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The essence of linkage-based imprinting detection: Comparing power, type 1 error, and the effects of confounders in two different analysis approaches

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Summary

Background and goal—The epigenetic phenomenon of imprinting is critical to understanding disease expression. However, it is hard to detect and can be species- and tissue-specific. One approach is to detect imprinting by taking advantage of linkage information. Although imprinting detection methods exist, the effects of potential confounders, such as heterogeneity, sex-specific penetrance, and differential sex-based ascertainment, have not been explored in depth. In this study we explored possible confounders using two different imprinting detection approaches. Our goal was to understand the *essence* of how imprinting and linkage interact and to elucidate the underlying issues in existing imprinting detection approaches.

Methods—One method (PP) models imprinting by maximizing lod scores with respect to parent-specific penetrances. The other method (DRF) approximates imprinting by maximizing two-point lods with respect to differential male-female recombination fractions. We compared power, type 1 error, and confounder effects in these two linkage-based imprinting detection methods using two-point linkage analysis for simplicity. We computer-simulated data, determining power and type 1 error for imprinting detection among datasets with detectable linkage. We generated data with and without imprinting, with and without heterogeneity, and with varying reduced penetrance, family and dataset size. We also examined non-imprinting situations that could mimic imprinting, e.g., sex-specific penetrances, and a scenario requiring a sex-specified affected parent for ascertainment.

Results—Without heterogeneity, PP had more imprinting-detecting power than DRF. Surprisingly, PP's power increased when parental affectedness status was ignored, but decreased with heterogeneity. With heterogeneity, type 1 error could increase dramatically for both methods. However, DRF's *power* also appeared to increase under heterogeneity, more than could be attributed to the inflated type 1 error. We determined the reasons behind these phenomena.

The presence of sex-specific penetrance increased false positives for PP but not for DRF. Ascertainment through an affected “mother” in unimprinted data did not lead to false positives with either method. For PP, increased information may depend on non-penetrant heterozygous individuals, arguing against using affected sib pairs or other affecteds-only methods.

Conclusions—The high type 1 error levels under some circumstances means methods must be used cautiously. Using differential recombination fractions to approximate imprinting has certain advantages and should be incorporated into future imprinting detection programs.

Keywords

Imprinting; linkage analysis; robust methods; lod score maximization; recombination fraction; computer simulation; epigenetic phenomena; confounders

1. Introduction

Imprinting is an epigenetic phenomenon by which the expression of an allele in an individual depends on which parent contributed that allele. Imprinting introduces an asymmetry between paternal and maternal marker transmission patterns, and can thus reduce the power to detect linkage when using classical approaches (Hanson et al., 2001). More importantly, the existence of imprinting potentially gives us critical information about mechanism, gene identification and expression. Imprinting can occur because of inactivation of either the paternal or maternal copy of a gene, possibly due to DNA methylation or to differences in chromatin structure (Hall, 1990, Ainscough et al., 1998, Bartolomei & Tilghman, 1997). Imprinting can also be tissue-specific, complicating studies of gene expression.

Angelman and Prader-Willi syndromes (Nicholls & Knepper, 2001) are two examples of human neurodevelopmental disorders caused by abnormalities in imprinting. In addition, imprinting has been demonstrated in a number of human diseases, including Beckwith-Wiedemann syndrome (DeBaun et al., 2002, Feinberg, 2004), Russell-Silver syndrome, and some complex genetic diseases as epilepsy, asthma and Alzheimer disease (Davies et al., 2005).

Imprinting has been recognized as a fundamental epigenetic mechanism regulating fetal growth (Reik & Walter, 2001) and the expression of genes involved in normal brain development (Keverne et al., 1996, Allen et al., 1995, Davies et al., 2005). In the mouse brain, maternal alleles have a much higher level of expression than paternal alleles in the telencephalon, and vice versa in the diencephalon (Keverne et al., 1996). These surprising observations indicate that genes of maternal origin control development of some brain regions while paternal genes control the development of other regions. Thus, while methods do exist to examine imprinting at the molecular level, one first needs to know *that* it exists so that one can examine the correct tissue.

Several statistical methods have been proposed to account for the parent-of-origin effect in segregation analysis (Haghighi & Hodge, 2002) and in linkage analysis (Hanson et al., 2001, Paterson et al., 1999, Greenberg et al., 2000, Strauch et al., 2003, Knapp & Strauch, 2004, Wu et al., 2005, Vincent et al., 2006) or to perform two-locus imprinting analyses specifically (Shete & Zhou, 2006, Strauch et al., 2000). However, to our knowledge, none of these studies has examined the effects of heterogeneity, ascertainment approaches, or other potential confounders such as sex-specific penetrance on the detection of imprinting, nor have any studies proposed an approximate method that may prove robust.

Using linkage methods, one can model imprinting using two different approaches. The first approach models parent-specific penetrances directly (Strauch *et al.*, 2000), whereas the second maximizes the lod scores separately over male and female recombination fractions, a method to detect imprinting indirectly (Smalley, 1993, Greenberg *et al.*, 2000).

In the current work we examine the detailed behavior of these two quite different approaches to detecting imprinting. We focus on the detection of imprinting in data sets that have sufficient information to demonstrate linkage; we do not attempt to demonstrate linkage and imprinting simultaneously. The first approach *directly* models imprinting. This means that the penetrance for an individual depends on which *parent* transmitted the disease gene to that individual. This approach, which we call the “Parent-specific Penetrance” (PP) method, maximizes lod scores with respect to the two parent-specific penetrances. The second approach *indirectly* models imprinting, by specifying separate male and female recombination fractions, as an “approximation” to imprinting. We call this the “Differential Recombination Fraction” (DRF) method. We wanted to investigate whether the DRF method, even though an approximation, would prove more robust to model misspecifications and confounders than the more-direct PP method, thus yielding insights into how imprinting and linkage interact. We compare their performances, in terms of power and type 1 error, and also explore how potential confounders such as heterogeneity, parent-specific ascertainment, and sex-specific penetrance affect their statistical properties.

2. Material and Methods

2.1. Notation

Before describing the models and the results in detail, we will define the parameters used in the data generation and data analyses:

f_{mat} = maternal-origin penetrance, defined as the penetrance in the offspring when the disease gene is inherited from the *mother* (independent of sex of offspring),

f_{pat} = paternal-origin penetrance, defined as the penetrance in the offspring when the disease gene is inherited from the *father*,

f_{female} = female-specific penetrance, defined as the disease gene penetrance in female *offspring* (i.e., unrelated to parent of origin)

f_{male} = male-specific penetrance, defined as the disease gene penetrance in male *offspring*

f_{common} = penetrance when there is no differential effect due to sex of parent or sex of offspring (i.e., $f_{\text{common}} = (f_{\text{mat}} + f_{\text{pat}})/2 = (f_{\text{female}} + f_{\text{male}})/2$).

