

Statistical Properties of Teng and Risch's Sibship Type Tests for Detecting an Association between Disease and a Candidate Allele

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Key Words

Family-based case-control design · TDT-like tests · Partial ascertainment approximation · Full ascertainment correction

Abstract

Risch and Teng [Genome Res 1998;8:1273–1288] and Teng and Risch [Genome Res 1999;9:234–241] proposed a class of transmission/disequilibrium test-like statistical tests based on the difference between the estimated allele frequencies in the affected and control populations. They evaluated the power of a variety of family-based and nonfamily-based designs for detecting an association between a candidate allele and disease. Because they were concerned with diseases with low penetrances, their power calculations assumed that unaffected individuals can be treated as a random sample from the population. They predicted that this assumption rendered their sample size calculations slightly conservative. We generalize their partial ascertainment conditioning by including the status of the unaffected sibs in the calculations of the distribution and power of the statistic used to compare the allele frequency in affected offspring to the estimated frequency in the parents, based on sibships with genotyped affected and unaffected sibs. Sample size formulas for our full ascertainment methods

are presented. The sample sizes for our procedure are compared to those of Teng and Risch. The numerical results and simulations indicate that the simplifying assumption used in Teng and Risch can produce both conservative and anticonservative results. The magnitude of the difference between the sample sizes needed by their partial ascertainment approximation and the full ascertainment is small in the circumstances they focused on but can be appreciable in others, especially when the baseline penetrances are moderate. Two other statistics, using different estimators for the variance of the basic statistic comparing the allele frequencies in the affected and unaffected sibs are introduced. One of them incorporates an estimate of the null variance obtained from an auxiliary sample and appears to noticeably decrease the sample sizes required to achieve a prespecified power.

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Introduction

It has been argued that the future of genetic dissection of complex diseases will require large-scale testing by association analysis [Lander, 1996; Risch and Merikangas, 1996; Risch, 2000]. The traditional epidemiological case-control design with unrelated controls is a common approach for investigating associations between candi-

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date genes and binary disease traits. However, such designs might produce spurious association due to population stratification [Li, 1972; Lander and Schork, 1994; Ewens and Spielman, 1995]. To protect against population stratification, many family-based association tests between a marker and a disease locus have been proposed that incorporate genotype information of parents and offspring [Falk and Rubinstein, 1987; Ott, 1989; Terwilliger and Ott, 1992; Spielman et al., 1993; Thomson, 1995; Schaid, 1996; Zhao et al., 1997; Knapp, 1999]. A prime example of a family-based association test is the transmission/disequilibrium test (TDT) [Spielman et al., 1993]. The TDT test relies on data on transmission of marker alleles from parents heterozygous for the marker to affected offspring. Ewens and Spielman [1995] showed that the TDT is robust to admixture and population stratification. Curnow et al. [1998] and Schaid [1998] have reviewed extensions of the TDT, and the implications of the completion of the Human Genome Project for family-based association studies are discussed by Zhao [2000].

Because parental genotypes may not be available, however, alternative family-based designs have been proposed based on a comparison of affected and unaffected siblings [Clark et al., 1956; Eaves and Meyer, 1994; Risch and Zhang, 1995; Curtis, 1997; Boehnke and Langefeld, 1998; Horvath and Laird, 1998; Witte et al., 1999; Slager and Schaid, 2001]. An appealing TDT-like statistic of this type was proposed by Teng and Risch [1999], namely $(\hat{p}_1 - \hat{p}_2)/\hat{\sigma}$, where \hat{p}_1 is the estimated frequency of the candidate allele in affected siblings, \hat{p}_2 is the estimated frequency of that allele in the parents, which is derived from the genotypes of affected and unaffected siblings, and $\hat{\sigma}^2$ is an estimator of the variance of $(\hat{p}_1 - \hat{p}_2)$. Teng and Risch [1999] calculated required sample sizes for this statistic under the simplifying assumption that the unaffected siblings could be regarded as a random sample from the population. They conjectured that this assumption would slightly overestimate required sample sizes. These calculations are of interest in their own right and also because they permitted Risch and Teng [1998] and Teng and Risch [1999] to compare the required sample sizes for a variety of family-based designs and designs using unrelated cases and controls.

To explore the region of accuracy of the assumptions underlying Teng and Risch [1999], we take into account the disease status of both the affected and unaffected sibs. This conditioning is called full ascertainment and the approximation of Teng and Risch which conditions only on the affecteds will be referred to as partial ascertainment. A second aspect of the paper is the use of two alter-

native estimators of the variance of $(\hat{p}_1 - \hat{p}_2)$. In particular, one that relies on an estimator of the null variance obtained from an auxiliary sample of the relevant population is shown to have a noticeable gain in power in many situations. The partial ascertainment approximation is valid when the penetrances and allele frequency are small. However, we observe that full ascertainment can have an appreciable impact on required sample sizes. Depending on the genetic model (baseline penetrance f_0 , allele frequency p , and mode of inheritance), the required number of sibships can be noticeably larger or smaller than reported in the table 3 of Teng and Risch [1999].

Methods

We consider a candidate locus with two alleles A and a. Following Teng and Risch [1999], sibships with r affected and s unaffected sibs without parental genotypes are classified into six exclusive and exhaustive groups: (I) all sibs are AA; (II) all sibs are aa; (III) all sibs are Aa; (IV) all sibs are either AA or Aa (but groups I or III are excluded); (V) all sibs are either Aa or aa (but groups II or III are excluded); and (VI) either both AA and aa are present in the sibship or all three genotypes, AA, Aa, and aa, are present in the sibship. Let $t = r + s$ be the sibship size. We denote the three penetrances by

$$f_2 = P(\text{Aff}|AA), \quad f_1 = P(\text{Aff}|Aa), \quad f_0 = P(\text{Aff}|aa),$$

where here and throughout 'Aff' denotes affected and 'Unaff' unaffected disease status. Following Teng and Risch [1999], we develop the distribution theory under the assumption that sibling phenotypes are conditionally independent given their respective genotypes. Under this assumption we test the null hypothesis $f_0 = f_1 = f_2$. In the discussion, we point out that this same null distribution holds for the weaker null hypothesis that parental mating types are independent of sibling phenotypes.

Full Ascertainment Conditioning

Following Teng and Risch [1999], we adopt the following notation to simplify the algebraic formulas:

$$c_{21} = \frac{f_2}{f_2 + f_1}, \quad c_{20} = \frac{f_1}{f_2 + f_1}, \quad c_{01} = \frac{f_1}{f_1 + f_0}, \quad c_{00} = \frac{f_0}{f_1 + f_0},$$

$$c_{12} = \frac{f_2}{f_2 + 2f_1 + f_0}, \quad c_{11} = \frac{2f_1}{f_2 + 2f_1 + f_0}, \quad c_{10} = \frac{f_0}{f_2 + 2f_1 + f_0},$$

$$d_{21} = \frac{1 - f_2}{(1 - f_2) + (1 - f_1)}, \quad d_{20} = \frac{1 - f_1}{(1 - f_2) + (1 - f_1)},$$

$$d_{01} = \frac{1 - f_1}{(1 - f_1) + (1 - f_0)}, \quad d_{00} = \frac{1 - f_0}{(1 - f_1) + (1 - f_0)},$$

$$d_{12} = \frac{1 - f_2}{(1 - f_2) + 2(1 - f_1) + (1 - f_0)},$$

$$d_{11} = \frac{2(1 - f_1)}{(1 - f_2) + 2(1 - f_1) + (1 - f_0)}, \quad d_{10} = \frac{1 - f_0}{(1 - f_2) + 2(1 - f_1) + (1 - f_0)}.$$

Table 1. Conditional mating type probabilities in families with r affected and s unaffected sibs

$G = (i,j)$	Conditional probability $m_{ij}^{(r,s)}$
(2,2)	$\frac{\binom{r+s}{r}}{K_{r,s}} f_2^r (1-f_2)^s g_{22}$
(2,1)	$\frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{2}\right)^r (f_2 + f_1)^r [(1-f_2) + (1-f_1)]^s g_{21}$
(1,2)	$\frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{2}\right)^r (f_2 + f_1)^r [(1-f_2) + (1-f_1)]^s g_{12}$
(2,0)	$\frac{\binom{r+s}{r}}{K_{r,s}} f_1^r (1-f_1)^s g_{20}$
(0,2)	$\frac{\binom{r+s}{r}}{K_{r,s}} f_1^r (1-f_1)^s g_{02}$
(1,1)	$\frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{4}\right)^r (f_2 + 2f_1 + f_0)^r \times [(1-f_2) + 2(1-f_1) + (1-f_0)]^s g_{11}$
(1,0)	$\frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{2}\right)^r (f_1 + f_0)^r [(1-f_1) + (1-f_0)]^s g_{10}$
(0,1)	$\frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{2}\right)^r (f_1 + f_0)^r [(1-f_1) + (1-f_0)]^s g_{01}$
(0,0)	$\frac{\binom{r+s}{r}}{K_{r,s}} f_0^r (1-f_0)^s g_{00}$

The c_{ij} 's and d_{ij} 's represent the conditional genotype probabilities of offspring given the parental mating types and offspring affection status. Let 2, 1, 0 denote the informative mating types: 2 - AA \times Aa (or Aa \times AA), 1 - Aa \times Aa, and 0 - Aa \times aa (or aa \times Aa). Then, the subscript i in c_{ij} or d_{ij} indicates the mating type and j indicates the number of A alleles the child received from the heterozygous parents. For example, $c_{21} = P\{C = AA | C = \text{Aff}, G = AA \times Aa\}$ and $d_{12} = P\{C = AA | C = \text{Unaff}, G = Aa \times Aa\}$.

