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Extreme Discordant Sib Pairs for Mapping Quantitative Trait Loci in Humans

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Analysis of differences between siblings (sib pair analysis) is a standard method of genetic linkage analysis for mapping quantitative trait loci, such as those contributing to hypertension and obesity, in humans. In traditional designs, pairs are selected at random or with one sib having an extreme trait value. The majority of such pairs provide little power to detect linkage; only pairs that are concordant for high values, low values, or extremely discordant pairs (for example, one in the top 10 percent and the other in the bottom 10 percent of the distribution) provide substantial power. Focus on discordant pairs can reduce the amount of genotyping necessary over conventional designs by 10- to 40 -fold.

The power of modern molecular methods for identifying Mendelian disease genes, such as those for cystic fibrosis, Huntington disease, and neurofibromatosis, has been amply demonstrated. The feasibility of these methods for identification of susceptibility genes for non-Mendelian disorders (such as diabetes, multiple sclerosis, and hypertension) remains to be seen. A major problem in searching for such loci is the lack of the simple one-to-one correspondence between gene effect (genotype) and disease outcome (phenotype) that is typical for the Mendelian case. Multiple loci may contribute to susceptibility, with complicated interaction effects among loci. For example, in the non-obese diabetic (NOD) mouse model of human insulin-dependent diabetes (IDDM), evidence for at least 10 susceptibility loci was obtained (*1*), and it appears from human studies that IDDM may be equally complex (*2*).

An important class of traits for study in human genetics are quantitative ones, in which the phenotype is measured on a continuous scale. These may either directly underlie disease classification (such as blood pressure and the associated disease, hypertension; or weight and obesity) or may be considered as a risk factor for a disease state (such as cholesterol and ischemic heart disease). One approach is to identify quantitative trait loci (QTL's) in an appropriate animal model system, and then search for similar associations in humans (*3*).

A problem heretofore in studying the genetics of quantitative traits in humans is the low power of linkage analysis to detect loci contributing to the trait. One commonly employed approach is the robust sib pair design first described by Haseman and Elston (*4*). In this method, the difference in trait values (such as height, weight, or blood pressure) for a pair of sibs is squared (D^2) and examined as a function of the number of alleles that the pair have derived from a common parent [identical by descent (ibd)] at a tested marker locus. When a locus contributing to the variation of the quantitative trait lies near the tested marker locus (in other words, there is linkage between the two loci), there will be a negative regression of D^2 on the number of alleles shared ibd; for sibs sharing two alleles ibd, D^2 will be small, while for sibs sharing no alleles ibd, D^2 will be large. This approach has also been extended to pedigree relationships other than sibs (*5*). However, Blackwelder and Elston (*6*) showed that the proportion of the total variance (heritability) in a trait attributable to a contributing locus would need to be large (~50%) to detect linkage in a reasonably-sized sample by sib pair analysis when the sibs are sampled at random (irrespective of their trait values). For example, 2953 pairs would be needed to detect linkage with 90% power for a locus that is responsible for 30% of the variation (30% heritability) (*6*). Extensions of the sib pair approach to allow for multipoint analysis with flanking marker loci have increased the power of this method (*7*). However, even with multipoint analysis, thousands of sib pairs are required to detect linkage to a locus that has a heritability of 25% (*8*).

The fact that power to detect linkage can be increased by using selected versus random samples has recently been noted (*9–10*). This approach is also based on sib pair analysis, but in this case one of the sibs is ascertained to have an extreme value (say, within the top 5 or 10% of the distribution); the second sib is selected at random. Again, regression is the statistical method employed. In this case, however, the value of

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the second (unselected) sib is regressed on the number of alleles shared ibd with the selected sib. In the presence of linkage, the mean value of the unselected sib regresses toward the population mean with decreasing ibd with the proband. Carey and Williamson (9) showed that sample sizes could be reduced dramatically to achieve the same power by ascertaining sib pairs through a proband as opposed to random pairs. This approach was further generalized to the multipoint setting by Cardon and Fulker (*11*), and used to suggest a QTL for reading disability (*12*).

