, LIME-mix and LL-LRT under the 8 simulation settings of relative risks. The vertical dotted lines divide each panel into 4 regions, from left to right, corresponding to (1) prevalence = 0.05 and AE hold; (2) prevalence = 0.15 and AE hold; (3) prevalence = 0.05 but neither AE nor MS holds; (4) prevalence = 0.15 but neither AE nor MS holds. Type I error rates are

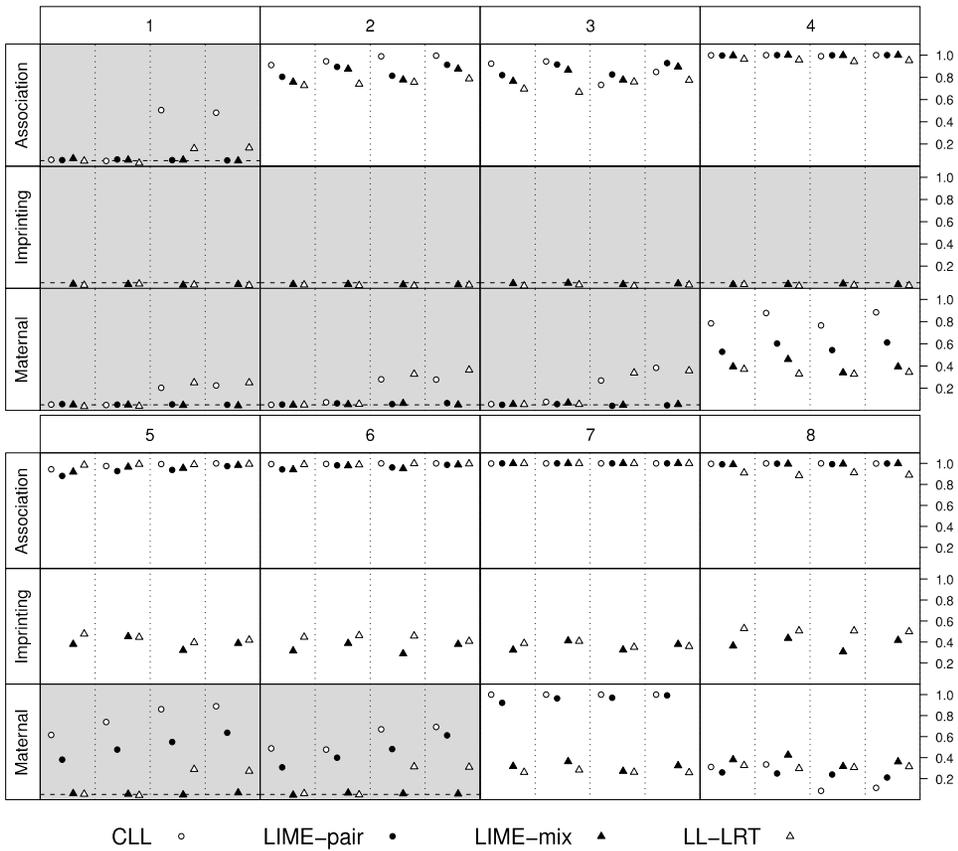


FIG. 2. Type I error rates and power of CLL, LIME-pair, LIME-mix and LL-LRT. Panels with gray background give type I error rates, whereas the rest show power. The vertical dotted lines divide each panel into 4 regions corresponding to, from left to right: (1) prevalence = 0.05, AE and MS holds; (2) prevalence = 0.15, MS and AE hold; (3) prevalence = 0.05, neither AE nor MS holds; (4) prevalence = 0.15, neither AE nor MS holds. Columns 1–8 correspond to the 8 simulation settings of relative risks in Table 2.

shown in the gray panels with a horizontal dashed line marking the nominal level of 0.05, while other panels show power. Type I error rates and power of CLL and LIME-pair are absent in the middle rows of the panels, since neither CLL nor LIME-pair includes the imprinting effect.

4.2.1. *Settings 1–4.* The imprinting effect is absent in simulation settings 1–4. Type I error rates for detecting the imprinting effect using LIME-mix and LL-LRT are all around the nominal level of 0.05. For detecting association and maternal effect, CLL and LL-LRT have inflated type I error rates in regions (3) and (4), in which neither AE nor MS holds, whereas LIME-pair and LIME-mix have correct type I error rates under all circumstances. The powers of CLL and LIME-pair are

higher than LIME-mix and LL-LRT, not surprisingly, since CLL and LIME-pair do not attempt to estimate the nonexistent imprinting effect and thus are more efficient. The power of CLL is higher than or about the same as LIME-pair in regions (1) and (2), in which AE holds and is correctly incorporated into CLL as a parameter constraint. However, the power of CLL is lower than that of LIME-pair in regions (3) and (4) under setting 3, a case in which the power for CLL is reduced because the constraint is wrongly imposed when the assumption does not hold. CLL has higher power than LIME-pair in setting 4 for detecting maternal effect. The power of LIME-mix is always higher than that of LL-LRT in detecting association and maternal effect in these four settings.