The possible parental mating types are represented by $G = (i,j)$, where i (j) is the number of A alleles carried by the first (second) parent. Clearly, $0 \leq i,j \leq 2$. For example, $G = (1,1)$ indicates that both parents are heterozygous with genotype Aa. The population frequency of mating type $G = (i,j)$ is $g_{ij} = P(G = (i,j))$. The conditional

probability of mating type $G = (i,j)$ given r affected and s unaffected sibs is $m_{ij}^{(r,s)} = P\{G = (i,j) | C_r = \text{Aff}, C_s = \text{Unaff}\}$, where the event that r sibs are affected is denoted by $C_r = \text{Aff}$, and the event that s sibs are unaffected is denoted by $C_s = \text{Unaff}$. Under the null hypothesis, the conditional probability $m_{ij}^{(r,s)}$ is just the unconditional probability g_{ij} . The derivations of the conditional mating type probabilities are given in Appendix A, and formulas for the conditional mating type probabilities are shown in table 1, where $K_{r,s} = P\{C_r = \text{Aff}, C_s = \text{Unaff}\}$. The formulas in table 1 are analogous to those in table 1 of Risch and Teng [1998] except that the additional effect of conditioning on $C_s = \text{Unaff}$ is accounted for.

For any given sibship, the genotype status can be regarded as a random vector $(j_2, j_1, j_0, k_2, k_1, k_0)$, where $j_i, i = 0, 1, 2$ is the number of affected sibs with i A alleles; similarly, $k_i, i = 0, 1, 2$ is the number of unaffected sibs with i A alleles. We have $j_2 + j_1 + j_0 = r$, and $k_2 + k_1 + k_0 = s$. The six groups into which the sibships were classified earlier are mutually exclusive. Hence, the group membership imposes constraints on the genotype vector. For example, the fact that a sibship belongs to group IV implies that $j_2 + k_2 \geq 1, j_1 + k_1 \geq 1$, and $j_0 = k_0 = 0$.

For group IV sibships, the conditional probability of the genotype vector given $C_r = \text{Aff}$ and $C_s = \text{Unaff}$ is (see Appendix B)

$$P\{j_2, j_1, 0, k_2, k_1, 0 | C_r = \text{Aff}, C_s = \text{Unaff}\} = \binom{r}{j_2} \binom{s}{k_2} [m_{(21)}^{(r,s)} c_{21}^j c_{20}^k d_{21}^{k_2} d_{20}^{k_1} + m_{(11)}^{(r,s)} c_{12}^j c_{11}^k d_{12}^{k_2} d_{11}^{k_1}], \quad (1)$$

where $m_{(21)}^{(r,s)} = m_{12}^{(r,s)} + m_{21}^{(r,s)}$ and c_{ij} and d_{ij} are defined at the beginning of the section. All other conditional probabilities along with the scores assigned to different groups in the proposed test statistic [Teng and Risch, 1999] defined below in equation (5) are provided in table 2. The other conditional probabilities in table 2 are derived by the methods used in Appendix B; details are available from the first author. Notice that d_{ij} 's reduce to 1/2 or 1/4 when $f_2 = f_1 = f_0$. Hence, the conditional probabilities given in table 2 generalize the conditional probabilities given in table 2 of Teng and Risch [1999]. For example, the conditional probability for a group IV sibship becomes

$$P\{j_2, j_1, 0, k_2, k_1, 0 | C_r = \text{Aff}\} = \binom{r}{j_2} \binom{s}{k_2} 2^{-s} [m_{(21)}^{(r)} c_{21}^j c_{20}^k + 2^{-k_2} m_{(11)}^{(r)} c_{12}^j c_{11}^k], \quad (2)$$

because when $f_2 = f_1 = f_0, d_{21} = 1/2, d_{20} = 1/2, d_{12} = 1/4, d_{11} = 1/2$, and $k_1 + k_2 = s$. Their assumption that unaffected individuals are a random sample of the population means that conditioning on the child being unaffected in the definition of d_{ij} is irrelevant, e.g.,

$$P\{C = AA | C = \text{Unaff}, G = Aa \times Aa\} = P\{C = AA | G = Aa \times Aa\} = \frac{1}{4}.$$

Thus, $d_{12} = 1/4$. Algebraically, this is equivalent to setting the factors $(1-f_i)$'s used in the d_{ij} 's and $m_{ij}^{(r,s)}$ at the null values $(1-f_2 = 1-f_1 = 1-f_0)$. Notice, however, that the factors, f_i , appearing in c_{ij} and in the formulas for $m_{ij}^{(r,s)}$ are not set equal to one another. Then, our $m_{ij}^{(r,s)}$, given in table 1, reduce to $m_{ij}^{(r)}$ in their table 1 [Risch and Teng, 1998].

Teng and Risch [1999] proposed a TDT-like statistic (5) for testing the null hypothesis $f_2 = f_1 = f_0$, using the scores given in table 2. These scores were derived from the difference, $\hat{p}_1 - \hat{p}_2$, between the

Table 2. Scores for T_{DS} statistic and conditional genotype vector probabilities

Group	Outcome	Score $S(\hat{p}_1 - \hat{p}_2)$	Probability
I	$(r, 0, 0, s, 0, 0)$	0	$m_{22}^{(r,s)} + m_{(21)}^{(r,s)} c_{21}^r d_{21}^s + m_{11}^{(r,s)} c_{12}^r d_{12}^s$
II	$(0, 0, r, 0, 0, s)$	0	$m_{00}^{(r,s)} + m_{(10)}^{(r,s)} c_{00}^r d_{00}^s + m_{11}^{(r,s)} c_{10}^r d_{10}^s$
III	$(0, r, 0, 0, s, 0)$	0	$m_{20}^{(r,s)} + m_{(21)}^{(r,s)} c_{20}^r d_{20}^s + m_{(10)}^{(r,s)} c_{01}^r d_{01}^s + m_{11}^{(r,s)} c_{11}^r d_{11}^s$
IV	$(j_2, j_1, 0, k_2, k_1, 0)$	$\frac{1}{4r} (j_2 - j_1)$	$\binom{r}{j_2} \binom{s}{k_2} [m_{(21)}^{(r,s)} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} + m_{11}^{(r,s)} c_{12}^{j_2} c_{11}^{j_1} d_{12}^{k_2} d_{11}^{k_1}]$
V	$(0, j_1, j_0, 0, k_1, k_0)$	$\frac{1}{4r} (j_1 - j_0)$	$\binom{r}{j_0} \binom{s}{k_0} [m_{(10)}^{(r,s)} c_{10}^{j_0} c_{00}^{j_1} d_{10}^{k_0} d_{00}^{k_1} + m_{11}^{(r,s)} c_{11}^{j_0} c_{10}^{j_1} d_{11}^{k_0} d_{10}^{k_1}]$
VI	$(j_2, j_1, j_0, k_2, k_1, k_0)$	$\frac{1}{2r} (j_2 - j_0)$	$m_{11}^{(r,s)} \binom{r}{j_2, j_1, j_0} \binom{s}{k_2, k_1, k_0} c_{12}^{j_2} c_{11}^{j_1} c_{10}^{j_0} d_{12}^{k_2} d_{11}^{k_1} d_{10}^{k_0}$

estimated frequency of allele A in the parents (\hat{p}_2) and the observed frequency of allele A in the affected sibs (\hat{p}_1). The maximum likelihood estimator \hat{p}_2 , under the null hypothesis, is

$$\hat{p}_2 = \frac{1}{n} \left[n_I + \frac{1}{2} n_{III} + \frac{3}{4} n_{IV} + \frac{1}{4} n_V + \frac{1}{2} n_{VI} \right], \quad (3)$$

where $n_I, n_{III}, n_{IV}, n_V$ and n_{VI} are the number of sibships in group I, III, IV, V and VI, respectively, and n is the total number of sibships. If the genotype vector for the i th sibship is $(j_2^i, j_1^i, j_0^i, k_2^i, k_1^i, k_0^i)$, then

$$\hat{p}_1 = \frac{1}{n} \sum_{i=1}^n \frac{j_2^i + \frac{1}{2} j_1^i}{r}. \quad (4)$$

For example, if the i th sibship is in group IV with genotype vector $(j_2, j_1, j_0, k_2, k_1, k_0)$ (for simplicity, the superscript i is eliminated), then, its contribution to \hat{p}_1 is $\frac{j_2 + 1/2 j_1}{r}$, and its contribution to \hat{p}_2 is $3/4$. Thus, the score for the i th sibship is

$$S_i(\hat{p}_1 - \hat{p}_2) = \frac{j_2 + \frac{1}{2} j_1}{r} - \frac{3}{4} = \frac{j_2 - j_1}{4r},$$

as in table 2. The form of the test statistic [Teng and Risch, 1999] is

$$T_{DS} = \frac{\sum_{i=1}^n S_i(\hat{p}_1 - \hat{p}_2)}{\sqrt{n\hat{\sigma}^2}}, \quad (5)$$

where the $S_i(\hat{p}_1 - \hat{p}_2)$, $i = 1, \dots, n$ are i.i.d. random variables. We denote the score for an ascertained sibship by $S(\hat{p}_1 - \hat{p}_2)$. The variance of $S(\hat{p}_1 - \hat{p}_2)$ is σ^2 . The statistic T_{DS} can be expressed in terms of the estimates in formulas (3) and (4) as

$$T_{DS} = \frac{\sqrt{n}(\hat{p}_1 - \hat{p}_2)}{\sqrt{\hat{\sigma}^2}}. \quad (5a)$$

Notice that different variance estimators, $\hat{\sigma}^2$, will create different test statistics, which are discussed later. When T_{DS} is large, the null

hypothesis will be rejected and an association between the candidate allele A and disease will be declared.