θ_{female} = female-specific recombination fraction

θ_{male} = male-specific recombination fraction

θ_{common} = recombination fraction without differential effect due to sex

α = heterogeneity parameter, defined as the proportion of families with disease that are linked to the marker

DSS = data set size, defined as the number of families in each dataset

N = number of datasets in a simulation.

Table 1 summarizes all the different penetrances and the situations in which they are used. For the rare individual who inherits a disease gene from both parents when there is imprinting (i.e., D/D homozygote), we used a penetrance of $1 - (1 - f_{\text{mat}})(1 - f_{\text{pat}})$. This is based on a “two-hit” model, whereby a homozygote’s probability of being unaffected is the

product of the probabilities that neither the maternal nor paternal alleles result in trait expression. While there are other ways to model imprinting in homozygotes, for the current studies, homozygotes are so rare that the value of the homozygote penetrance makes no difference in the result.

2.2. Data Generation (GMs = Generating Models)

We modified our extensively tested genetic simulation program (Greenberg *et al.*, 1998) to generate family data under the two paradigms described below. The first is True Imprinting (Paradigm A), while the other paradigm (B) includes data generated without imprinting but in the presence of specific factors that may misleadingly mimic imprinting: sex-specific penetrance (B1) and parent-specific ascertainment (B2). The “detection” of imprinting for B1 and B2 represents false positives.

2.2.1. Paradigm A: True imprinting—We focused on the *power* to detect imprinting and also investigated type 1 error in imprinting detection, i.e., the frequency with which imprinting is falsely detected. In the simulations, the probability of disease in an offspring depended on which parent contributed the disease allele. We define “maternal-origin penetrance” (f_{mat}) as the penetrance in the offspring when the disease gene is inherited from the mother; the corresponding paternal penetrance is f_{pat} . We generated family data using four different combinations of these parameters: $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.0), (0.5, 0.0), (0.9, 0.9)$ and $(0.5, 0.5)$. Thus, for imprinting, we set “maternal” penetrance f_{mat} to either 0.9 or 0.5, while “paternal” penetrance f_{pat} was always zero, whereas for data generated without imprinting we set both “maternal” and “paternal” penetrance to either 0.9 or 0.5 (f_{common}).

Additionally, we examined the effect of heterogeneity on imprinting detection and type 1 error as follows: we let a specified proportion $(1-\alpha)$ of the families be affected with disease caused by a second, unlinked, unimprinted locus. We examined generating α -values of 0.5 and 0.7.

2.2.2. Paradigm B: Data generated in the presence of factors that may misleadingly mimic imprinting

Paradigm B1: Sex-specific penetrance: Affectedness status depends on the sex of the offspring, not on the sex of the transmitting parent. We set f_{female} to 0.9 (or to 0.5) and f_{male} to 0.0.

Paradigm B2: Parent-specific ascertainment: Ascertainment of a family is through an offspring if and only if a parent of the specified sex is affected. Families were generated with a specified penetrance value, $f_{\text{common}} = 0.9$ or 0.5, but families are ascertained only if the parent of the designated sex (“mother”) is affected.

2.3. Simulation Details

In all simulations, in order to be at risk for the disease, an individual must have inherited the disease allele from the “mother”. Penetrances are as described below and in Table 1. The gene frequency was set to 0.01 and the recombination fraction was set to zero. In separate experiments, we simulated nuclear families and pedigrees. For nuclear families, we used different ascertainment schemes, including requiring either at least one, two, or three affected family members. For extended multi-generation (usually, three-generation) pedigrees we required at least six affected members.

For each simulation (i.e., each parameter combination), we generated 75,000 families, and randomly sampled them (with replacement) in datasets of 10 to 50 families each. The data set sizes (DSS) were 10 to 50 families, to allow us to study the power to detect imprinting as

a function of sample size. The number of data sets (N) for a simulation was always greater than 3000, except for the simulations of extended pedigrees, for which $N=1500$. For type 1 error calculations, we systematically varied the data set sizes between 10 and 50 families, and each simulation consisted of no less than 5000 data sets. Families had between 2 and 10 sibs per generation, as determined by a standard family size distribution (Cavalli-Sforza & Bodmer, 1971). We also simulated data with affected sib pairs only. All matings were fully informative ($AB \times CD$) and disease allele frequency was always 0.01.

We tested each dataset for linkage, and only datasets that reached a nominally significant lod score for detecting *linkage* were used to calculate the power to detect imprinting. We discarded datasets that did not reach the specified lod score, and kept sampling until we reached the desired N . That is, we did not try to simultaneously detect linkage and imprinting, because our focus is on imprinting detection once linkage has been established.

For datasets that were going to be analyzed by the PP method, lod scores for linkage detection were first maximized with respect to both recombination fraction (θ) and overall penetrance. We required these datasets to have max lods ≥ 3.3 in order to be included in the study. The reasoning behind choosing a lod of 3.3 cut-off is as follows: to determine the best estimate of f_{mat} , f_{pat} for the PP method under the null hypothesis of “no imprinting”, we maximize the lod score subject to the constraint of $f_{mat}=f_{pat}$. Previous work had shown that maximizing the lod score over penetrance, while assuming a fixed mode of inheritance, leads to increased type 1 error. We used the common penetrance-maximized value to determine whether linkage exists for these experiments. Correcting for that requires using a lod score cut-off of 3.3 instead of 3.0 (Hodge *et al.*, 1997).

For datasets analyzed by the DRF method, lod scores for linkage detection were maximized with respect to only one parameter: θ_{common} . Thus, we required these datasets to have max lods ≥ 3.0 in order to be included in the study.

2.4. Analysis Models (AMs)

We analyzed the simulated data using the two different methods that are the focus of this study: the PP method, which maximizes the lod scores over differential maternal and paternal penetrances, and the DRF method, which maximizes the lod scores over independent male-female recombination fractions. The assumed penetrance used in the DRF analysis was set to f_{common} . The disease allele frequency used in the analysis was 0.01, the same as in the generating models.

We determined the proportion of datasets yielding positive results for detecting imprinting (with appropriate degrees of freedom, as discussed below) as:

$$\% \text{ of datasets for which } \chi^2 \geq \text{critical value}$$

This proportion yielded the *power* to detect imprinting when the datasets had been generated under imprinting, and *type I error* (i.e. the frequency with which imprinting is falsely detected) when they had been generated under no imprinting (i.e., maternal and paternal penetrances set equal). The test statistics for concluding imprinting versus no imprinting are derived separately for the PP and DRF analysis methods (below).

2.4.1. Parent-specific Penetrance approach (PP method)—We modified the two-point linkage program LIPED (Ott, 1974) to calculate lod scores for 99 pairs of assumed parent-specific penetrances where $f_{mat} = 0.0$ to 0.9, and $f_{pat} = 0.0$ to 0.9, excluding the (0.0, 0.0) combination.