4.2.2. *Settings 5–8.* The imprinting effect is present in the simulation settings 5–8, and maternal effect is present in the latter two settings. Under settings 5 and 6, CLL and LIME-pair have a lot of false positives for maternal effects that are actually due to the imprinting effect because of confounding [Hager, Cheverud and Wolf (2008)]. LL-LRT also has inflated type I error rates in regions (3) and (4) due to its MS assumption being violated, whereas LIME-mix has correct type I error rates in all regions. Due to the strong association signal in these four settings, all four methods have about the same power in detecting association. LL-LRT has higher power in detecting the imprinting effect than LIME-mix, but LIME-mix has higher power in detecting maternal effect than LL-LRT. Due to the confounding between imprinting and maternal effect, the paternal imprinting in setting 7 “magnifies” the signal of maternal effect if imprinting effect is not properly accounted for. As such, CLL and LIME-pair have a higher power for detecting the maternal effect, but they miss the imprinting effect completely. On the other hand, setting 8 depicts maternal imprinting, and thus CLL and LIME-pair have a lower power for detecting the “reduced” maternal effect. This represents the worse case scenario; not only is the imprinting effect missed completely but there is also reduced power for detecting maternal effect.

4.3. *Sensitivity analysis.* Since the proposed method LIME-mix needs the disease prevalence in the population as a known constant, we conduct a sensitivity analysis to investigate the impact of using a misspecified prevalence in our model. A constant that is 5% higher, 5% lower, 20% higher or 20% lower than the true prevalence in the simulation setting is used as the prevalence in LIME-mix. The mixtures of triads/pairs simulated under the 8 settings of relative risks are analyzed again using LIME-mix with these inaccurate prevalences. Relative biases of LIME-mix using inaccurate prevalence are plotted versus relative biases of LIME-mix using the true prevalence in Figure 3.

When the disease is rare (with a true prevalence of 0.05), we can see from Figure 3(a) that all points fall almost exactly on the dashed line with slope 1, indicating that the estimates of the relative risks using the inaccurately specified prevalence are actually very close to those using the true prevalence. That is, the results are

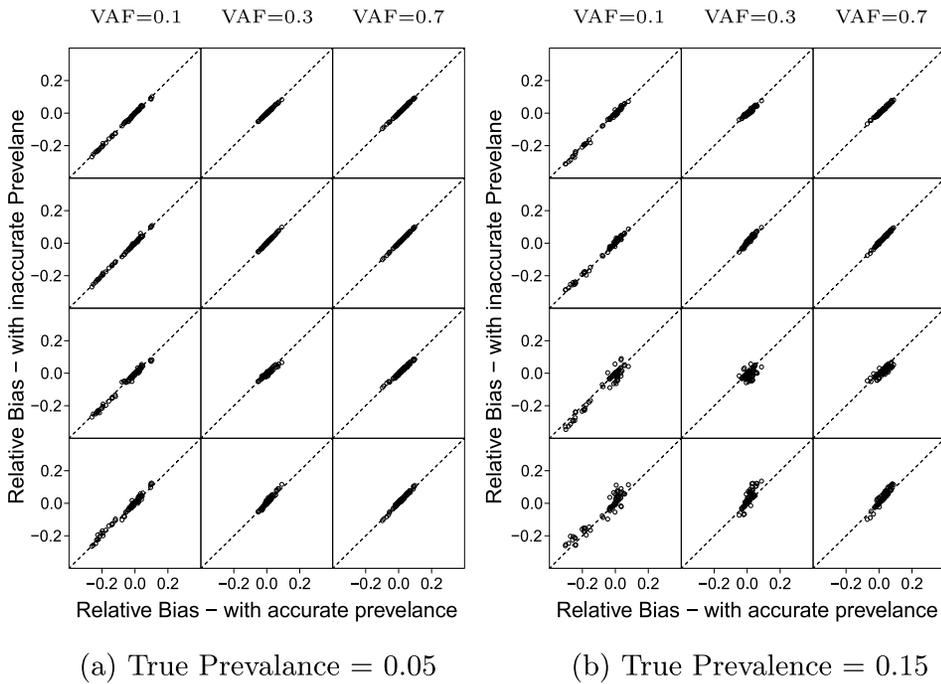


FIG. 3. Sensitivity analysis of the bias with a misspecified prevalence when (a) the true prevalence = 0.05 (rare disease) and (b) the true prevalence = 0.15 (common disease). In both figures (a) and (b), three variant allele frequencies (VAF) are considered and plotted in the three columns. The four rows correspond to four different misspecified prevalences. Row 1: prevalence is 5% higher than the true value; row 2: prevalence is 5% lower than the true value; row 3: prevalence is 20% higher than the true value; row 4: prevalence is 20% lower than the true value.