To obtain the sample size required to achieve prespecified type I and type II errors, we need the expected value and variance of the statistic (5) under both the null and the alternative hypotheses. The expectation of the score for an ascertained sibship is

$$\begin{aligned} v_a &= E_{H_1}(S(\hat{p}_1 - \hat{p}_2)) \\ &= \frac{m_{(21)}^{(r,s)}}{4} [c_{21} - c_{20} + c_{20}^r d_{20}^s - c_{21}^r d_{21}^s] \\ &\quad + \frac{m_{(10)}^{(r,s)}}{4} [c_{01} - c_{00} + c_{00}^r d_{00}^s - c_{01}^r d_{01}^s] \\ &\quad + \frac{m_{(11)}^{(r,s)}}{4} [2(c_{12} - c_{10}) - (c_{12} + c_{11})^r (d_{12} + d_{11})^s \\ &\quad + (c_{10} + c_{11})^r (d_{10} + d_{11})^s + c_{10}^r d_{10}^s - c_{12}^r d_{12}^s]. \end{aligned} \quad (6)$$

The derivation of formula (6) is in Appendix C. By setting $f_2 = f_1 = f_0$ in formula (6), it is seen that $E_{H_0}(S(\hat{p}_1 - \hat{p}_2)) = 0$.

Formula (6) yields the expectation of $S(\hat{p}_1 - \hat{p}_2)$ given in formula (7) of Teng and Risch when the factors $(1 - f_i)$'s are set equal to $(1 - f_0)$ for the unaffected siblings; then the d_{ij} 's again reduce to $1/2$ or $1/4$, and $m_{ij}^{(r,s)}$ equal $m_{(21)}^{(r,s)}$. Their result is

$$\begin{aligned} v_{aTR} &= E_{H_1}(S(\hat{p}_1 - \hat{p}_2)) \\ &= \frac{m_{(21)}^{(r,s)}}{4} \left[c_{21} - c_{20} + \left(\frac{1}{2}\right)^s (c_{20}^r - c_{21}^r) \right] \\ &\quad + \frac{m_{(10)}^{(r,s)}}{4} \left[c_{01} - c_{00} + \left(\frac{1}{2}\right)^s (c_{00}^r - c_{01}^r) \right] \\ &\quad + \frac{m_{(11)}^{(r,s)}}{4} \left[2(c_{12} - c_{10}) - \left(\frac{3}{4}\right)^s (c_{12} + c_{11})^r \right. \\ &\quad \left. + \left(\frac{3}{4}\right)^s (c_{10} + c_{11})^r \left(\frac{1}{4}\right)^s c_{10}^r - \left(\frac{1}{4}\right)^s c_{12}^r \right]. \end{aligned} \quad (6a)$$

We also obtain the variance of $S(\hat{\rho}_1 - \hat{\rho}_2)$ under the alternative hypothesis as:

$$\begin{aligned} \sigma_a^2 &= \text{Var}_{H_1}(S(\hat{\rho}_1 - \hat{\rho}_2)) = E_{H_1}(S(\hat{\rho}_1 - \hat{\rho}_2))^2 - v_a^2 \\ &= \frac{m_{(21)}^{(r,s)}}{16r} [r - 4(r-1)c_{21}c_{20} - r(c_{20}^r d_{20}^s + c_{21}^r d_{21}^s)] \\ &\quad + \frac{m_{(10)}^{(r,s)}}{16r} [r - 4(r-1)c_{01}c_{00} - r(c_{00}^r d_{00}^s + c_{01}^r d_{01}^s)] \\ &\quad + \frac{m_{(11)}^{(r,s)}}{16r} [r(c_{11} - 3c_{12})(c_{12} + c_{11})^{r-1}(d_{12} + d_{11})^s \\ &\quad + r(c_{11} - 3c_{10})(c_{10} + c_{11})^{r-1}(d_{10} + d_{11})^s \\ &\quad - rc_{12}^r d_{12}^s - 2rc_{11}^r d_{11}^s - rc_{10}^r d_{10}^s \\ &\quad + 4r(c_{12}^2 + c_{10}^2) - 8rc_{10}c_{11}] - v_a^2 \end{aligned} \quad (7)$$

The derivation of the variance formula (7) is similar to that of (6) and an outline is given in Appendix D.

Using the arguments similar to those for formula (6a), we obtain the variance of $S(\hat{\rho}_1 - \hat{\rho}_2)$ under Teng and Risch's original partial ascertainment approximation as

$$\begin{aligned} \sigma_{aTR}^2 &= \text{Var}_{H_1}(S(\hat{\rho}_1 - \hat{\rho}_2)) = E_{H_1}(S(\hat{\rho}_1 - \hat{\rho}_2))^2 - v_{aTR}^2 \\ &= \frac{m_{(21)}^{(r)}}{16r} \left[r - 4(r-1)c_{21}c_{20} - r\left(\frac{1}{2}\right)^s (c_{20}^r + c_{21}^r) \right] \\ &\quad + \frac{m_{(10)}^{(r)}}{16r} \left[r - 4(r-1)c_{01}c_{00} - r\left(\frac{1}{2}\right)^s (c_{00}^r + c_{01}^r) \right] \\ &\quad + \frac{m_{(11)}^{(r)}}{16r} \left[r\left(\frac{3}{4}\right)^s (c_{11} - 3c_{12})(c_{12} + c_{11})^{r-1} \right. \\ &\quad \left. + r\left(\frac{3}{4}\right)^s (c_{11} - 3c_{10})(c_{10} + c_{11})^{r-1} \right. \\ &\quad \left. - r\left(\frac{1}{4}\right)^s c_{12}^r - 2r\left(\frac{1}{2}\right)^s c_{11}^r - r\left(\frac{1}{2}\right)^s c_{10}^r \right. \\ &\quad \left. + 4r(c_{12}^2 + c_{10}^2) - 8rc_{12}c_{10} \right. \\ &\quad \left. + 16c_{12}c_{10} + 4c_{12}c_{11} + 4c_{10}c_{11} \right] - v_{aTR}^2 \end{aligned} \quad (7a)$$

The variance formula (7a) is different from the corresponding formula (9) in Teng and Risch [1999], as v_{aTR}^2 in (7a) involves second-order terms in the conditional probabilities, $m_{ij}^{(r)}$, which are not included in formula (9) of Teng and Risch [1999]. However, the sample sizes obtained using (7a) are the same as those given in table 3 of Teng and Risch [1999]. This suggests that their sample size calculations used the correct variance.

To obtain the variance of $S(\hat{\rho}_1 - \hat{\rho}_2)$ under the null hypothesis, we set $f_2 = f_1 = f_0$ in formula (7) yielding:

$$\begin{aligned} \sigma_0^2 &= \text{Var}_{H_0}(S(\hat{\rho}_1 - \hat{\rho}_2)) \\ &= \frac{g_{(21)}}{16r} \left[1 - r\left(\frac{1}{2}\right)^{t-1} \right] + \frac{g_{(10)}}{16r} \left[1 - r\left(\frac{1}{2}\right)^{t-1} \right] \\ &\quad + \frac{g_{(11)}}{8r} \left[1 - r\left\{ \frac{1}{3}\left(\frac{3}{4}\right)^t + \left(\frac{1}{4}\right)^t + \left(\frac{1}{2}\right)^t \right\} \right], \end{aligned} \quad (8)$$

where $g_{(21)} = g_{12} + g_{21}$ and $g_{(10)} = g_{10} + g_{01}$.

Required Number of Sibships for Various Statistics

The Statistic of Teng and Risch

The use of statistic (5) requires an estimator of σ^2 . Teng and Risch use

$$\hat{\sigma}_{TR}^2 = \frac{\rho_1}{16n} (n_{IV} + n_V) + \frac{\rho_2}{8n} n_{VI}, \quad (9)$$

where

$$\begin{aligned} \rho_1 &= \frac{\frac{1}{r} - \left(\frac{1}{2}\right)^{t-1}}{1 - \left(\frac{1}{2}\right)^{t-1}}, \\ \rho_2 &= \frac{\frac{1}{r} \left[1 - \left(\frac{1}{2}\right)^t - \left(\frac{3}{4}\right)^t + \left(\frac{1}{4}\right)^t \right] + \left(\frac{3}{8}\right)^{t-1} - \frac{1}{3}\left(\frac{3}{4}\right)^t - \left(\frac{1}{2}\right)^t - \left(\frac{1}{4}\right)^t}{\left[1 - \left(\frac{1}{2}\right)^{t-1} \right] \left[1 + \left(\frac{1}{2}\right)^t - 2\left(\frac{3}{4}\right)^t \right]}. \end{aligned}$$

Using the law of large numbers and calculations similar to those in Appendix C, it can be shown that $\hat{\sigma}_{TR}^2$ converges to $\sigma_*^2 = E\hat{\sigma}_{TR}^2$, where

$$\begin{aligned} \sigma_*^2 &= \frac{\rho_1}{16} \{ m_{(21)}^{(r,s)} [1 - c_{20}^r d_{20}^s - c_{21}^r d_{21}^s] + m_{(10)}^{(r,s)} [1 - c_{01}^r d_{01}^s - c_{00}^r d_{00}^s] \} \\ &\quad + \frac{\rho_1}{16} m_{(11)}^{(r,s)} \{ (c_{12} + c_{11})^r (d_{12} + d_{11})^s + (c_{10} + c_{11})^r (d_{10} + d_{11})^s \\ &\quad - 2c_{11}^r d_{11}^s - c_{12}^r d_{12}^s - c_{10}^r d_{10}^s \} \\ &\quad + \frac{\rho_2}{8} m_{(11)}^{(r,s)} \{ (1 - (c_{12} + c_{11})^r (d_{12} + d_{11})^s \\ &\quad - (c_{10} + c_{11})^r (d_{10} + d_{11})^s + c_{11}^r d_{11}^s) \}. \end{aligned} \quad (10)$$

It is worth noting that $\sigma_*^2 = \sigma_0^2$ under the null hypothesis, but $\sigma_*^2 \neq \sigma_a^2$ under the alternative hypothesis. The required number of sibships to achieve the prespecified significance level α and power $1 - \beta$ is given by

$$n = \frac{(\sigma_* z_{1-\alpha} + \sigma_a z_{1-\beta})^2}{v_a^2}. \quad (11)$$

This is the sample size formula for the Teng and Risch statistic with the full ascertainment correction.