For each parent-specific penetrance pair, the lod score was maximized over the common recombination fraction θ_{common} in order to determine if there was sufficient evidence to detect linkage.

For the null hypothesis, the lod scores were maximized subject to the constraint $f_{\text{mat}}=f_{\text{pat}}$, whereas for the alternative hypothesis, lods were maximized over all $f_{\text{mat}}, f_{\text{pat}}$ combinations.

The test statistic for the PP method is

$$\text{Maxlod}(f_{\text{mat}}, f_{\text{pat}}; \theta_{\text{male}} = \theta_{\text{female}}) - \text{Maxlod}(f_{\text{mat}}, f_{\text{pat}}; \theta_{\text{male}} = \theta_{\text{female}})] * 4.605$$

This statistic, representing the difference of two “mod” scores (Hodge & Elston, 1994), does not have a known theoretical distribution. However, heuristically its behavior appears to be close to that of a χ^2 with one degree of freedom, becoming somewhat more conservative as information or sample size increases, as we confirmed in extensive simulations (see Results).

For analysis, it was not obvious what penetrance should be applied to the *parents*. In fact, we discovered that parental affectedness status is best ignored (see below).

2.4.2. Differential Recombination Fraction approach (DRF method)—We maximized the lod score assuming independent θ_{male} and θ_{female} and compared that lod score to the lod score maximized under the constraint of $\theta_{\text{male}} = \theta_{\text{female}}$ (the null hypothesis). Note that when analyzing independent θ_{male} and θ_{female} , we make no overt assumption about parent of origin of the disease gene. Under complete imprinting, the parent contributing the silenced gene contributes no information for linkage (at least for nuclear families). Therefore, the expectation is that there will be little variation in lod score with respect to recombination fraction value for that parent.

We used 49 recombination fraction combinations: $\theta = 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5$ in all combinations for males and females.

The test statistic for the DRF method is

$$\chi^2 = [\text{Maxlod}(\theta_{\text{male}}, \theta_{\text{female}}) - \text{Maxlod}(\theta_{\text{male}} = \theta_{\text{female}})] * 4.605$$

with one degree of freedom.

3. Results

3.1. PP Method

Power to detect imprinting—Figure 1A shows the power to detect imprinting as a function of data set size at full imprinting with almost full penetrance, i.e., $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.0)$. The x-axis shows the number of families/data set (10 to 50), and the y-axis, the percentage of data sets in which imprinting was detected (power to detect imprinting). At DSS=30 families, the PP method correctly detected imprinting approximately 50% of the time and at a DSS = 50, 80% of the time.

We also examined two levels of heterogeneity, $\alpha = 0.7$ and $\alpha = 0.5$. The effect of heterogeneity on imprinting detection among those data sets capable of detecting linkage was modest. With 70% of families linked and a DSS=50, the power to detect imprinting among the data sets in which linkage was detected declined from 80% to 75%. The power to detect imprinting declined to 60% when $\alpha = 0.5$.

Surprisingly, when the generating parental penetrances were reduced to (0.5, 0.0), the power to detect imprinting actually appeared to increase compared to when no heterogeneity was present. Figure 1B shows that at the lower penetrance, the power to detect imprinting exceeded 90% with 20-family data sets and reached 100% at DSS=50. In trying to determine why the reduced penetrance case appeared to have more power to detect imprinting than the almost-full penetrance case, we discovered that it was not the reduced penetrance per se that caused the increase in power, but the fact that there were more unaffected X unaffected matings when $f_{\text{mat}} = 0.5$ compared to $f_{\text{mat}} = 0.9$. When we set parental affectedness status to “unknown”, the power to detect imprinting was similar at either f_{mat} we examined. We discuss this below.

The presence of heterogeneity combined with reduced penetrance caused a decline in power to detect imprinting (Figure 1B). At $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.0)$ and $\alpha = 0.7$, the power declined from 100% (without heterogeneity) to 60% power. At $\alpha = 0.5$, the power declined to 40%. We contrasted the above results from full nuclear families with those from families with affected sib pairs (ASPs), in order to observe the contribution of the unaffected sibs to the imprinting information. Power to detect imprinting dropped by about 20% – 50% when only ASPs are used (Table 2).

Thus, the power to detect imprinting for the PP method could reach values over 80%. With increasing heterogeneity, the power to detect imprinting declined. (As we will show, this is one of the apparent differences between the PP and DRF methods of detecting imprinting).

Type 1 error—We also examined the behavior of the PP method when parent-specific penetrances were set to $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.9)$ and $(0.5, 0.5)$ (i.e., no imprinting), with and without heterogeneity, and as a function of sample size and of ascertainment requirements (Figure 2). Nuclear families were ascertained through at least 1, or 2 or 3 affected members. To test the effects of more extreme ascertainment specifications, extended pedigrees were ascertained through at least 6 affected members. The x-axis shows the number of families/data set (10 to 50, to produce graphs similar to the ones for power), and the y-axis, the percentage of data sets reaching a 5% significance level (type 1 error).

For nuclear families and no heterogeneity in the generating model, the likelihood ratio test either was conservative (at penetrance = 0.9, Figure 2A) or else yielded type 1 error rates close to the expected 5% level (at penetrance = 0.5, Figure 2B), although the test became conservative as the family size/ascertainment requirements increased. When 50% heterogeneity was present (Figure 2C), type 1 error levels were elevated (8–10% as opposed to the expected 5%) at penetrances of 0.9. At reduced penetrance of 0.5 with heterogeneity present (Figure 2D), type 1 error was close to the nominal 5%, even when ascertainment occurred through three affected members. The effect of ascertainment on type 1 error in the presence of heterogeneity was notable when we examined multi-generation pedigrees (Figure 2E–F), rising to levels close to 20% at 0.9 penetrance and 50% heterogeneity at large data set size. Note that in Figure 2C, the families with more information (three affected members) also appeared to show higher type 1 error.

3.2. DRF Method

Power to detect imprinting—Figure 3A shows the power to detect imprinting when the generating penetrance was (0.9, 0.0). At the largest tested data set size, about 35% of the data sets showed significant evidence of imprinting when there was no heterogeneity.

Power actually *increased* when heterogeneity was present. When 50% of the families were linked to the marker ($\alpha=0.5$) at $f_{\text{mat}}, f_{\text{pat}} = (0.9, 0.0)$, power to detect imprinting with DSS=50 was greater than 95%. When 70% of the families were linked to the marker at $f_{\text{mat}}, f_{\text{pat}} =$

(0.9, 0.0), power was still 75% at DSS=50. (We discuss the reason power appears to increase under heterogeneity in the Discussion). Figure 3B shows the equivalent power when the generating penetrance was (0.5, 0.0). At DSS=50 and no heterogeneity, the power was 50%. When 70% of the families were linked to the marker, power was 80%. Power was less with $f_{\text{mat}}, f_{\text{pat}} = (0.5, 0.0)$ because the combination of heterogeneity and lower reduced penetrance severely decreases the amount of information available for both linkage and imprinting.