rather insensitive to the misspecification of population prevalence. On the other hand, when the disease is more common (true prevalence being 0.15), the points still scatter around the dashed line with slope 1, as we can see from Figure 3(b), although there is more scattering when the prevalence is incorrectly specified to be 20% higher/lower than the true value. Overall, the results are reasonably insensitive to the specification of population prevalence. Estimated prevalences are often obtained through large scale population studies and would, in those cases, not deviate greatly from their true values. As such, our results seem to suggest that LIME would give reasonably accurate estimates by using population prevalences estimated from external sources when the same population and diagnostic criteria have been studied.

5. Discussion. In this paper we propose a partial likelihood approach for detecting imprinting and maternal effects simultaneously using case-control families. The crucial role played by imprinting and maternal effects in complex human diseases has been increasingly explored in the last few years, as simply focusing on

sequence variation has been proven to be insufficient for studying disease etiology. As such, there is an immediate need for robust and efficient statistical methods for detecting imprinting and maternal effects, and our contribution is one step in this direction. Our proposed method possesses a number of novel features compared to existing ones in the literature. We augment the traditional case-parent triads design to a family-based case-control design by recruiting control-parent triads as well. This differs from Weinberg and Umbach (2005) in that they only genotype the parents of the controls, leading to a case-parent triad/control-parent design. Further, we allow for missing fathers considering the fact that fathers are often harder to recruit in family-based studies. Thus, this design also differs from that of Vermeulen et al. (2009), as the current design also recruits control-parent triads and case-mother pairs. By recruiting control families of the same structure as case families, we create “internal matches” stratified by the familial genotypes. As such, we can extract from the full likelihood of the retrospective design a partial likelihood component that can be thought of as the products of likelihoods from stratified prospective designs. Through this conditional on the familial genotypes, the nuisance parameters with respect to the population mating type probabilities (i.e., probabilities of parental genotype combinations) are no longer involved in the partial likelihood. As such, it is no longer necessary to make any assumption about mating type probabilities; such assumptions are strong and usually unrealistic but are made in the literature in an effort to reduce the number of parameters for the full likelihood approach. This alleviates the problem of over-parametrization that often plagues existing methods. Furthermore, our formulation takes into account the fact that control families have unaffected children, making it applicable to both rare and common diseases as opposed to just rare diseases in some existing methods. However, we note that the mother-child data type in which both the mother and the child are heterozygotes cannot be included in the analysis, an issue that will be discussed further below.

Through simulation with a variety of settings, including some adopted from the literature, we demonstrate the robustness of LIME to violation of assumptions on absence of imprinting effects, rarity of disease and mating type probabilities. First, by utilizing a mixture of case/control triad families and case/control pair families, imprinting and maternal effects can be studied jointly to address the confounding issue faced by approaches that assume the absence of imprinting effect when detecting maternal effects. As such, LIME has correct type I error rate compared to those approaches. If, however, there is a priori and unequivocal information that imprinting effect is indeed absent, then a method that assumes the absence of imprinting effect will usually lead to gain in power for detecting maternal effect. In this situation, a version of LIME that assumes the absence of imprinting (by setting $R_{im} = 1$) is recommendable given its robust and efficient features.

Second, regardless of whether the disease is common or rare, LIME has very little bias in the estimates of the model parameters. In contrast, CLL, which assumes the disease being rare, has much larger biases, even when the disease is indeed

rare, since rare disease is a necessary but not a sufficient condition for CLL to be valid.