To obtain the sample size for Teng and Risch's original procedure with the partial ascertainment approximation, we use their formula (8) for the expectation of $\hat{\sigma}_{TR}^2$. We denote their parameter by σ_{*TR}^2 . Again it can be obtained from (10) by setting the factors $(1 - f_i) = (1 - f_0)$, $i = 1, 2$ in the d_{ij} 's and the $m_{ij}^{(r,s)}$'s. Then, the sample size formula for the original procedure of Teng and Risch is

$$n = \frac{(\sigma_{*TR} z_{1-\alpha} + \sigma_a z_{1-\beta})^2}{v_{aTR}^2}. \quad (11a)$$

The Statistic Utilizing the Empirical Variance of the Score

A different statistic can be formed by substituting the sample variance of the scores into (5). The sample variance of the scores $S_i = S_i(\hat{\rho}_1 - \hat{\rho}_2)$ is

$$\hat{\sigma}_S^2 = \frac{1}{n-1} \sum_{i=1}^n (S_i - \bar{S})^2, \text{ where } \bar{S} = \frac{1}{n} \sum_{i=1}^n S_i,$$

which is used in place of $\hat{\sigma}^2$ in the denominator of (5). When the null (alternative) hypothesis holds, $\hat{\sigma}_3^2$ converges to σ_0^2 (σ_a^2). As in (11) the required number of sibships is given by

$$n = \frac{\sigma_a^2 (z_{1-\alpha} + z_{1-\beta})^2}{v_a^2} \quad (12)$$

When $\sigma_a^2 > \sigma_0^2$, the test statistic based on $\hat{\sigma}_3^2$ requires fewer sibships to achieve a prespecified significance level and power compared to the method of Teng and Risch [1999], using $\hat{\sigma}_{TR}^2$.

The previous result incorporates full ascertainment. The corresponding sample size formula based on the partial ascertainment approximation is

$$n = \frac{\sigma_{aTR}^2 (z_{1-\alpha} + z_{1-\beta})^2}{v_{aTR}^2} \quad (12a)$$

The Statistic Incorporating an Auxiliary Sample

A third statistic can be based on a consistent estimator of the variance of the general score under the null hypothesis, $\hat{\sigma}_0^2$. We describe two methods for accomplishing this.

First, the null variance, σ_0^2 , is a function of the unconditional mating probabilities: $g_{(21)}$, $g_{(10)}$, and g_{11} . Under Hardy-Weinberg equilibrium, these unconditional mating probabilities are functions of the allele frequency, p . This frequency can be estimated from an independent auxiliary sample, e.g., a sample survey of the general population for the candidate gene as in Steinberg et al. [2001]. Of course, one does not need the Hardy-Weinberg equilibrium if one estimates the mating type frequencies from a random sample of sibships, which can be obtained from a survey as in Steinberg et al. [2001].

An alternate auxiliary sample estimates the mating type probabilities from a random sample of sibships. Estimates of the mating type probabilities, g_{ij} , are obtained by adapting formula (4) of Teng and Risch [1999] and equating observed proportions of different groups of sibships to their theoretical probabilities. This leads to the estimating equations:

$$\begin{aligned} g_{(21)} \left[1 - \left(\frac{1}{2}\right)^{t-1} \right] + g_{11} \left[\left(\frac{3}{4}\right)^t - \left(\frac{1}{2}\right)^t - \left(\frac{1}{4}\right)^t \right] &= \frac{n_{IV}^0}{n}, \\ g_{(10)} \left[1 - \left(\frac{1}{2}\right)^{t-1} \right] + g_{11} \left[\left(\frac{3}{4}\right)^t - \left(\frac{1}{2}\right)^t - \left(\frac{1}{4}\right)^t \right] &= \frac{n_V^0}{n}, \\ g_{11} \left[1 + \left(\frac{1}{2}\right)^t - 2\left(\frac{3}{4}\right)^t \right] &= \frac{n_{VI}^0}{n}. \end{aligned} \quad (13)$$

We use the notation n_{IV}^0 , n_V^0 and n_{VI}^0 to emphasize that these counts must be obtained from a sample of sibships that is *not* ascertained based on affection status.

Thus, they are different from n_{IV} , n_V and n_{VI} in the formula for $\hat{\sigma}_{TR}^2$. Equations (13) give the estimators for the unconditional mating probabilities:

$$\begin{aligned} \hat{g}_{11} &= \frac{n_{VI}^0}{n \left[1 + \left(\frac{1}{2}\right)^t - 2\left(\frac{3}{4}\right)^t \right]}, \\ \hat{g}_{(10)} &= \frac{n_V^0 - \hat{g}_{11} \left[\left(\frac{3}{4}\right)^t - \left(\frac{1}{2}\right)^t - \left(\frac{1}{4}\right)^t \right]}{1 - \left(\frac{1}{2}\right)^{t-1}}, \end{aligned}$$

$$\hat{g}_{(21)} = \frac{\frac{n_{IV}^0}{n} - \hat{g}_{11} \left[\left(\frac{3}{4}\right)^t - \left(\frac{1}{2}\right)^t - \left(\frac{1}{4}\right)^t \right]}{1 - \left(\frac{1}{2}\right)^{t-1}}$$

Substituting these into (8) yields

$$\hat{\sigma}_0^2 = \frac{\rho_1}{16n} (n_{IV}^0 + n_V^0) + \frac{\rho_2}{8n} n_{VI}^0 \quad (14)$$

where ρ_1 and ρ_2 are given in (9).

Since $\hat{\sigma}_0^2$ obtained from either type of auxiliary sample converges to σ_0^2 under either the null or alternative hypothesis, the required number of sibships for the corresponding test with significance level α and power $1 - \beta$ is

$$n = \frac{(\sigma_0 z_{1-\alpha} + \sigma_a z_{1-\beta})^2}{v_a^2} \quad (15)$$

The sample size formula based on partial ascertainment approximation is

$$n = \frac{(\sigma_{0TR} z_{1-\alpha} + \sigma_{aTR} z_{1-\beta})^2}{v_{aTR}^2} \quad (15a)$$

Numerical Results

The required numbers of sibships are calculated as in Teng and Risch [1999], using $\alpha = 5 \times 10^{-8}$ ($z_{1-\alpha} = 5.33$) and $1 - \beta = 80\%$ ($z_{1-\beta} = 0.8416$). Table 3 provides comparisons between full and partial ascertainment for the three different statistics.

Four different genetic models are considered: (1) dominant: $f_2 = \gamma^2 f_0$, $f_1 = f_2$; (2) recessive: $f_2 = \gamma^2 f_0$, $f_1 = f_0$; (3) multiplicative: $f_2 = \gamma^2 f_0$, $f_1 = \gamma f_0$, and (4) additive: $f_2 = \gamma^2 f_0$, $f_1 = 1/2(f_2 + f_0)$. Teng and Risch's partial ascertainment approximation only depends on the relative risks, $\frac{f_1}{f_0}$ and $\frac{f_2}{f_0}$; baseline penetrance plays no role. They indicate that this should be a close approximation to full ascertainment when the penetrances are low. The domain of applicability of this approximation was explored in an extensive set of calculations based on the sample size formulas ((11), (11a), (12), (12a), (15), (15a)). Table 3 reports several situations when $r = 1$ that are illustrative of the general results (available from the first author). The required sample sizes for a 0.05 level test to have 80% power for various sibship configurations when $f_0 = 0.025$, 0.05, and 0.10 and $\gamma = 2$ are given. The results when $f_0 = 0.025$ show that partial ascertainment yields a very good approximation to full ascertainment. For larger f_0 , however, the approximation is less accurate. For example, for a dominant genetic model with allele frequency $p = 0.05$, $r = 1$, $s = 1$, and $f_0 = 0.05$, the required numbers of sibships using the Teng and Risch statistic is 19% more than the value

Table 3. Number of required sibships^a computed from the partial ascertainment approximation and exactly (full ascertainment) for scores standardized by $\hat{\sigma}_{TR}$, $\hat{\sigma}_S$ and $\hat{\sigma}_0$

Ascertainment	$\hat{\sigma}_{TR}$ (Teng-Risch statistic) r = 1		$\hat{\sigma}_S$ (empirical variance) r = 1		$\hat{\sigma}_0$ (auxiliary variance estimate) r = 1	
	s = 1	s = 2	s = 1	s = 2	s = 1	s = 2
<i>Dominant, p = 0.05</i>						
Partial	643	431	610	395	364	242
Full						
$f_0 = 0.025$	591	419	558	384	335	243
$f_0 = 0.05$	541	409	508	373	307	245
$f_0 = 0.100$	447	391	415	356	255	258
<i>Recessive, p = 0.05</i>						
Partial	79,752	61,577	79,719	62,445	73,335	57,432
Full						
$f_0 = 0.025$	75,594	61,946	77,561	62,819	69,643	58,073
$f_0 = 0.05$	71,547	62,448	71,514	63,329	66,047	58,859
$f_0 = 0.100$	63,786	63,960	63,753	64,865	59,140	60,993
<i>Multiplicative, p = 0.05</i>						
Partial	2,540	1,716	2,507	1,689	1,862	1,263
Full						
$f_0 = 0.025$	2,379	1,663	2,346	1,637	1,754	1,246
$f_0 = 0.05$	2,224	1,610	2,191	1,584	1,650	1,229
$f_0 = 0.100$	1,928	1,505	1,895	1,480	1,450	1,199
<i>Additive, p = 0.05</i>						
Partial	1,491	1,003	1,458	970	1,007	678
Full						
$f_0 = 0.025$	1,390	973	1,357	940	944	672
$f_0 = 0.05$	1,292	942	1,259	910	883	667
$f_0 = 0.100$	1,107	883	1,074	852	767	661

Dominant: $f_2 = \gamma^2 f_0, f_1 = f_2$; recessive: $f_2 = \gamma^2 f_0, f_1 = f_0$; multiplicative: $f_2 = \gamma^2 f_0, f_1 = \gamma f_0$; additive: $f_2 = \gamma^2 f_0, f_1 = \frac{1}{2}(f_2 + f_0)$. Here, $\gamma = 2$.