In all these methods, the outcome (dependent variable) is the quantitative trait value for an unselected sib or the squared difference for a sib pair (D^2), while the predictor (independent) variable is the number of alleles shared ibd at the marker locus or loci. However, it is more natural to view number of alleles ibd at the marker locus as the outcome (dependent variable) and the sib trait values as independent (predictor) variables. This is particularly important because sib pairs can be chosen for analysis based on their trait values but not based on their marker information. Thus, to maximize the power to detect linkage with a QTL, it is key to ascertain sib pairs in an optimal way through their trait values and only use those pairs likely to be most informative.

Estimating Expected IBD by Decile

We consider a trait composed of a single gene effect with residual variation that has both genetic (or shared environmental) and unique (unshared environmental) components.

Let x_{1i} and x_{2i} be the observed trait values for the first and second sibs, respectively, in the ith sib pair. We assume the general model (*4*)

$$x_{1i} = \mu + g_{1i} + e_{1i},$$

$$x_{2i} = \mu + g_{2i} + e_{2i},$$

where μ is the overall mean and g_{ji} and e_{ji} are the genetic and environmental effects, respectively. We assume that one locus, A, determines g_{ji} and that two alleles, A_1 and A_2, are involved in this locus with gene (population) frequencies p and q, respectively. Then, following Falconer (*13*), let

$$g_{ji} = a \text{ for an } A_1A_1 \text{ individual}$$

$$= d \text{ for an } A_1A_2 \text{ individual}$$

$$= -a \text{ for an } A_2A_2 \text{ individual}$$

In general, it is not necessary to assume a normal distribution for e_{ji}; however, for simplicity, our numerical computation is done for the normal distribution case. Without loss of generality, we assume that e_{1i} and e_{2i} have variance σ_e^2 of 1 and correlation ρ. The residual correlation reflects the possibility of other genetic or shared environmental determinants. The terms σ_a^2 and σ_d^2 are the additive and dominant components of the genetic variance σ_g^2 of locus A, namely,

$$\sigma_a^2 = 2pq[a - d(p - q)]^2 \text{ and } \sigma_d^2 = (2pqd)^2$$

The heritability due to this locus is $H = \sigma_g^2/(\sigma_g^2 + 1)$.

First, we break the trait value into 10 consecutive intervals (deciles) $I_1, \ldots, I_{10}$ within each of which the probability of a random individual falling is 0.1. The following methods apply to any number of divisions, however. Ignoring order, there are six combinations of genotypes for sib pairs. In Table 1 we give the conditional probability for each combination given ibd (columns 2 to 4) and also define the conditional probability of one sib's phenotype falling in the hth decile and the other's in the lth decile (columns 5 to 6), assuming no residual correlation ($\rho = 0$).

Here, f_{jh} is the probability that an individual's phenotype falls in the hth decile given he(she) is genotype j, where j corresponds to the number of A_1 alleles in the genotype (j = 2 for A_1A_1, 1 for A_1A_2 and 0 for A_2A_2). Denote by $O(h,l)$ the outcome event that one sib's phenotype falls in the hth decile and the other sib's in the lth decile. Let G_k represent the pair of genotypes as enumerated in Table 2, where $k = 1, \ldots, 6$. Then, let $D_i = P(\pi = i \text{ and } O(h,l))$, or

$$D_i = P(\pi = i) \sum_{k=1}^{6} P(G_k \mid \pi = i)\, P(O(h,l) \mid G_k) \quad (1)$$

and

$$D = \sum_{i=0}^{2} D_i$$

where $P(G_k \mid \pi = i)$ is given in columns 2 through 4 of Table 1. $P(O(h,l)|G_k)$ is given in columns 5 and 6, and $P(\pi = 2) = P(\pi = 0) = 1/4$ and $P(\pi = 1) = 1/2$. Then