Finally, mating symmetry is commonly assumed for many imprinting and/or maternal effects detection methods [Ainsworth et al. (2011), Shi et al. (2008), Weinberg, Wilcox and Lie (1998), Weinberg (1999), Zhou et al. (2009)]. However, when this assumption is violated, there can be large biases and greatly inflated type I error rates, whereas LIME is not affected at all by departure from such assumptions (Figures 1 and 2). In particular, when there is population substructure, the assumption of HWE is violated even if there is HWE in each of the subpopulations. Therefore, population substructure will likely exert a large effect on methods that assume some mating frequency distributions. In contrast, because the partial likelihood is independent of the mating type parameters, LIME may not be as sensitive to such population substructure. The non-HWE scenario considered in our simulation may be viewed as a mixture of two subpopulations, one is in HWE and the other is inbred. Indeed, CLL and LL-LRT are highly sensitive, whereas LIME is robust to this type of population stratification. However, will LIME still be robust if the disease prevalences also differ in the subpopulations? To investigate this, we consider the following three scenarios of population substructure in which a population consists of two distinct subpopulations each in HWE: (a) the two populations differ in their allele frequencies ($VAF = 0.1$ or 0.3) but with the same disease prevalence of 0.05 ; (b) the two populations have the same allele frequency ($VAF = 0.3$) but different prevalences (0.05 or 0.15); (c) the two populations have different allele frequency ($VAF = 0.1$ or 0.3) and also different prevalences (0.05 or 0.15). Under disease risk settings 1–4 (Table 2; imprinting absence), LIME has smaller relative biases in all parameter estimates and smaller type I error rates under all scenarios, and they are smaller (some are much smaller) than those from CLL. For disease settings 5–8 (Table 2; imprinting present), LIMEs estimates have larger biases, especially for scenario (b), but they are, in the majority of cases, much smaller than those from CLL. This indicates that LIME is sensitive to population stratification when there is imprinting effect, but it is certainly more robust than the alternative method even under the situation where the disease prevalences are different in addition to different frequencies in the subpopulations.

To compare with the performance of CLL, we consider LIME-pair in addition to LIME-mix. In real data applications, only LIME-mix will be used unless there are no triads in the sample (i.e., all the fathers are missing in all the families). This is an unlikely scenario, but if this does happen, then LIME-pair is recommendable over CLL due to its robust feature, although we note that LIME-pair, like CLL, also assumes the absence of imprinting effect.

The need to specify disease prevalence deserves further discussion. Since the prevalence is in fact a function of the disease model parameters and mating type probabilities, it would be attractive if one can make use of this fact without the need to specify this parameter. However, in order to use the current argument, the partial likelihood needs to be free of the mating type probabilities, which will no longer be

true if we express $P(D)$ in terms of the other parameters. Fortunately, as we have pointed out earlier, estimated prevalences are often obtained through large scale population studies and would usually not deviate greatly from their true values. Overall, even if the prevalence is misspecified as much as 20% away from the true value, the estimates are still quite close to those had the prevalence been specified correctly, reaffirming the robustness of the proposed procedure. However, for rarer diseases, the deviations between the true and estimated prevalences may be larger. Thus, we further investigate the effect of greater prevalence misspecification for a disease with a prevalence of 1%. We use a prevalence of 0.2%, 5% (representing 500% decrease or increase) and 0.8%, 1.2% (20% decrease or increase). Our results show that LIME remains robust with a 20% increase/decrease misspecification. Even with a 500% decrease, the parameter estimates closely track those using the true prevalence. A 500% increase leads to more sensitivity, but the procedure is still quite robust if the variant allele frequency is moderate.

In LIME, the mother-child data type in which both the mother and the child are heterozygous—the (1, 1) data type—is not included in the analysis since the nuisance parameters are tangled up with the risk parameters of interest (Table 1), and therefore its inclusion will render it impossible to adopt the partial likelihood approach. This exclusion, however, will lead to loss of information. Since we cannot evaluate its effect on LIME directly, we use CLL as a surrogate by analyzing the data without the (1, 1) data type and compare the results with those from the earlier analysis where (1, 1) is included. The power is slightly lower for most of the tests, although power actually increases for some; overall, the average power drop is 0.056, small but appreciable. This amount may be viewed as an upper bound; the effect on LIME is expected to be much smaller unless most of the data are from pairs, not triads. This finding of small effect is not surprising considering the parent asymmetry test (PAT) of [Weinberg \(1999\)](#). Therein, five triad categories, including (1, 1, 1) (all three people in the family are heterozygous), were omitted from the model, but PAT is competitive in power for imprinting test compared to methods that use all categories.

Although fathers are usually harder to recruit than mothers, this does not imply 100% participation rate from mothers. As such, there will likely be child-father pairs in a study as well, but such data cannot be utilized in our partial likelihood approach since the model parameters and nuisance parameters cannot be factored as in child-mother pairs.

Finally, in this paper we assume a single affected child for each case family. In genetic diseases, there may be familial aggregation and, therefore, it is not unlikely that there may be multiple affected siblings in a family. If each family is being recruited through single ascertainment (i.e., through an affected/unaffected child), then additional information from the siblings (even from the control families) may be used in the partial likelihood formulation. This may lead to substantial power gain, and thus warrants further investigation.

Acknowledgments. We would like to thank the Editors and two anonymous referees for their constructive comments and suggestions, which have led to improved presentation and content of the paper.

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