^a Computed for size 5×10^{-8} and power 0.80.

calculated for complete ascertainment. For $f_0 = 0.10$, the partial ascertainment approximation is 44% too high. The difference increases as the baseline penetrance increases (table 3). In some cases, the sample sizes computed under the partial ascertainment approximation are twice the sample size sizes required for the full ascertainment procedure when an auxiliary sample is used to estimate $\hat{\sigma}_0^2$. For instance, from an unreported result, in a dominant model with $p = 0.20, r = 2, s = 1$, and $f_0 = 0.100$, the sample size for partial ascertainment is 200 while only 116 sibships are needed for full ascertainment.

Whether or not the partial ascertainment approximation is conservative depends on the genetic model, allele frequency, and sibship structure. The partial ascertainment procedure can be anti-conservative. For example,

using the auxiliary sample estimate of $\hat{\sigma}_0^2$ in formula (5), under a recessive model with $r = 3, s = 4, p = 0.05$ and $f_0 = 0.100$, the sample size from the partial ascertainment approximation is 1,965 (result not shown) while sample size required for full ascertainment is 3,637 (see table 5). Usually, when the baseline penetrance is $f_0 = 0.100$ the partial ascertainment approximation is poor.

The results in table 3 provide insight into the relative sample size requirements for full ascertainment for statistics based on $\hat{\sigma}_{TR}^2, \hat{\sigma}_S^2$ and $\hat{\sigma}_0^2$. Standardizing equation (5) by $\hat{\sigma}_S$ leads to slightly smaller sample size requirements than standardizing by $\hat{\sigma}_{TR}$ for the dominant, multiplicative, and additive models. Standardizing by $\hat{\sigma}_0$ leads to very considerable reductions in numbers of required sibships for dominant, multiplicative and additive models

Table 4. The required number of sibships for 80% power with a one-sided $\alpha = 5 \times 10^{-8}$ level test standardized by the empirical variance estimate $\hat{\sigma}_s^2$

	r=1		r=2			r=3		
	s=1	s=2	s=1	s=2	s=3	s=2	s=3	s=4
<i>Dominant</i>								
p = 0.05								
$f_0 = 0.025$	558	384	233	148	125	79	66	61
$f_0 = 0.050$	508	373	220	145	128	77	66	62
$f_0 = 0.100$	415	356	193	142	141	72	67	68
p = 0.02								
$f_0 = 0.025$	372	247	269	164	132	153	121	105
$f_0 = 0.050$	324	221	241	147	118	136	106	91
$f_0 = 0.100$	236	174	185	114	94	102	78	66
p = 0.70								
$f_0 = 0.025$	4,670	3,341	6,134	3,790	2,930	5,461	3,775	2,972
$f_0 = 0.050$	3,798	2,634	4,981	2,981	2,238	4,260	2,874	2,206
$f_0 = 0.100$	2,321	1,482	3,035	1,670	1,161	2,341	1,474	1,054
<i>Recessive</i>								
p = 0.05								
$f_0 = 0.025$	75,561	62,819	19,348	14,728	13,184	4,229	3,777	3,546
$f_0 = 0.050$	71,514	63,329	18,885	15,183	14,229	4,417	4,102	3,997
$f_0 = 0.100$	63,753	64,865	17,932	16,307	16,912	4,881	4,951	5,246
p = 0.20								
$f_0 = 0.025$	1,935	1,542	654	476	424	194	173	164
$f_0 = 0.050$	1,809	1,529	630	480	446	197	181	176
$f_0 = 0.100$	1,569	1,515	581	495	503	204	202	211
p = 0.70								
$f_0 = 0.025$	362	246	297	190	158	196	167	153
$f_0 = 0.050$	317	219	270	171	140	177	147	130
$f_0 = 0.100$	232	168	215	133	107	136	106	89
<i>Multiplicative</i>								
p = 0.05								
$f_0 = 0.025$	2,346	1,637	1,015	683	590	351	310	293
$f_0 = 0.050$	2,191	1,584	980	676	596	351	315	302
$f_0 = 0.100$	1,895	1,480	910	661	612	351	327	324
p = 0.20								
$f_0 = 0.025$	769	546	431	284	239	185	156	141
$f_0 = 0.050$	702	514	406	272	233	179	152	139
$f_0 = 0.100$	576	453	354	248	221	167	145	136
p = 0.70								
$f_0 = 0.025$	783	551	596	391	328	326	281	257
$f_0 = 0.050$	668	480	538	354	297	303	257	232
$f_0 = 0.100$	464	347	420	278	233	250	206	181
<i>Additive</i>								
p = 0.05								
$f_0 = 0.025$	1,357	940	578	383	329	197	172	162
$f_0 = 0.050$	1,259	910	556	378	333	196	174	166
$f_0 = 0.100$	1,074	852	511	371	347	194	180	180
p = 0.20								
$f_0 = 0.025$	595	412	368	236	195	176	145	129
$f_0 = 0.050$	537	383	342	222	186	166	137	123
$f_0 = 0.100$	429	326	290	193	167	146	121	110
p = 0.70								
$f_0 = 0.025$	1,165	826	927	610	509	508	431	391
$f_0 = 0.050$	982	709	828	547	456	471	395	354
$f_0 = 0.100$	661	492	627	416	345	386	315	274

Dominant: $f_2 = \gamma^2 f_0, f_1 = f_0$; recessive: $f_2 = \gamma^2 f_0, f_1 = f_0$; multiplicative: $f_2 = \gamma^2 f_0, f_1 = \gamma f_0$; additive: $f_2 = \gamma^2 f_0, f_1 = \frac{1}{2}(f_2 + f_0)$. Here, $\gamma = 2$.

Table 5. The required number of sibships for 80% power with a one-sided $\alpha = 5 \times 10^{-8}$ level test standardized by a consistent estimator of the null variance, σ_0^2 , from an auxiliary sample

	r = 1		r = 2			r = 3		
	s = 1	s = 2	s = 1	s = 2	s = 3	s = 2	s = 3	s = 4
<i>Dominant</i>								
p = 0.05								
$f_0 = 0.025$	355	243	80	55	49	24	21	20
$f_0 = 0.050$	307	245	75	55	52	23	21	20
$f_0 = 0.100$	255	258	65	56	62	22	22	24
p = 0.20								
$f_0 = 0.025$	322	226	178	117	99	100	82	73
$f_0 = 0.050$	279	206	156	105	90	88	71	63
$f_0 = 0.100$	204	170	116	82	74	65	53	48
p = 0.70								
$f_0 = 0.025$	5,043	3,622	6,393	3,984	3,107	5,778	4,017	3,183
$f_0 = 0.050$	4,047	2,814	5,106	3,077	2,325	4,430	2,995	2,305
$f_0 = 0.100$	2,384	1,521	2,971	1,639	1,138	2,317	1,447	1,024
<i>Recessive</i>								
p = 0.05								
$f_0 = 0.025$	69,943	58,073	15,524	11,850	10,571	2,770	2,432	2,253
$f_0 = 0.050$	66,047	58,859	15,174	12,311	11,561	2,924	2,693	2,611
$f_0 = 0.100$	59,140	60,993	14,453	13,449	14,135	3,310	3,390	3,637
p = 0.20								
$f_0 = 0.025$	1,608	1,291	428	318	283	110	97	91
$f_0 = 0.050$	1,508	1,293	411	322	300	111	101	97
$f_0 = 0.100$	1,316	1,309	377	336	348	115	113	118
p = 0.70								
$f_0 = 0.025$	423	292	351	233	195	272	229	206
$f_0 = 0.050$	361	252	306	199	163	230	186	160
$f_0 = 0.100$	251	181	220	138	109	153	113	89
<i>Multiplicative</i>								
p = 0.05								
$f_0 = 0.025$	1,754	1,246	535	369	323	146	129	122
$f_0 = 0.050$	1,650	1,229	518	370	335	147	134	130
$f_0 = 0.100$	1,450	1,199	484	375	364	151	146	150
p = 0.20								
$f_0 = 0.025$	650	474	296	203	174	118	100	91
$f_0 = 0.050$	596	452	278	195	171	115	98	91
$f_0 = 0.100$	496	412	242	181	168	107	95	91
p = 0.70								
$f_0 = 0.025$	877	620	690	460	387	422	360	327
$f_0 = 0.050$	737	528	607	403	335	376	313	277
$f_0 = 0.100$	494	364	445	292	238	282	222	186
<i>Additive</i>								
p = 0.05								
$f_0 = 0.025$	994	672	262	180	159	71	63	60
$f_0 = 0.050$	883	667	252	181	165	72	65	63
$f_0 = 0.100$	767	661	232	184	184	73	70	73
p = 0.20								
$f_0 = 0.025$	507	363	252	170	144	115	96	87
$f_0 = 0.050$	460	342	233	160	139	109	91	83
$f_0 = 0.100$	371	303	196	142	129	96	82	77
p = 0.70								
$f_0 = 0.025$	1,288	917	1,049	697	583	623	527	476
$f_0 = 0.050$	1,072	771	918	609	505	560	464	410
$f_0 = 0.100$	700	512	662	434	351	425	335	281

Dominant: $f_2 = \gamma^2 f_0, f_1 = f_2$; recessive: $f_2 = \gamma^2 f_0, f_1 = f_0$; multiplicative: $f_2 = \gamma^2 f_0, f_1 = \gamma f_0$; additive: $f_2 = \gamma^2 f_0, f_1 = \frac{1}{2}(f_2 + f_0)$. Here, $\gamma = 2$.

to more modest reductions for recessive models (table 3). The calculations in table 3 for $\hat{\sigma}_0^2$ ignore the possible costs of obtaining auxiliary data to estimate $\hat{\sigma}_0^2$, however. Note the similarity in sample sizes required by the Teng-Risch statistic with full ascertainment conditioning and the statistic based on $\hat{\sigma}_S^2$.