$$P(\pi = i \mid O(h,l)) = D_i/D \quad (2)$$

The above conditional probability of π given $O(h,l)$ can also be calculated for the situation of a positive residual correlation between sibs ($\rho > 0$) in a similar fashion. For this case, we need to employ a bivariate normal distribution as follows. Let $\phi(x; \mu)$ represent a normal density function with mean μ and variance 1; let $\phi(x, y; \mu_1, \mu_2, \rho)$ correspond to a bivariate normal density function for random variables X and Y, where μ_1 is the mean of X and μ_2 is the mean of Y, each variable has variance 1, and ρ is the correlation between X and Y. Then

$$P(O(h, l) \mid G_k) = \int_{u_1}^{u_2} \int_{t_1}^{t_2} \phi(x, y; \mu_1, \mu_2, p)\, dxdy \quad (3)$$

where

$u_1 = F^{-1}(h/10 \text{ and } u_2 = F^{-1}((h + 1)/10)$ demark the hth decile,

$t_1 = F^{-1}(l/10 \text{ and } t_2 = F^{-1}((l + 1)/10)$ demark the lth decile,

$\mu_1 = a, d, \text{ or } -a$ as the first sib's genotype is A_1A_1, A_1A_2, or A_2A_2

μ_2 is defined similarly according to the second sib's genotype, and

$$F(x) = \int_{-\infty}^{x} [p^2\phi(w; a) + 2pq\phi(w; d) + q^2\phi(w; -a)]dw$$

is the cumulative distribution function for the population distribution of the trait. The conditional probability $P(\pi = i|O(h,l))$ can then be calculated using formula 2, but replacing formula 3 for $P(O(h,l)|G_k)$ in formula 1.

Power Calculations

By means of the above formulas, we can calculate the expected proportion of alleles shared ibd for each combination of deciles for a sib pair, its deviation from the null value of 1/2 and hence the power to detect linkage. Assume a sample of n fully infor-

Table 1. Six sib-pair genotype combinations with associated probabilities of trait outcomes when $\rho = 0$.

Genotypes	Probability of genotypes given			Probability of trait outcomes given	
	$\pi = 2$	$\pi = 1$	$\pi = 0$	$h = l$	$h \neq l$
A_1A_1,A_1A_1	p^2	p^3	p^4	f_{2h}^2	$2f_{2h}f_{2l}$
A_1A_1,A_1A_2	0	$2p^2q$	$4p^3q$	$f_{2h}f_{1h}$	$f_{2h}f_{1l} + f_{2l}f_{1h}$
A_1A_1,A_2A_2	0	0	$2p^2q^2$	$f_{2h}f_{0h}$	$f_{2h}f_{0l} + f_{2l}f_{0h}$
A_1A_2,A_1A_2	$2pq$	pq	$4p^2q^2$	f_{1h}^2	$2f_{1h}f_{1l}$
A_1A_2,A_2A_2	0	$2pq^2$	$4pq^3$	$f_{0h}f_{1h}$	$f_{0h}f_{1l} + f_{0l}f_{1h}$
A_2A_2,A_2A_2	q^2	q^3	q^4	f_{0h}^2	$2f_{0h}f_{0l}$

mative sib pairs (genotypes of both parents are known, marker heterozygosity of 1). For the ith sib pair let X_{1i} be a random variable representing the number of alleles (1 or 0) shared ibd from the father; define X_{2i} similarly for the mother. Let $Z_2 = P(X_{1i} = 1, X_{2i} = 1)$, $Z_1 = P(X_{1i} = 1, X_{2i} = 0) + P(X_{1i} = 0, X_{2i} = 1)$, $Z_0 = P(X_{1i} = 0, X_{2i} = 0)$, and $\tau = Z_2 + \frac{1}{2}Z_1$. Define $\bar{X} = \frac{1}{2n}\sum_{i=1}^{n}(X_{1i} + X_{2i})$. Then $\bar{X}$ is approximately a normal random variable with mean τ and variance $(\tau(1 - 2\tau) + Z_2)/(2n)$.