Thus, under homogeneity, the power to detect imprinting was higher with the PP method than with the DRF method. But heterogeneity appeared to increase the power to detect imprinting for the DRF method, while it decreased the power for the PP method.

Type 1 error—The frequency of false positives fell below the nominal 5%. Ascertainment through multiple affected offspring in nuclear families did not affect the false positive rate (only $f_{\text{mat}} = f_{\text{pat}} = .5$) is shown in Figure 4A). However, there was a notable increase in false positives when there was heterogeneity. Figure 4B shows the false positive rate ($f_{\text{mat}} = f_{\text{pat}} = 0.9$) for families ascertained through 1, 2 or 3 affected when $\alpha=50\%$. With heterogeneity present, type 1 error increased as the information per data set increased, just as happened for the PP method (Figures 2C–D). Figure 4C shows the equivalent false positive rates for reduced penetrance ($f_{\text{mat}} = f_{\text{pat}} = 0.5$), which were well over the nominal 5% level for DSS=50 for datasets ascertained through at least 3 affected members.

Similarly, Figures 4D–E show multi-generation pedigrees, but now ascertained through at least 6 affected members (members, not necessarily offspring). In contrast to what happened in the nuclear family simulations, here the type 1 error rate remained constant with DSS. The level of false positives was 10% when the parent-specific penetrance was (0.5, 0.5), with 50% heterogeneity, and 14 % when heterogeneity was 50% and parent-specific penetrance was (0.9, 0.9). (In the limiting cases where $\theta = 0$ and $\alpha=0$, there is little or no statistical variation, and a false positive is unlikely. Large pedigrees with no heterogeneity make the estimation of θ highly reliable, even with reduced penetrance.)

We noted above that heterogeneity appeared to increase the power to detect imprinting, particularly in the larger (50-family) datasets. For example, when we decrease the heterogeneity parameter α from 1.0 to 0.5, observed power rises from about 35% to almost 100% for generating $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.0)$ (Figure 3A). It rises from approximately 45% to 80% power for $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.0)$ when α goes from 1 to 0.7 (Figure 3B). (Apparently the information loss at the lower penetrance of 0.5 offsets any apparent increase due to heterogeneity. Recall that only datasets in which linkage can be detected before testing for imprinting are included.) How much of this apparent power increase is real, as opposed to resulting from the inflated false positive rate? We can get a rough idea, since our false positive rates were inflated *at most* from 5% to 14%. First, we determine that a type 1 error of 14% would correspond to a corrected cutoff value, $c_{\text{corr}} = 2.07$, based on the χ^2 (1 d.f.) distribution of our DRF test statistic. Second, we determine the appropriate noncentrality parameter λ for the examples above. For the first example, the original observed power of 35% corresponds to $\lambda = 2.48$, so the power inflation due to the inflated type 1 error equals $P[X \geq 2.07 \mid \chi^2(1, 2.48)] \approx .56$. But power went up to almost 1.00 when we incorporated 50% heterogeneity, so it seems reasonable to conclude that much of that increase (i.e., the extent of 1.00 over 0.56) was indeed due to the heterogeneity. Similarly, for the second example, the original observed power of 45% corresponds to $\lambda = 3.36$, so the power inflation due to inflated type 1 error is given by $P[X \geq 2.07 \mid \chi^2(1, 3.36)] \approx .65$, whereas with heterogeneity power went up to about 0.80. Remember that these two examples are using the worst case of type 1 error equaling 0.14. Thus we suggest that not all of the power increase is due to the inflated false positive rates.

3.3. Factors that may misleadingly mimic imprinting

Sex-Specific Penetrance—We generated data in which penetrance was sex-specific rather than parent-specific. We set one sex-specific penetrance (“male”) to 0.0, and the other (“female”) to 0.9 or 0.5. When analyzed using the PP method, these datasets of size 50 showed substantial (false) evidence for imprinting: 72% and 15% of data sets, for female penetrance of 0.9 and 0.5, respectively (Figure 5). Thus, using the PP method, it was possible to falsely conclude that *imprinting* existed, when in actuality, the penetrance of the disease was greater in one sex than in the other. In contrast, sex-specific penetrance did not lead to increased false positives for the DRF method (data not shown).

Parent-Specific Ascertainment—Finally, we modeled a scenario in which disease affects the sexes equally, but only when the “mother” is affected do affected offspring come to the attention of a study. Could such skewed ascertainment lead to false evidence of imprinting? The disease penetrance (f_{common}) used to generate and analyze the data was either 0.9 or 0.5. For these experiments, although ascertainment was always through affected offspring, the only families that were ascertainable were those in which the mother was affected. Such ascertainment did not lead to an increase in the probability of falsely detecting imprinting for either PP or DRF (results not shown).

4. Discussion

We have used comparisons between the PP (direct) and DRF (indirect) approaches to elucidate some of the underlying essential issues involved in detecting imprinting via linkage analysis. We have shown that each approach reveals something about imprinting detection. Perhaps the greatest problem for both methods is the relatively high rate of type 1 error when there is unrecognized heterogeneity. Certainly the most surprising and counterintuitive observations involved the effects of heterogeneity and of reduced penetrance: Heterogeneity appeared to *increase* DRF’s ability to detect imprinting, and reduced penetrance seemed to have the same effect on the PP approach. We now discuss each of these observations in turn, then conclude with implications and future work.

4.1. Effects of heterogeneity on imprinting detection (DRF)

4.1.1. Effects on type 1 error—As noted in the Results, heterogeneity raises both type 1 error for both methods and, for DRF, increases the power to detect imprinting. The increase in type 1 error can be explained by the following reasoning: Heterogeneity tends to disrupt the location of the maxima (Greenberg and Abreu 2001), making it more probable that the location of the global maximum will occur “off the diagonal” even when there is, in fact, no imprinting. That can lead to statistically significant differences between the null and alternative hypothesis maxlods. If the information content of the dataset is high, that can lead to an increase in “gain” (to use a term from electronics), allowing the detection of differences between the null and alternative hypotheses that reflect meaningless differences in estimates of (f_{mat} , f_{pat}), and especially (θ_{male} , θ_{female}). Thus “noise” is amplified. We suggest that if one required a minimum distance between the “positions” of the maxima under the null and the alternative, this would filter out much of the false positive signal. This could be especially helpful for the DRF approach, in which power can increase under heterogeneity, as we discuss below.