Because one may wish to test for an association either based only on a sample of sibships or based on a sample of sibship and auxiliary data to estimate $\hat{\sigma}_0^2$, we provide more extensive sample size tables for the statistic (5) standardized by $\hat{\sigma}_S$ (table 4) and the statistic standardized by $\hat{\sigma}_0$ (table 5). Standardizing by $\hat{\sigma}_0$ requires smaller sample sizes than standardizing by $\hat{\sigma}_S$ when the frequency is in a low to moderate range. When the allele frequency is high, the procedure using the empirical variance, $\hat{\sigma}_S^2$, requires somewhat fewer sibships, as reflected in the results for $p = 0.70$. The results in tables 4 and 5 also highlight the difficulty of detecting a rare recessive trait even using the most powerful design. Larger sibships are more informative (tables 4, 5), but they are harder to obtain.

Sample sizes in tables 4 and 5 imply that the sibship structure $r = 2$ and $s = 3$ is more informative than the sibship structure $r = 3$ and $s = 2$ when the allele frequency is high $p = 0.70$. This pattern also holds for the sibship structures $r = 1, s = 2$ and $r = 2, s = 1$. These results suggest that when the allele frequency (p) is high, unaffected individuals are rare and hence more informative. The sample sizes needed by the Teng-Risch statistic with full ascertainment are very similar to those in table 4. Only when $p = 0.70$ were the sample sizes needed by the Teng-Risch statistic with full ascertainment noticeably less than the corresponding values in table 4 (details are available from the first author).

Simulations

The sample sizes given in tables 3–5 were based on large sample theory. To assess their applicability for realistic sample sizes, we conducted simulations for the discordant sib pair case, $r = s = 1$. First, the accuracy of the mean and variance of the various statistics given in formulas (6), (6a), (7), (7a), and (10) were confirmed and then the power of the tests was studied.

For each genetic model we generated a set of sibship genotypes, assuming Hardy-Weinberg equilibrium, for a sufficient number of sib pairs that it was virtually certain that the number of discordant pairs would be larger than the required number computed from equation (11) for the Teng-Risch statistic with full ascertainment. Given each

subject's genotype and the penetrance parameters, and assuming that the affection status of each subject is conditionally independent of all other subject's phenotypes, given his or her genotype, we generated a random affection status (phenotype) for each member of each sibship. We generated such phenotypes sequentially, sibship by sibship, until the required number of sibships with discordant phenotypes was obtained. From this sample of discordant pairs, we computed each of the three statistics with $\hat{\sigma}_{TR}$, $\hat{\sigma}_S$ and $\hat{\sigma}_0$ in equation (5) as well as estimates of parameters in table 6. The size (α), power ($1 - \beta$) (table 7) and average of the parameter estimates (table 6) were obtained from 100,000 simulated data sets for low penetrance ($f_0 = 0.001$), which requires a large sample of sibships to accumulate the required numbers of discordant phenotypes. For $f_0 = 0.10$, we generated 1,000,000 data sets. Different simulated data sets were obtained for each choice of f_0 and genetic model in tables 6 and 7. Thus estimated parameter values and powers in tables 6 and 7 are correlated across columns but independent from row to row. The results in each row of tables 6 and 7 are based on the same simulated data. As an example, consider the dominant model with $p = 0.05$, $f_0 = 0.10$ and $\gamma = 2$. According to formula (11), one needs 447 discordant sib pairs to achieve 80% power. For each simulated data set, we generated 4,500 sib pairs according to the joint sib pair probability. Then we determined the affection status of the individuals in successive sib pairs, stopping once there were 447 discordant pairs. This process was repeated 1,000,000 times.

In order to estimate $\hat{\sigma}_0^2$ from an auxiliary sample, we generated genotypes and phenotypes as described above for an unselected sample of 1,000 sib pairs and applied formula (14). An independent auxiliary sample was generated for each of the genetic models and choice of f_0 studied in tables 6 and 7.

Theoretical calculations of v_a , σ_a^2 and σ_{*TR}^2 and their partial ascertainment approximations v_{aTR} , σ_{aTR}^2 and σ_{*TR}^2 show excellent agreement for $f_0 = 0.001$, but differences are evident, especially between v_a and v_{aTR} , for $f_0 = 0.1$ (table 6). Simulations agree well with the exact formulas for full ascertainment (table 6). Simulations at the null hypothesis yielded average estimates of $\hat{\sigma}_0^2$ that agreed with theory to 5 decimal places for $\hat{\sigma}_{TR}^2$, $\hat{\sigma}_S^2$ and $\hat{\sigma}_0^2$ (results not shown).

The accuracy of the required sample sizes is of greater practical importance. From equation (11), we calculated the sample sizes needed for the Teng and Risch statistic under full ascertainment to achieve 80% power with a one-sided $\alpha = 5 \times 10^{-8}$ level test. For these sample sizes,

Table 6. Theoretical parameter values and average estimates from simulations

Penetrance	Full ascertainment			Partial ascertainment			Simulated		
	v_a	σ_a^2	σ_*^2	v_{aTR}	σ_{aTR}^2	σ_{*TR}^2	v_a	$\hat{\sigma}_a^2$	$\hat{\sigma}_*^2$
Dominant, $p = 0.05$									
$f_0 = 0.100$	0.03155	0.01055	0.01152	0.02618	0.01098	0.01167	0.03115	0.01055	0.01152
$f_0 = 0.001$	0.02623	0.01098	0.01166	0.02618	0.01098	0.01167	0.02620	0.01097	0.01166
Recessive, $p = 0.70$									
$f_0 = 0.100$	0.06274	0.02394	0.02787	0.04464	0.02147	0.02346	0.06274	0.02394	0.02787
$f_0 = 0.001$	0.04476	0.02149	0.02349	0.04464	0.02147	0.02346	0.04475	0.02148	0.02348
Multiplicative, $p = 0.20$									
$f_0 = 0.100$	0.03970	0.02383	0.02541	0.03333	0.24444	0.02555	0.03970	0.02384	0.02541
$f_0 = 0.001$	0.03339	0.02444	0.02555	0.03333	0.02444	0.02555	0.03340	0.02445	0.02556
Additive, $p = 0.20$									
$f_0 = 0.100$	0.04587	0.02368	0.02578	0.03750	0.02422	0.02563	0.04586	0.02368	0.02578
$f_0 = 0.001$	0.03757	0.02421	0.02563	0.03750	0.02422	0.02563	0.03759	0.02422	0.02563

Dominant: $f_2 = \gamma^2 f_0, f_1 = f_2$; recessive: $f_2 = \gamma^2 f_0, f_1 = f_0$; multiplicative: $f_2 = \gamma^2 f_0, f_1 = \gamma f_0$; additive: $f_2 = \gamma^2 f_0, f_1 = \frac{1}{2}(f_2 + f_0)$. Here, $\gamma = 2$.

Table 7. Simulated power (and expected power in parentheses) of the statistics standardized by $\hat{\sigma}_{TR}^2, \hat{\sigma}_S^2$ and $\hat{\sigma}_0^2$ ^a

Penetrances	n	Teng-Risch variance $\hat{\sigma}_{TR}^2$	Empirical variance $\hat{\sigma}_S^2$	Variance from auxiliary sample $\hat{\sigma}_0^2$
Dominant, $p = 0.05$				
$f_0 = 0.100$	447	0.8485 (0.8003)	0.8870 (0.8605)	0.9919 (0.9921)
$f_0 = 0.001$	641	0.8279 (0.8005)	0.8594 (0.8432)	0.9918 (0.9922)
Recessive, $p = 0.70$				
$f_0 = 0.100$	264	0.8370 (0.8022)	0.9010 (0.8981)	0.8442 (0.8460)
$f_0 = 0.001$	442	0.8281 (0.8016)	0.8675 (0.8622)	0.7002 (0.7016)
Multiplicative, $p = 0.20$				
$f_0 = 0.100$	609	0.8180 (0.8004)	0.8497 (0.8453)	0.9271 (0.9284)
$f_0 = 0.001$	868	0.8148 (0.8001)	0.8362 (0.8320)	0.9277 (0.9293)
Additive, $p = 0.20$				
$f_0 = 0.100$	462	0.8233 (0.8012)	0.8629 (0.8595)	0.9324 (0.9344)
$f_0 = 0.001$	827	0.8150 (0.8009)	0.8437 (0.8408)	0.9304 (0.9311)

Dominant: $f_2 = \gamma^2 f_0, f_1 = f_2$; recessive: $f_2 = \gamma^2 f_0, f_1 = f_0$; multiplicative: $f_2 = \gamma^2 f_0, f_1 = \gamma f_0$; additive: $f_2 = \gamma^2 f_0, f_1 = \frac{1}{2}(f_2 + f_0)$. Here, $\gamma = 2$.

^a The statistics are given by equation (5) with three different choices for $\hat{\sigma}^2$.

the expected power for tests based on $\hat{\sigma}_S$ and $\hat{\sigma}_0$ in equation (5) were calculated by solving equation (12) and (15) for β . Unreported simulations under the null hypothesis $f_2 = f_1 = f_0$ confirm that all three tests have nominal size. Under various alternatives shown in table 7, the simulated values agree to two decimal places with expected

power for the statistic standardized by $\hat{\sigma}_0$. The simulated power exceeds the expected power very slightly for the statistics standardized by $\hat{\sigma}_S$ and $\hat{\sigma}_{TR}$. The most extreme discrepancy occurred for the statistic standardized by $\hat{\sigma}_{TR}$ with $f_0 = 0.10$ in a dominant model; in this case the simulated power was 0.8485 compared to an expected power

of 0.8003. These slight excesses, though demonstrable in such large simulations, would not seriously compromise the study design. Potential reasons for these slight discrepancies may be due to: (1) The sample sizes may not be adequate for the normal approximation to hold, especially as the variance is estimated. In unreported simulations with larger numbers of sibships these discrepancies were smaller. (2) The estimator of the variance may be correlated with the score in the numerator. Our experience in a similar situation has been that the rate of convergence to normality is slower.

These simulations indicated that the statistic based on $\hat{\sigma}_0^2$ is more powerful than that based $\hat{\sigma}_S^2$ except for recessive models, in agreement with tables 4 and 5. Both these statistics tend to be more powerful than the one based on $\hat{\sigma}_{TR}^2$.