The null hypothesis is H_o: $\tau = 1/2$. We are generally interested in a one-sided alternative (either $\tau > 1/2$ or $\tau < 1/2$), so we employ the power for a one-sided test of a normal random variable, namely

$$\Phi\left(\frac{Z_\alpha/2 + |\tau - 1/2|\sqrt{2n}}{\sqrt{\tau(1 - 2\tau) + Z_2}}\right)$$

where Φ is the cumulative standard normal distribution function, and Z_α is the normal deviate corresponding to an α type 1 error probability (in other words, the significance level).

Hence, the required number of sib pairs to obtain a power of $1 - \beta$ (the probability of rejecting the null hypothesis) is

$$\frac{1}{2}\left(\frac{Z_{1-\beta}\sqrt{\tau(1 - 2\tau) + Z_2} - Z_\alpha/2}{\tau - 1/2}\right)^2 \quad (4)$$

We also compare the required sample sizes for our selected sib-pair approach to that of a random sib pair design analyzed by Haseman-Elston statistics. In that approach, the linear regression model $E(D_j^2|\pi) = \beta_0 + \beta_1\pi$ is applied, where D_j^2 is the squared difference of trait values for the jth sib pair, $\beta_0 = \sigma_e^2 + 2\sigma_g^2$ and $\beta_1 = -(2\sigma_g^2 + 2n_1(n_2 - n_0)\sigma_d^2)/(4n_0n_2 + n_0n_1 + n_1n_2)$. Here, n_i is the number of sib pairs sharing i genes ibd ($i = 0$, 1 or 2). From reference (4), the estimate $\hat{\beta}$ for β_1 is

$$\hat{\beta}_1 + \frac{(n_0 + n_1/2)\sum_{j=1}^{n_2} D_{2j}^2 + (n_0 - n_2)\sum_{j=1}^{n_1} \frac{1}{2}D_{1j}^2 - (n_2 + n_1/2)\sum_{j=1}^{n_0} D_{0j}^2}{n_0n_2 + \frac{1}{4}n_1n_0 + \frac{1}{4}n_1n_2} \quad (5)$$

where the D_{ij}^2 correspond to the squared sib-pair differences for those pairs sharing i genes ibd. For a large number of sib pairs, we expect that $n_2 = n_0 = n/4$ and $n_1 = n/2$. Under this assumption, formula (5) simplifies to

$$\hat{\beta}_1 = \frac{4}{n}\sum_{j=1}^{n/4}(D_{2j}^2 - D_{0j}^2), \quad (6)$$

and is approximately normally distributed with mean $-2\sigma_g^2$, and variance $(4\xi + 8\sigma_e^2(1 - \rho))$, where ρ is the residual correlation between sibs, and ξ is the variance of D_{0j}^2, which is $2\sigma_e^4 + 8\sigma_e^2\sigma_g^2 - 4\sigma_g^2 + 4pq[p^2(a - d)^4 + q^2(a + d)^4 + 8a^4pq]$. Under the null hypothesis ($\sigma_g^2 = 0$), $\hat{\beta}_1$, is distributed with mean 0 and variance $16\sigma_e^2(1 - \rho)/n$. The rejection region for the null hypothesis is $\hat{\beta}_1 < 4Z_\alpha\sigma_e^2(1 - \rho)/\sqrt{n}$, and the power is given by

$$\Phi\left(\frac{2Z_\alpha\sigma_e^2(1 - \rho) + \sigma_g^2\sqrt{n}}{\sqrt{\xi + 2\sigma_e^4(1 - \rho)^2}}\right)$$

Thus, to obtain a power of $1 - \beta$, the required number of sib pairs is

$$\left(\frac{Z_{1-\beta}\sqrt{\xi + 2\sigma_e^4(1 - \rho)^2} - 2Z_\alpha\sigma_e^2(1 - \rho)}{\sigma_g^2}\right)^2 \quad (7)$$