We also examined the effect on type 1 error of increasing the minimum number of affected siblings required to include a family in the study. There was virtually no effect on the results unless heterogeneity was present and, even then, effects appeared to be relatively minor except when we ascertained large pedigrees (mostly three-generation pedigrees, with at least six affected).

4.1.2. Effects on power—More surprising is what heterogeneity does for DRF when imprinting exists, namely, increases the lod score difference between H_0 and H_1 . This leads to increased evidence for *imprinting* even while heterogeneity lowers the evidence in favor of *linkage*. Moreover, the resulting increase in the power to detect imprinting seems to extend over and above the increase due to inflation of type 1 error, as discussed above. This initially counterintuitive finding becomes clearer if we consider the particular characteristics of the lod score distribution as a function of θ_{male} and θ_{female} . Table 3 illustrates how this occurs with a typical dataset, chosen from one of our simulations to be representative. Table 3A shows lod scores from a data set containing 25 families, generated with no linkage and no imprinting (assuming dominant inheritance, penetrance $f_{\text{common}} = 99\%$). Table 3B, in contrast, shows the lod score pattern for a data set with 50 *linked* families, generated with $(f_{\text{mat}}, f_{\text{pat}}) = (1.0, 0.0)$. Table 3C shows the two tables added together, producing a data set with an α of .67.

For the 25 unlinked families (Table 3A), the lod score pattern is such that scores are lowest (most negative) in the lower left corner, where the assumed θ_{male} and θ_{female} are lowest; the lod scores increase to zero as one moves to the upper right. However, in moving horizontally or vertically from the lower left corner, the lod scores approach, but do not reach, zero. In fact, substantial negative lod scores remain at both the upper left and lower right corners, i.e., at both $(\theta_{\text{male}}, \theta_{\text{female}}) = (0.5, 0.01)$ and $(0.01, 0.5)$. The pattern when linkage but no imprinting exists (not shown) is similar to the pattern in Table 3A but reversed in sign. In Table 3B, showing the 50 linked and imprinted families, the disease gene always comes from one parent (“mother” in this example). There is little variation in the lod score with respect to θ_{male} (y-axis) because the father contributes no information to the lod score; the disease allele origin is always maternal. To produce a heterogeneous data set, the two above tables are combined on a cell-by-cell basis, yielding the lod scores in Table 3C.

Recall that the imprinting evidence is based on the *difference* between the global maximum lod score (the maximum over the whole table) and the maximum under the null hypothesis (the maximum of the values when $\theta_{\text{male}} = \theta_{\text{female}}$, i.e. along the diagonal of the table). In the absence of heterogeneity (Table 3B), the difference in lod score between the alternative and null hypotheses is $5.69 - 5.55$, a difference of 0.14, which is much less than when heterogeneity exists (Table 3C), $3.48 - 1.32$, a difference of 2.16.

Thus, although heterogeneity degrades the evidence for *linkage* it actually strengthens the evidence for imprinting. It would be ideal if this apparently paradoxical behavior could be incorporated into imprinting-detection methods.

4.2. The apparent effect of reduced penetrance on imprinting detection (PP)

Some additional surprising results concerned the effect of reducing the generating penetrance when there is imprinting. For the PP approach, reduced penetrance degrades linkage evidence but appears to strengthen imprinting evidence. We observed this apparent increase in imprinting detection power when the parent-specific penetrances went from $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.0)$ to $(0.5, 0.0)$. This is in contrast to what happens with heterogeneity, which has a positive effect on imprinting detection for the DRF approach but not PP. We also observed that deleting parental affectedness status has the effect of decreasing linkage evidence but increasing imprinting evidence for PP. We attribute the apparent increase in imprinting detection with reduced penetrance to the presence of more unaffected X unaffected matings, essentially equivalent to ignoring parental affectedness status.

4.2.1 Reduced penetrance and imprinting detection—Tables 4A and 4B show representative datasets generated with two different maternal (f_{mat}) penetrances, 0.9 and 0.5 (f_{pat} was always 0), then analyzed by PP. In Table 4A, the max lod score under H_0 is about

86. When f_{mat} is 0.5 (Table 4B), the max lod score under H_0 is considerably less (maxlod = 36.). However, the difference in the max lods for H_1 and H_0 when f_{mat} is 0.9 is only 0.8, whereas the difference when $f_{\text{mat}} = .5$ is 6.0, meaning more information favoring imprinting ($\chi^2 = 3.68$ at almost full f_{mat} vs $\chi^2 = 27.6$ at reduced f_{mat}). This is similar to what we saw when we examined nuclear families with two affected sibs, comparing those with only the two affected sibs to those with additional unaffected siblings. The information for imprinting was higher for families with more unaffected siblings.

However, we also noted that the penetrance values assumed to apply to the *parental generation* appear to affect imprinting detection. Apparently, the reduced penetrance case has more power to detect imprinting than the fuller penetrance case simply because there are more unaffected X unaffected matings when penetrance is lower. This idea is supported by the observation that ignoring parental affectedness status altogether enhances the ability to detect imprinting regardless of the value assumed for penetrance applied to parents. This may seem counterintuitive, since it is well known that ignoring parental affectedness status reduces power to detect *linkage*. But detecting imprinting is different because one is using the segregation of the marker alleles to provide information about whether a parent of a specific sex supplied the disease allele.

4.2.2 Parental affectedness status and imprinting detection in the PP method

—It appears that when we code the parental affectedness status as “unknown”, this forces the lod score calculation to include all at-risk mating types, exaggerating differences between the null hypothesis maxlod and the alternative hypothesis maxlod. In tables 4C and 4D, in which parental affectedness status is set to “unknown”, we see slightly lower lod scores than in the “parental status known” tables, 4A and B, but much more imprinting support. But unlike the comparable tables 4A and B, 4C, done at higher penetrance, shows more statistical support for imprinting ($\chi^2 = 61.7$ when $f_{\text{mat}} = 0.9$) than 4D ($\chi^2 = 48.35$ when $f_{\text{mat}} = 0.5$). We explain this phenomenon as follows:

Let A represent the disease allele, a the normal allele, and we assume the frequency of A is so low that we can ignore the $Aa \times Aa$ mating type (mt). All mts are ordered, with the Father always listed first. Thus, given that we ascertained through affected children, the only possible genotypic mts that the investigator needs to consider are $aa \times Aa$ (mt 1) (Mother carries disease allele) and $Aa \times aa$ (mt 2) (Father carries disease allele). Our dataset contains only the second type (since we generate families with $f_{\text{pat}} = 0$) but the naïve analysis does not know this and must allow for both mts. The important point is that mt 1 contains *no information* for imprinting. If one had a dataset consisting only of mt 1, one could estimate f_{mat} , but there is no information regarding f_{pat} and therefore no information about the difference between those two parameters. In contrast, mt 2 does contain imprinting information, because the likelihood must give equal prior weight to the possibilities that the father or the mother is contributing the disease allele to the affected children. Then the transmission patterns of alleles in the families reveal how much more frequently the disease allele is coming from the mother than from the father. Moreover, this will essentially be equally true for mts in which both parental phenotypes are coded as “unknown.” Now, consider what happens when we analyze the data with parental affectedness information (Method 1) vs. without (Method 2). Under Method 1, the mt 1 families contribute no imprinting information, whereas the mt 2 families do. Under Method 2, in contrast, all parental phenotypes are treated as “unknown,” so now all families in the dataset contribute imprinting information.