Discussion

A critical assumption underlying the original investigation of the properties of the TDT-like statistic proposed by Teng and Risch [1999] is that unaffected sibs are like a random sample of the general population. Those authors asserted that this assumption should 'nearly' hold when the locus-specific penetrances are low. By incorporating the status of the unaffected sibs in calculating the distribution of the Teng-Risch statistic, calculated from equation (5) with $\hat{\sigma}_{TR}^2$, we are able to assess when the key assumption holds. The numerical results and simulations based on full ascertainment indicate that their partial ascertainment conditioning is a reasonable approximation when the penetrances are low. Depending on the genetic model and other parameters, the partial ascertainment approximation can either be conservative or anticonservative.

We present sample sizes (tables 4, 5) for designing studies in which sibships of fixed size are sampled conditional on the number of affected sibs. Because the family sizes are fixed, such sampling is conditioned on both the number of affected and unaffected sibs ('full ascertainment'). Table 4 is useful when one plans to test for an association using data only from these sampled sibships and based on the empirical variance estimator $\hat{\sigma}_S^2$. Table 5 is useful when one has access to data from an auxiliary random sample for estimating σ_0^2 . Risch and Teng [1998] concluded that using unrelated controls is more efficient than family controls. Our calculations support this conclusion for studies of diseases with low penetrance.

The procedure based on an auxiliary sample has the advantage that it requires a smaller sample of the sibships

with affected members (tables 3, 5). As such sibships may be difficult to accrue, the cost saving may be appreciable. Unless population-based data are available for estimating σ_0^2 , however, there will also be a cost to obtaining an auxiliary sample of randomly selected individuals, sibships or parent pairs to estimate σ_0^2 . Moreover, one must evaluate whether the general population sample used to estimate σ_0^2 is representative of the source population for the sampled sibships with affected members. General population surveys, such as in Steinberg et al. [2001], may have sufficient coverage, when data for several years are used, to estimate the allele and genotype frequencies for the source population. While the auxiliary sample approach does not require Hardy-Weinberg equilibrium when parental mating types are estimated from a survey, if the gene affects the age of onset of a fatal disease, then the genotype frequencies in the offspring may differ slightly from their Mendelian expectation based on the parental genotypes.

The approach discussed here compared the candidate allele frequency in affected sibs to the estimated frequency in their parents. Genotype data on unaffected as well as affected sibs are needed for this analysis. Witte et al. [1999] and other authors cited in the introduction proposed alternative analyses for such data.

In practice, one may have sibships of several (K) different structures, say (r_u, s_u) , $u = 1, \dots, K$. One then has a statistic $T_u = \sum_{i=1}^{n_u} S_i(\hat{p}_1 - \hat{p}_2)$ with estimated variance $n_u \hat{\sigma}_u^2$ for each type (u). A common approach uses

$$T_{comb} = \frac{\sum_{u=1}^K T_u}{\sqrt{\sum_{u=1}^K n_u \hat{\sigma}_u^2}},$$

although a weighted sum of the T_u 's can be more efficient against a particular alternative.

A reviewer raised the question of what null hypothesis is being tested with the Teng-Risch type of statistic. Following Teng and Risch [1999], we derived our distribution theory under the assumption that phenotypes of siblings are conditionally independent given their respective genotypes. Thus, the strong null hypothesis is $f_0 = f_1 = f_2$ and conditional independence. However, the same null distribution holds under the 'weak null hypothesis' that parental mating types are independent of sibling phenotypes. The strong null hypothesis implies the weak null hypothesis but not vice versa. In certain applications, the weak null hypothesis might be satisfied but not the strong one. For example, if one is genotyping a marker that is not linked to the disease gene, the weak null hypothesis should be satisfied because the disease is independent of the candidate gene, but the conditional independence condition might not be satisfied because the disease gene

might be circulating in the family. Similarly, if an unmeasured environmental factor ('random effect') independent of marker genotype influences phenotype, the weak null hypothesis, not the strong one, is likely to hold. In order to calculate power in this article, however, we have relied on the conditional independence assumption, as would be appropriate for the study of a candidate disease gene.

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Appendix A: Derivations of $m_{ij}^{(r,s)}$

We give the derivation for $m_{21}^{(r,s)}$. The derivations for all other $m_{ij}^{(r,s)}$ follow similar logic and are available from the first author. Using conditional independence, we have

$$\begin{aligned}
 m_{21}^{(r,s)} &= P\{G = (2,1) | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= \frac{P\{C_r = \text{Aff}, C_s = \text{Unaff} | G = (2,1)\} g_{21}}{P\{C_r = \text{Aff}, C_s = \text{Unaff}\}} \\
 &= \frac{\binom{r+s}{r} [P\{C_1 = \text{Aff} | G = (2,1)\}]^r [P\{C_1 = \text{Unaff} | G = (2,1)\}]^s}{K_{r,s}} \\
 &= \frac{\binom{r+s}{r}}{K_{r,s}} [P\{C_1 = \text{Aff} | C_1 = AA\} P\{C_1 = AA | G = (2,1)\} \\
 &\quad + P\{C_1 = \text{Aff} | C_1 = Aa\} P\{C_1 = Aa | G = (2,1)\}]^r \\
 &\quad \times [P\{C_1 = \text{Unaff} | C_1 = AA\} P\{C_1 = AA | G = (2,1)\} \\
 &\quad + P\{C_1 = \text{Unaff} | C_1 = Aa\} P\{C_1 = Aa | G = (2,1)\}]^s \\
 &= \frac{\binom{r+s}{r}}{K_{r,s}} \left(\frac{1}{2}\right)^r (f_2 + f_1)^r [(1 - f_2) + (1 - f_1)]^s, \quad (16)
 \end{aligned}$$

where $K_{r,s} = P\{C_r = \text{Aff}, C_s = \text{Unaff}\}$. Here, ' $C_1 = \text{Aff}$ ' is the event that one offspring is affected, and ' $C_1 = AA$ ' is the event that the genotype of the offspring is AA. It can be verified that the conditional mating type probabilities ($m_{ij}^{(r,s)}$) are the unconditional mating type probabilities (g_{ij}) under the null hypothesis ($f_2 = f_1 = f_0$). The nine conditional mating type probabilities are given in table 1.

Appendix B: Derivations of the Conditional Genotype Probabilities

We give the derivation of the conditional genotype probability for group IV. The derivations for all other groups (see table 2) are similar and are available from the first author. The conditional genotype probability of group IV can be decomposed as

$$\begin{aligned}
 &P\{j_2, j_1, 0, k_2, k_1, 0 | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= P\{j_2, j_1, 0, k_2, k_1, 0, G = (2, 1) \text{ or } (1, 2) | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &\quad + P\{j_2, j_1, 0, k_2, k_1, 0, G = (1, 1) | C_r = \text{Aff}, C_s = \text{Unaff}\}.
 \end{aligned}$$

We have

$$\begin{aligned}
 &P\{j_2, j_1, 0, k_2, k_1, 0, G = (2, 1) \text{ or } (1, 2) | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= m_{(21)}^{(r,s)} P\{j_2, j_1, 0, k_2, k_1, 0 | G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= m_{(21)}^{(r,s)} \frac{P\{j_2, j_1, 0, k_2, k_1, 0, G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\}}{P\{G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\}}
 \end{aligned}$$

$$\begin{aligned}
 &= \frac{m_{(21)}^{(r,s)} \binom{r+s}{r}}{P\{G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\}} \\
 &\quad \times P\{C_r = \text{Aff}, C_s = \text{Unaff} | j_2, j_1, 0, k_2, k_1, 0\} \\
 &\quad \times P\{j_2, j_1, 0, k_2, k_1, 0 | G = (2, 1)\} p\{G = (2, 1)\} \\
 &= \frac{m_{(21)}^{(r,s)} \binom{r+s}{r} f_2^j f_1^j (1 - f_2)^{k_2} (1 - f_1)^{k_1} \binom{r}{j_2} \binom{s}{k_2} \left(\frac{1}{2}\right)^r g_{21}}{P\{G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\}}.
 \end{aligned}$$

Using the conditional independence, we could show

$$\begin{aligned}
 &P\{G = (2, 1), C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= \binom{r+s}{r} \left(\frac{1}{2}\right)^r (f_2 + f_1)^r [(1 - f_2) + (1 - f_1)]^s.
 \end{aligned}$$

Hence

$$\begin{aligned}
 &P\{j_2, j_1, 0, k_2, k_1, 0, G = (2, 1) \text{ or } (1, 2) | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= m_{(21)}^{(r,s)} \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1}. \quad (17)
 \end{aligned}$$

Similarly,

$$\begin{aligned}
 &P\{j_2, j_1, 0, k_2, k_1, 0, G = (1, 1) | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= m_{(11)}^{(r,s)} \binom{r}{j_2} \binom{s}{k_2} c_{12}^{j_2} c_{11}^{j_1} d_{12}^{k_2} d_{11}^{k_1}. \quad (18)
 \end{aligned}$$

Adding (17) and (18) together, we obtain

$$\begin{aligned}
 &P\{j_2, j_1, 0, k_2, k_1, 0 | C_r = \text{Aff}, C_s = \text{Unaff}\} \\
 &= \binom{r}{j_2} \binom{s}{k_2} [m_{(21)}^{(r,s)} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} + m_{(11)}^{(r,s)} c_{12}^{j_2} c_{11}^{j_1} d_{12}^{k_2} d_{11}^{k_1}]. \quad (19)
 \end{aligned}$$

Appendix C: Derivations of Expectation v_a

Let $P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\}$ denote the general term of the conditional genotype probability for group IV. Similar notation will be used for other groups as well. Then,

$$\begin{aligned} v_a &= E_{H_1}(S(\hat{p}_1 - \hat{p}_2)) \\ &= \sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &+ \sum_V \left(\frac{j_1 - j_0}{4r} \right) P\{V|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &+ \sum_{VI} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\}, \end{aligned}$$

where the summation \sum_{IV} is over all possible configurations within group IV.