In Table 2 we have provided the sample sizes necessary to detect linkage at a significance level α of 0.05 with 80% power ($= 1 - \beta$) for an additive genetic model with $a = 1$, $d = 0$, and $\sigma_e^2 = 1$. Table 2A corresponds to a gene frequency p of 0.20, while for Table 2B, $p = 0.40$. In each table, the upper triangle corresponds to a residual correlation $\rho = 0$, and the bottom triangle to $\rho = 0.4$. For the major locus in Table 2A, the heritability H due to this locus is 0.242; in Table 2B, it is 0.480. Each column and row corresponds to a decile of the trait distribution (1 = lowest 10th percentile, 10 = highest 10th percentile). The numbers in Table 2A also apply for the same model with an allele frequency $p = 0.80$, but with the deciles reversed (for example, 1 replaces 10). The same applies to Table 2B, but for an allele frequency $p = 0.60$.

The most striking observation is that the power to detect linkage is concentrated in the pairs at the three corners of the triangle—those concordant for high values, low values, or the extremely discordant pairs. Pairs involving individuals with intermediate values (between 30th to 70th percentile) provide little information for linkage analysis. It is also clear from the tables that the uniformly best strategy in the additive case (in which a heterozygote for genes underlying a continuous trait has a phenotype halfway between the homozygotes) is to take the most discordant pairs (in other words, those with one sib in decile 1 and the other in decile 10). To expand the sample size, an alternative would be to take the top and bottom 20th percentiles, or the top 10% and bottom 30%, although this leads to somewhat reduced power. The latter strategy would be most useful when a disease is defined by extreme values of a continuous trait (for example hypertension and the trait of blood pressure). In this example, hypertension patients (in the top 10th percentile of blood pressure) could be sampled, and those with a sib in the bottom 30th percentile of blood pressure identified). Pairs concordant for high trait values will be useful when the allele frequency (p)

Table 2. Number of sib pairs required to detect linkage by decile for the additive model. Above the diagonal, $\rho = 0$; below the diagonal, $\rho = 0.4$. *, greater than 999; **, greater than 9999; ***, greater than 99999. (**A**) is for $p = 0.2$, and (**B**) is for $p = 0.4$.

A

decile	1	2	3	4	5	6	7	8	9	10	decile
	478	693	*	*	*	***	*	*	277	62	1
		*	*	*	*	***	**	*	458	102	2
1	632		*	*	**	***	**	*	780	168	3
2	*	*		*	**	***	**	*	*	310	4
3	*	*	*		**	***	***	**	*	747	5
4	**	*	*	*		***	***	***	***	*	6
5	*	**	*	*	*		**	**	**	**	7
6	878	**	**	*	*	*		*	*	*	8
7	283	*	*	***	*	*	*		635	259	9
8	115	346	*	*	***	*	*	*		73	10
9	49	110	224	523	*	**	*	*	539		
10	19	31	48	77	136	302	*	***	647	121	

B

decile	1	2	3	4	5	6	7	8	9	10	decile
	126	228	464	*	**	*	543	185	77	28	1
		407	787	*	**	**	*	465	179	60	2
1	176		*	*	**	***	*	*	405	118	3
2	430	389		*	**	***	**	**	*	260	4
3	*	662	700		**	**	***	***	*	808	5
4	**	*	*	*		**	**	**	**	**	6
5	617	***	*	*	*		*	*	*	*	7
6	181	*	***	*	*	*		*	889	550	8
7	79	361	*	***	*	*	*		397	185	9
8	40	122	381	*	***	*	*	643		71	10
9	21	49	109	286	*	***	*	614	323		
10	10	18	31	54	108	285	*	*	383	107	

for high values is low. However, when the allele frequency for high values is large, such pairs will be far less useful (for example, when $p = 0.8$, 661 pairs in the top 10% are necessary, compared to only 84 pairs in the top and bottom 10%). A similar argument applies for low trait values.