One limitation of this study is that we chose to keep the true paternally-derived penetrance at zero. Had we explored all possible penetrance combinations, the evidence for imprinting would have been a function of ($f_{\text{mat}}, f_{\text{pat}}$) differences when neither was zero.

4.2.3 Sex-specific Penetrance—One confounder appears to cause false positives when using the PP approach: differential male-female penetrance. However, inclusion of parental affectedness status plays a role in this observation also. When we set the parental affectedness status to “unknown”, the high false positive rate decreased, comparably to decreasing the penetrance (data not shown).

4.2.4 The Unaffected Sibs in DRF and PP—Absent potential confounding factors such as heterogeneity or sex-specific penetrance, the PP method has greater power to detect imprinting than the DRF approach, but only when there are unaffected sibs present.

Unaffected sibs contain the most information for penetrance (Table 2). For the DRF method, the presence of additional sibs, affected or not, proportionally increases the lod score under both the null hypothesis (no imprinting: $\theta_{\text{male}} = \theta_{\text{female}}$) and at the alternative hypothesis ($\theta_{\text{male}} \neq \theta_{\text{female}}$); the result is that, while the evidence for linkage increases proportionally, the evidence for imprinting changes little (when imprinting exists) (data not shown). In contrast, using the PP method, the evidence for imprinting (when it is present) increases with the addition of more siblings (i.e. more information about penetrance). However, in the absence of confounders, and when only ASPs are analyzed, there is little difference between the DRF and PP methods.

4.3. Implications and future work

4.3.1. Impetus for DRF approach—As we have indicated, our goal in this study is to explore different approaches to imprinting detection, to see what they can reveal about the essence of imprinting and about how evidence for imprinting interacts with evidence for linkage.

The Differential Recombination Fraction (DRF) approach intrigued us and seemed worth investigating. The original impetus came from our studies of the genetics of common epilepsy, in which we observed exactly the same kinds of differences between lod scores maximized over independent male-female recombination fraction and lod scores maximized over $\theta_{\text{male}} = \theta_{\text{female}}$ as are illustrated in Table 3. With the epilepsy data, we explored a number of possible explanations for that result, concluding it was most likely due to imprinting (Greenberg *et al.*, 2000), a suggestion first proposed by Smalley (1993). This conclusion was later supported by a population-based family study (Pal *et al.*, 2006).

4.3.2. Extending two-point to multipoint (MP)—We performed our study using two-point linkage analysis because we wanted to use a simple, unambiguous system. The DRF approach, in its simple form here, requires examining male and female recombinations fractions. Thus, two-point analysis, which involves only one recombination fraction between marker and trait, enabled us to easily compare how the DRF and PP methods responded to imprinting. The next step will be to extend these insights to MP analysis.

But there is an additional issue concerning MP linkage analysis that investigators should keep in mind. It is widely understood in our field that the probability distributions of MP lods are not well characterized. It is less well known that apparently for MP analysis, type 1 error consistently *decreases* as the sample size or amount of linkage information increases, as we have shown elsewhere (Hodge *et al.*, 2008). This phenomenon arises because MP analysis necessarily examines only a finite region of the space of all possible genetic distances. There are ways to deal with these problems when performing a MP analysis (e.g., bootstrapping, simulations methods). However, precisely for a study such as the current one, which is estimating the effects of confounders (heterogeneity, sex-specific penetrance) on type 1 error in imprinting detection, this uncertainty in the behavior of type 1 error makes it difficult to yield readily interpretable results.

In any case, it is relevant to recall that there is no *inherent* difference between two-point and MP linkage analysis. Both methods examine marker sharing information and both give the same types of results. MP methods are invaluable when we deal with SNPs, since SNPs are only bi-allelic. However, if one had fully polymorphic markers, the only disadvantage of two-point analysis would be that a recombination fraction of, say, $\theta = 0.05$ does not tell us on which side of the marker the disease gene is to be found, a disadvantage overcome by performing several two-point analyses. It can be said that the goal of multipoint analysis using SNPs is to “convert” many low-polymorphism SNPs into highly polymorphic markers so as to extract the maximum information from the data. The point is that there is no reason to expect that the behavior of multipoint data under imprinting would differ *fundamentally* from what we have observed here.

The DRF approach, with its potential advantages over the PP method, can be applied to haplotypes, recoded as a single polymorphic marker, that emerge from MP analysis and from sequencing data. There is a MP linkage program that uses *published* sex-specific recombination fractions in calculating lod scores (Strauch et al., 2006). Because one is using a sex-specific map constructed from a general population, the effect of imprinting for a specific trait would not be taken into account in such a calculation. However, if the population sex-specific map is known, the *differences* from that map might be useful in an expanded DRF-type analysis that could reflect imprinting. The fact that the DRF approach offers the opportunity to view the same data in a different way could be of great advantage when trying to demonstrate the existence of imprinting.

4.3.3. Conclusions and future work—As stated, our goal was to investigate the essential relationship between imprinting and linkage, not to suggest that investigators should stop using established programs. From all the different experiments we performed in this study, it appears that the evidence for imprinting comes not directly from strength of the “linkage” evidence, *per se*, but from the *differences* in lod scores calculated under two different sets of assumptions. This seems to hold true for both the PP and DRF approaches: In the DRF approach, imprinting evidence comes from the differences in evidence for maternal vs. paternal recombination, whereas in the PP method, imprinting evidence appears to come from non-penetrant heterozygous individuals.

With that in mind, we summarize conclusions that will prove helpful for future investigations of imprinting:

Concerning unrecognized heterogeneity – This complication inflates type 1 error for both approaches. However, DRF seems able to take this complication turn it into an advantage – increased power – as discussed above, whereas heterogeneity reduced power for the PP approach.

Concerning reduced penetrance – The PP approach has more power to detect imprinting than the DRF method when families contain unaffected sibs (*except* when heterogeneity is present). This suggests that ASP data are not ideal for testing for imprinting. Ignoring parental affectedness status increases the ability to detect imprinting using the PP approach without apparently increasing the false positive rate, at least under the circumstances that we explored, in which only data sets that had sufficient information to detect linkage were analyzed for imprinting.