First, we compute the summation for group IV:

$$\begin{aligned} &\sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &= \sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) \left[\binom{r}{j_2} \binom{s}{k_2} m_{(21)}^{(r,s)} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \right. \\ &\quad \left. + \binom{r}{j_2} \binom{s}{k_2} m_{(11)}^{(r,s)} c_{12}^{j_2} c_{11}^{j_1} d_{12}^{k_2} d_{11}^{k_1} \right]. \end{aligned}$$

Because the six groups are mutually exclusive, we have the following constraints for group IV: $j_2 + k_2 \geq 1$, $j_1 + k_1 \geq 1$, $j_2 + j_1 = r$, and $k_2 + k_1 = s$. Hence, $j_2 - j_1 = 2j_2 - r$, and

$$\begin{aligned} &\sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) \binom{r}{j_2} \binom{s}{k_2} m_{(21)}^{(r,s)} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \\ &= \frac{m_{(21)}^{(r,s)}}{4r} \left[\sum_{k_2=0}^s \sum_{j_2 \leq r+s-1-k_2}^{j_2 \geq 1-k_2} 2j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \right. \\ &\quad \left. - \sum_{k_2=0}^s \sum_{j_2 \leq r+s-1-k_2}^{j_2 \geq 1-k_2} r \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \right]. \end{aligned}$$

Notice that

$$\begin{aligned} &2 \sum_{k_2=0}^s \sum_{j_2 \leq r+s-1-k_2}^{j_2 \geq 1-k_2} j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \\ &= 2 \left[I_{\{k_2=0\}} \sum_{j_2=1}^r j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{r-j_2} d_{21}^{k_2} d_{20}^{s-k_2} \right. \\ &\quad \left. + \sum_{k_2=1}^{s-1} \sum_{j_2=0}^r j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{r-j_2} d_{21}^{k_2} d_{20}^{s-k_2} \right. \\ &\quad \left. + I_{\{k_2=s\}} \sum_{j_2=1}^{r-1} j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{r-j_2} d_{21}^{k_2} d_{20}^{s-k_2} \right] \\ &= 2 \left[\sum_{k_2=0}^s \sum_{j_2=0}^r j_2 \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{r-j_2} d_{21}^{k_2} d_{20}^{s-k_2} - rc_{21}^r d_{21}^s \right] \\ &= 2 [rc_{21} - rc_{21}^r d_{21}^s] \end{aligned} \tag{20}$$

where $I_{\{k_2=0\}}$ is an indicator function. Similarly,

$$\begin{aligned} &\sum_{k_2=0}^s \sum_{j_2 \leq r+s-1-k_2}^{j_2 \geq 1-k_2} r \binom{r}{j_2} \binom{s}{k_2} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \\ &= r - rc_{20}^r d_{20}^s - rc_{21}^r d_{21}^s. \end{aligned} \tag{21}$$

Adding (20) and (21), we have

$$\begin{aligned} &\sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) \binom{r}{j_2} \binom{s}{k_2} m_{(21)}^{(r,s)} c_{21}^{j_2} c_{20}^{j_1} d_{21}^{k_2} d_{20}^{k_1} \\ &= \frac{m_{(21)}^{(r,s)}}{4} [c_{21} - c_{20} + c_{20}^r d_{20}^s - c_{21}^r d_{21}^s]. \end{aligned} \tag{22}$$

Repeating the above algebraic procedures with the notice that $c_{12} + c_{11} + c_{10} = 1$, we obtain

$$\begin{aligned} &\sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) \binom{r}{j_2} \binom{s}{k_2} m_{(11)}^{(r,s)} c_{12}^{j_2} c_{11}^{j_1} d_{12}^{k_2} d_{11}^{k_1} \\ &= \frac{m_{(11)}^{(r,s)}}{4} [(c_{12} - c_{11})(c_{12} + c_{11})^{r-1} (d_{12} + d_{11})^s - c_{12}^r d_{12}^s + c_{11}^r d_{11}^s]. \end{aligned} \tag{23}$$

Thus, we have

$$\begin{aligned} &\sum_{IV} \left(\frac{j_2 - j_1}{4r} \right) P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &= \frac{m_{(21)}^{(r,s)}}{4} [c_{21} - c_{20} + c_{20}^r d_{20}^s - c_{21}^r d_{21}^s] \\ &+ \frac{m_{(11)}^{(r,s)}}{4} [(c_{12} - c_{11})(c_{12} + c_{11})^{r-1} (d_{12} + d_{11})^s - c_{12}^r d_{12}^s + c_{11}^r d_{11}^s]. \end{aligned} \tag{24}$$

Similar computations for group V give

$$\begin{aligned} &\sum_V \left(\frac{j_1 - j_0}{4r} \right) P\{V|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &= \frac{m_{(10)}^{(r,s)}}{4} [c_{01} - c_{00} + c_{00}^r d_{00}^s - c_{01}^r d_{01}^s] \\ &+ \frac{m_{(11)}^{(r,s)}}{4} [(c_{11} - c_{10})(c_{11} + c_{10})^{r-1} (d_{10} + d_{11})^s - c_{11}^r d_{11}^s + c_{10}^r d_{10}^s]. \end{aligned} \tag{25}$$

For group VI, we have

$$\begin{aligned} &\sum_{VI} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &= \sum_{\text{Overall}} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &- \sum_I \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &- \sum_{II} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &- \sum_{III} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\ &- \sum_{IV} \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \end{aligned}$$

$$\begin{aligned}
& - \sum_V \left(\frac{j_2 - j_0}{2r} \right) P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& = \frac{m_{11}^{(r,s)}}{2} [(c_{12} - c_{10}) - (c_{12}^r d_{12}^s - c_{10}^r d_{10}^s) - c_{12} (c_{12} + c_{11})^{r-1} (d_{12} + d_{11})^s \\
& \quad + c_{10} (c_{10} + c_{11})^{r-1} (d_{10} + d_{11})^s - c_{10}^r d_{10}^s + c_{12}^r d_{12}^s], \quad (26)
\end{aligned}$$

where the first summation on the right hand side of (26) is over all possible configurations of $(j_2, j_1, j_0, k_2, k_1, k_0)$.

Adding (24), (25), and (26) together, we obtain v_a .

Appendix D: Derivations of Variance σ_a^2

The algebraic steps are very similar to the computation of the expectation in Appendix C.

$$\begin{aligned}
E_{H_i}(S(\hat{\beta}_1 - \beta_2))^2 & = \sum_{IV} \left(\frac{j_2 - j_1}{4r} \right)^2 P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& \quad + \sum_V \left(\frac{j_1 - j_0}{4r} \right)^2 P\{V|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& \quad + \sum_{VI} \left(\frac{j_2 - j_0}{2r} \right)^2 P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\}.
\end{aligned}$$

The three terms in the right hand side of the above formula are: for group IV

$$\begin{aligned}
& \sum_{IV} \left(\frac{j_2 - j_1}{4r} \right)^2 P\{IV|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& = \frac{m_{(21)}^{(r,s)}}{16r} [r - 4(r-1)c_{21}c_{20} - r(c_{20}^r d_{20}^s + c_{21}^r d_{21}^s)]
\end{aligned}$$

$$\begin{aligned}
& + \frac{m_{11}^{(r,s)}}{16r} [4c_{12}c_{11}(c_{12} + c_{11})^{r-2}(d_{12} + d_{11})^s \\
& \quad + 4rc_{12}^2(c_{12} + c_{11})^{r-2}(d_{12} + d_{11})^s \\
& \quad - 4rc_{12}(c_{12} + c_{11})^{r-1}(d_{12} + d_{11})^s \\
& \quad + r(c_{12} + c_{11})^r(d_{12} + d_{11})^s - rc_{11}^r d_{11}^s - rc_{12}^r d_{12}^s], \quad (27)
\end{aligned}$$

for group V,

$$\begin{aligned}
& \sum_V \left(\frac{j_1 - j_0}{4r} \right)^2 P\{V|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& = \frac{m_{(10)}^{(r,s)}}{16r} [r - 4(r-1)c_{01}c_{00} - r(c_{00}^r d_{00}^s + c_{01}^r d_{01}^s)] \\
& \quad + \frac{m_{11}^{(r,s)}}{16r} [4c_{10}c_{11}(c_{10} + c_{11})^{r-2}(d_{10} + d_{11})^s \\
& \quad + 4rc_{10}^2(c_{10} + c_{11})^{r-2}(d_{10} + d_{11})^s \\
& \quad - 4rc_{10}(c_{10} + c_{11})^{r-1}(d_{10} + d_{11})^s \\
& \quad + r(c_{10} + c_{11})^r(d_{10} + d_{11})^s - rc_{11}^r d_{11}^s - rc_{10}^r d_{10}^s], \quad (28)
\end{aligned}$$

and for group VI,

$$\begin{aligned}
& \sum_{VI} \left(\frac{j_2 - j_0}{2r} \right)^2 P\{VI|C_r = \text{Aff}, C_s = \text{Unaff}\} \\
& = \frac{m_{11}^{(r,s)}}{4r} [c_{12}(c_{11} + c_{10}) + rc_{12}^2 + 2c_{12}c_{10} - 2rc_{12}c_{10} + c_{10}(c_{12} + c_{11}) \\
& \quad + rc_{10}^2 - c_{12}c_{11}(c_{12} + c_{11})^{r-2}(d_{12} + d_{11})^s \\
& \quad - rc_{12}^2(c_{12} + c_{11})^{r-2}(d_{12} + d_{11})^s - c_{10}c_{11}(c_{10} + c_{11})^{r-2}(d_{10} + d_{11})^s \\
& \quad - rc_{10}^2(c_{10} + c_{11})^{r-2}(d_{10} + d_{11})^s]. \quad (29)
\end{aligned}$$

Adding (27), (28), and (29) together, we obtain $E^{H_i} S(\hat{\beta}_1 - \beta_2)^2$.

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