It should also be noted from these tables that when there is a positive residual correlation (for example, when several genes interact to generate the heritability) the power of concordant pairs to detect the genes decreases, whereas the power of discordant pairs increases, because, when there is a positive residual correlation, discordant pairs have an increased probability of being genotypically discordant at the locus of interest. When this residual correlation is large the increase in power can be sizeable, resulting in a reduction of necessary sample size of threefold.

For comparative purposes, if one analyzed random sib pairs with the Haseman-Elston approach, with the model in Table 2A, 1,082 sib pairs would be required at $\rho = 0$, or 780 sib pairs at $\rho = 0.40$; using only the most discordant pairs (top 10% and bottom 10%), we would require, for $\rho = 0$, only 61 pairs (one eighteenth as many), or for $\rho = 0.40$, only 19 pairs (one forty-first as many).

Table 3 gives similar results for a dominant model ($a = d = 1$, $\sigma_e^2 = 1$) with the allele frequency p equal to 0.2 (Table 3A) and 0.6 (Table 3B). Again, $\alpha = 0.05$ and $1 - \beta = 0.80$; the upper triangle corresponds to $\rho = 0$ and the lower triangle to $\rho = 0.4$. As before, symmetry indicates that these tables also apply to the recessive case for allele frequencies $q = 0.8$ and 0.4, where the deciles are inverted in order. The heritabilities due to the loci of interest in these two tables are 0.480 and 0.350, respectively.

The pattern in Table 3 is similar to that in Table 2 in that again the power to detect linkage is found in sib pairs lying in the three corners of the triangle. However, which corner provides the greatest power now depends on the allele frequency. For a low frequency dominant allele ($p = 0.2$), the discordant pairs are most useful, including the top and bottom 20th percentile. In this case, sibs concordant for high values are also useful; those concordant for low values are less so. When there is a significant residual correlation ($\rho = 0.4$), the power of discordant pairs is increased; the power of pairs concordant for high values is now reduced slightly as it is for pairs concordant for low values.

For a dominant allele, the pattern begins to change as p increases. As long as the allele frequency is not too large ($p < 0.6$), discordant pairs are still powerful. Again, residual correlation increases the power for this group. When the residual correlation is large ($\rho = 0.4$), the discordant pairs are most powerful, even at high allele frequencies ($p = 0.6$). Pairs concordant for low values are informative while those concordant for high values are not. In this case, the power for these pairs is slightly reduced. Thus the discordant pairs are always informative, whereas concordant pairs are unpredictable. These conclusions also apply to the recessive case. We also note that in this case, as in the additive case, the use of discordant sib pairs is vastly more efficient than using random pairs. For low allele frequencies, the sample size ratio is about 12 to 1, whereas for high allele frequency it ranges from 7 to 1 to 40 to 1 depending on the degree of residual correlation.

Comparison of Double Proband with Single Proband Designs

Carey and Williamson (9) suggested that the power to detect linkage with a QTL could be increased by sampling sib pairs through probands with extreme values. In their design, the second sib is selected at random, irrespective of his/her trait value. The second sib's trait value is regressed on ibd with the proband sib at a marker locus. Under linkage, those second sibs sharing two alleles ibd with the proband should show the highest mean trait value, whereas those with no alleles ibd should regress close to the population mean, depending on the amount of residual correlation. We refer to this method as the single proband sib pair (SPSP) approach.

By contrast, we focus on sib pairs in which selection has occurred on both sibs' trait values. Hence, we refer to this method as double proband sib pair (DPSP) analysis. The results in Tables 2 and 3 suggest that SPSP analysis is still inefficient due to the inclusion of many sibs with intermediate values. When the second sib falls in the 20th to 70th percentiles, there is generally little deviation from the null expectation of ibd with the proband. This finding would suggest that sampling only the corners of the table, in particular the discordant pairs, would lead to far greater efficiency.