Concerning issues of ascertainment: The PP approach has to deal either with issues of ascertainment, if one uses the likelihood ratio to test for the existence of imprinting, or with the uncertain distribution of the test statistic, which we approached in this work as the difference in mod scores. Other investigators have not yet investigated the effects of this

complication. The DRF approach is more robust with respect to ascertainment. On the other hand, it is reassuring that neither method is affected by the parent-specific ascertainment scheme we tested, although it is disturbing that the PP approach seems susceptible to false positives when sex-specific penetrance is present.

The DRF approach, as used here, functions in areas of the genome where $\theta_{\text{male}} = \theta_{\text{female}}$. For areas where that is not true, the approach would need to be modified to incorporate known male-female recombination differences.

In conclusion, we have posed a series of quite complicated questions in this study, and of necessity the extensive studies we have done cannot definitively answer all the questions. Each approach has its advantages and disadvantages. It would be ideal if future imprinting-detection methods could incorporate the best features of both approaches.

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Figure 1.

Power curves for PP method. The graphs show probability of detecting imprinting, as a function of the generating imprinting penetrances, heterogeneity (α), and sample size. Families had to have at least two affected children in order to be ascertained.

1A. Power vs. sample size and heterogeneity parameter, for generating $(f_{mat}, f_{pat}) = (0.9, 0.0)$.

1B. Same as 1A, except $(f_{mat}, f_{pat}) = (0.5, 0.0)$.

Figure 2.

Type 1 error curves for PP method, showing effects of ascertainment criterion (Asc), penetrance, heterogeneity (α), and sample size. “Asc = x ” means that at least x affected offspring were required in order for a family to be ascertained.

2A. Type 1 error vs. sample size and ascertainment criterion, for generating $(f_{mat}, f_{pat}) = (0.9, 0.9)$.

2B. Same as 2A, except $(f_{mat}, f_{pat}) = (0.5, 0.5)$.

2C. Type 1 error vs. sample size and ascertainment criterion, for $(f_{mat}, f_{pat}) = (0.9, 0.9)$ and heterogeneity $\alpha = .5$.

2D. Same as 2C, except $(f_{mat}, f_{pat}) = (0.5, 0.5)$.

2E. Type 1 error vs. sample size and heterogeneity (α), for $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.9)$, in multi-generation pedigrees ascertained to have at least six affected members.

2F. Same as 2E, except $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.5)$.

Figure 3.

Power curves for DRF method, showing effects of penetrance, heterogeneity (α), and sample size. The data were analyzed maximizing the lod scores over $\theta_{male} = \theta_{female}$ for the null hypothesis and for all combinations of $(\theta_{male}, \theta_{female})$ for the alternative hypothesis. Families had to have at least two affected children in order to be ascertained.

3A. Power vs. sample size and heterogeneity parameter, for generating $(f_{mat}, f_{pat}) = (0.9, 0.0)$.

3B. Same as 3A, except $(f_{mat}, f_{pat}) = (0.5, 0.0)$.

Figure 4.

Type 1 error curves for DRF method, showing effects of ascertainment criterion (Asc), penetrance, heterogeneity (α), and sample size. “Asc = x ” means that at least x affected offspring were required in order for a family to be ascertained.

4A. Type 1 error vs sample size and ascertainment criterion, for $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.5)$ and $\alpha=1.0$.

4B. Type 1 error vs sample size and ascertainment criterion, for $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.9)$ and $\alpha=0.5$.

4C. Same as 4B, except $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.5)$ and $\alpha=0.5$.

4D. Type 1 error vs sample size and α , for $(f_{\text{mat}}, f_{\text{pat}}) = (0.5, 0.5)$, in multi-generation pedigrees ascertained to have at least six affected members.

4E. Same as 4D, except $(f_{\text{mat}}, f_{\text{pat}}) = (0.9, 0.9)$.

Figure 5.
Probability of falsely detecting imprinting when there is only sex-specific penetrance, but no imprinting, vs. sample size, when data are analyzed by the PP method.

Table 1

Penetrances for Different Scenarios

Scenario	Offspring	Offspring genotype ^I			
		d / d	d / D	D / d	D / D
GM: No imprinting, no sex effect	Female	0	f_{common}	f_{common}	f_{common}
"	Male	0	f_{common}	f_{common}	f_{common}
GM: No imprinting, with sex effect	Female	0	f_{female}	f_{female}	f_{female}
"	Male	0	f_{male}	f_{male}	f_{male}
GM: Imprinting	Female	0	f_{pat}	f_{mat}	$1 - (1 - f_{\text{mat}})(1 - f_{\text{pat}})$
"	Male	0	f_{pat}	f_{mat}	$1 - (1 - f_{\text{mat}})(1 - f_{\text{pat}})$
AM: PP	Female	0	$\hat{f}_{\text{pat}}$	$\hat{f}_{\text{mat}}$	$1 - (1 - \hat{f}_{\text{mat}})(1 - \hat{f}_{\text{pat}})$
"	Male	0	$\hat{f}_{\text{pat}}$	$\hat{f}_{\text{mat}}$	$1 - (1 - \hat{f}_{\text{mat}})(1 - \hat{f}_{\text{pat}})$
AM: DRF	Female	0	f_{common}	f_{common}	f_{common}
"	Male	0	f_{common}	f_{common}	f_{common}

^I First allele is maternally inherited, second one is paternally inherited
GM = Generating Model, AM = analysis model
 $f_{\text{common}} = (f_{\text{mat}} + f_{\text{pat}})/2 = (f_{\text{female}} + f_{\text{male}})/2$.

Table 2

Power to detect imprinting in nuclear families with two affected sibs

	Nuclear families with two affected sibs and....	
Data set size	w/ add'l unaffected sibs	no add'l unaffected sibs
10	.07	0.0
20	.28	.12
30	.48	.24
40	.67	.40
50	.79	.58

Table 3
Illustration of how heterogeneity increases the evidence for imprinting in the DRF method

Lod Score tables of A) data generated with no linkage and analyzed with independent male-female θ , B) linked data generated with imprinting and analyzed with independent male-female θ , C) The cell-wise sum of A and B.

	0.5	-2.22	-2.43	-2.28	-1.73	-1.03	-0.34	0.00	0.5	5.69	5.08	4.30	2.78	1.40	0.38	0.00
θ_{male}	0.4	-2.42	-2.63	-2.48	-1.93	-1.23	-0.54	-0.20	0.4	5.69	5.07	4.30	2.78	1.40	0.39	0.01
	0.3	-2.85	-3.06	-2.91	-2.36	-1.66	-0.97	-0.63	0.3	5.68	5.06	4.29	2.77	1.40	0.39	0.02
	0.2	-3.33	-3.54	-3.39	-2.84	-2.14	-1.45	-1.11	0.2	5.65	5.04	4.27	2.75	1.38	0.38	0.01
	0.1	-3.81	-4.02	-3.88	-3.32	-2.62	-1.93	-1.59	0.1	5.61	4.99	4.22	2.70	1.33	0.33	-0.04
	0.05	-4.04	-4.26	-4.11	-3.55	-2.86	-2.17	-1.82	0.05	5.58	4.96	4.18	2.65	1.28	0.28	-0.09
	0.01	-4.23	-4.44	-4.29	-3.74	-3.04	-2.35	-2.01	0.01	5.55	4.93	4.14	2.61	1.23	0.22	-0.16
	0.01	0.05	0.1	0.2	0.3	0.4	0.5	0.01	0.05	0.1	0.2	0.3	0.4	0.5		
θ_{female}																
A																
+																
B																
0.5																
0.4																
0.3																
0.2																
0.1																
0.05																
0.01																
=																
C																
θ_{male}																
0.5																
0.4																
0.3																
0.2																
0.1																
0.05																
0.01																
0.01																
0.05																
0.1																
0.2																
0.3																
0.4																
0.5																
θ_{female}																

Table 4
Behavior of PP method under two different generating f_{mat} either including parental affectedness status or classifying parental affectedness as “unknown” ($f_{pat}=0$)

On diagonal the maximum lod score for H_0 and off-diagonal the maximum lod score for H_1 are in **bold**. χ^2 represents the evidence for imprinting

<i>A Parental Affectedness Known; Generating $f_{mat}=9$</i>												
f_{pat}	0.9	2	9.8	34.8	48.6	57.2	63.8	69.9	75.7	81.4	85.7	
	0.8	1.7	12.1	36.9	49.9	57.7	64.0	69.9	75.8	81.3	85.7	
	0.7	1.4	15.3	39.5	51.2	58.4	64.2	70.1	75.8	81.3	85.7	
	0.6	1.1	19.1	42.2	52.7	59.0	64.7	70.2	75.9	81.3	85.7	
	0.5	0.9	23.6	45.2	53.9	59.9	65.3	70.3	76.0	81.4	85.7	
	0.4	0.8	29.5	48.0	54.9	60.7	65.8	70.9	76.1	81.5	85.8	
	0.3	0.7	36.6	49.9	56.3	61.8	66.8	71.7	76.4	81.7	86.0	
	0.2	0.6	43.3	52.2	57.9	63.0	67.8	72.6	77.0	81.8	86.2	
	0.1	0.6	48.3	54.6	59.5	64.0	68.5	73.0	77.4	82.0	86.4	
	0.0		51.8	55.8	60	64.3	68.8	73.2	77.6	82.1	86.5	
$\chi^2 = 3.68$, Difference = 0.8												
<i>B Parental Affectedness Known; Generating $f_{mat}=5$</i>												
f_{pat}	0.9	1.1	2.2	11.7	19.6	24.3	27.5	28.9	27.9	24.7	22.4	
	0.8	1.6	3.4	12.1	19.7	24.4	27.4	28.7	27.8	24.2	22.1	
	0.7	1.9	4.4	12.9	20.4	24.8	27.8	28.9	28.0	24.0	21.8	
	0.6	2.1	5.6	15.1	22.1	26.2	28.7	29.7	28.4	24.4	21.7	
	0.5	2.4	7.2	17.4	24.0	27.7	29.9	30.5	29.1	25.0	21.8	
	0.4	2.6	9.2	20.1	26.1	29.3	31.1	31.5	29.9	25.9	22.3	
	0.3	2.7	12.0	23.3	28.4	30.8	32.3	32.4	31.1	27.1	23.3	
	0.2	2.7	17.4	27.1	30.4	32.3	33.4	33.4	32.4	28.6	25.0	
	0.1	2.6	25.6	30.0	31.9	33.6	34.5	34.7	33.6	30.5	27.4	
	0		29.7	32.8	33.6	35.0	35.9	35.8	35.6	33.4	29.8	
$\chi^2 = 3.68$, Difference = 0.8												
f_{mat}												
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9		

<i>B Parental Affectedness Known; Generating $f_{mat}=5$</i>												
$\chi^2 = 27.6$, Difference = 6.0												
f_{mat}												
<hr/>												
<i>C Parental Affectedness Unknown; Generating $f_{mat}=9$</i>												
f_{pat}	0.9	0.8	0.8	1.0	6.4	24.3	38.5	50.0	59.4	66.9	72.2	
	0.8	0.7	0.7	1.1	9.3	26.9	40.7	51.8	60.7	67.9	72.8	
	0.7	0.6	0.6	1.2	13.0	30.3	43.7	54.7	63.3	69.3	73.7	
	0.6	0.5	0.5	1.4	17.7	34.7	48.6	59.0	66.3	71.6	74.9	
	0.5	0.4	0.5	2.2	24.1	41.8	54.6	63.1	69.3	73.8	76.9	
	0.4	0.4	0.6	10.0	33.6	50.3	60.0	66.6	71.8	75.9	79.0	
	0.3	0.4	1.0	22.2	46.1	57.3	64.0	69.4	73.9	77.7	81.1	
	0.2	0.5	4.8	42.1	55.2	61.6	66.6	71.2	75.5	79.3	83.1	
	0.1	0.6	38.3	53.7	59.0	63.5	68.0	72.4	76.6	80.8	84.7	
	0.0		51.7	55.8	59.9	64.2	68.5	72.9	77.1	81.5	85.6	
<hr/>												
f_{mat}												
$\chi^2 = 61.7$, Difference = 13.4												
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<i>D Parental Affectedness Unknown; Generating $f_{mat}=5$</i>												
f_{pat}	0.9	0.7	1.0	2.9	13.5	20	23.8	26.2	26.5	24.3	22.2	
	0.8	1.1	1.6	2.8	13.0	19.5	23.3	25.8	26.4	24.4	22.4	
	0.7	1.6	2.0	3.7	13.0	19.3	23.2	25.7	26.3	24.6	22.5	
	0.6	1.9	2.2	5.0	14.1	20.2	23.7	26.0	26.6	24.9	22.7	
	0.5	2.1	2.9	7.3	16.9	22.7	25.6	26.9	27.2	25.5	23.2	
	0.4	2.4	3.4	11.1	20.4	25.4	27.6	28.6	28.4	26.5	24.2	
	0.3	2.6	3.9	16.4	24.5	28.0	30.2	30.7	30.2	28.2	25.6	
	0.2	2.7	7.8	23.3	28.6	31.3	32.8	33.0	32.5	30.6	27.4	
	0.1	2.6	21.9	29.0	32.1	33.9	34.9	35.0	35.0	33.3	29.6	
	0		29.7	31.8	33.7	35.2	36.1	36.1	36.1	34.7	31.0	
<hr/>												
f_{mat}												
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9												

<i>D Parental Affectedness Unknown; Generating $f_{ma}=5$</i>
$\chi^2=48.35$, Difference = 10.5