To compare power, we use results presented in (9) for 240 sib pairs ascertained through single probands in the top decile, with the other sib at random. Their results were generated by simulations. For the DPSP approach, we consider three different strategies, two for discordant pairs and one for concordant pairs: (i) T1B1, one sib in top decile, the other sib in bottom decile; (ii) T1B3, one sib in top decile, the other sib in bottom 30%; (iii) T1T1, both sibs in top decile. We also assume 240 sib pairs, and calculate power as described above. Carey and Williamson considered additive, dominant and recessive alleles for high trait values with $p = 0.2$, 0.4, 0.6 and 0.8, with the residual correlation $\rho = 0.2$. We evaluated the same cases. By fix-

Table 3. Number of sib pairs required to detect linkage by decile for dominant model. Above the diagonal, $\rho = 0$; below the diagonal, $\rho = 0.4$. *, greater than 999; **, greater than 9999; ***, greater than 99999. (**A**) $p = 0.2$, and (**B**) $p = 0.6$.

A

decile	1	2	3	4	5	6	7	8	9	10	decile
	260	277	310	391	665	*	*	103	29	15	1
		294	330	417	711	*	*	114	33	18	2
1	270		371	470	806	*	*	138	42	23	3
2	310	295		598	*	*	*	197	63	37	4
3	446	334	335		*	**	*	417	148	93	5
4	*	474	397	398		**	**	*	*	972	6
5	**	*	678	508	485		**	*	966	707	7
6	246	*	*	*	675	520		314	162	125	8
7	49	155	560	*	*	681	345		89	71	9
8	21	36	64	151	817	*	343	156		58	10
9	14	17	23	36	80	546	*	145	84		
10	12	13	14	17	24	52	416	577	96	62	

B

decile	1	2	3	4	5	6	7	8	9	10	decile
	25	79	*	386	113	68	52	45	41	38	1
		366	**	*	*	737	597	531	495	474	2
1	32		***	***	***	**	**	**	**	**	3
2	227	214		**	**	**	**	*	*	*	4
3	829	*	*		*	*	*	*	*	*	5
4	106	***	*	*		*	*	*	*	*	6
5	54	*	*	*	*		*	*	*	*	7
6	37	572	***	*	*	*		*	*	*	8
7	30	258	**	*	*	*	*		*	*	9
8	25	141	*	**	*	*	*	*		*	10
9	22	82	770	***	*	*	*	*	*		
10	19	43	189	*	***	*	*	*	*	*	

ing $\sigma_e^2 = 1$ and allowing a to vary, we obtain the different heritabilities H due to the locus considered.

The results of this comparison for the additive case are given in Fig. 1. From these figures, the dramatic increase in power when using a DPSP strategy is apparent. It is clear that the discordant pairs always have highest power, especially for the T1B1 group. The pairs concordant for high values (T1T1) also perform well except when the allele frequency for high values is large (0.8). In any event, it is clear that if the T1B1 strategy is used at low heritability, the power is three to four times greater than for the SPSP strategy. Even for the T1B3 strategy the power is double that of SPSP. The situation for a dominant allele is similar. The power is dramatically higher for a T1B1 strategy across all gene frequencies, but is especially so, when the gene frequency is large (≥0.6). For gene frequencies less than 0.6, T1B3 also performs well, but when the allele frequency is higher (0.8), power is reduced, although still considerably greater than for the SPSP strategy. For a recessive allele, the findings are again the same, except that when the allele frequency is low (0.2), there is little difference between the T1B1 and SPSP strategies. In this case, the T1T1 strategy is best, because these pairs are likely to share two alleles ibd.

In Table 4 we give the number of sib pairs required by a T1B1 or T1B3 strategy to achieve the same power as the SPSP strategy, as a function of heritability (H) due to the locus of interest and the allele frequency (p). These numbers were calculated with formula (4), in which τ and Z_2 are determined for a sib pair falling in the deciles of interest, and 1-β is the power of the SPSP strategy. It is apparent from this table that the reduction in necessary sample size is usually dramatic. For the T1B1 strategy, there is generally a 6- to 40-fold reduction in necessary sample size. The only exception is for a rare recessive allele, where the sample sizes are comparable.

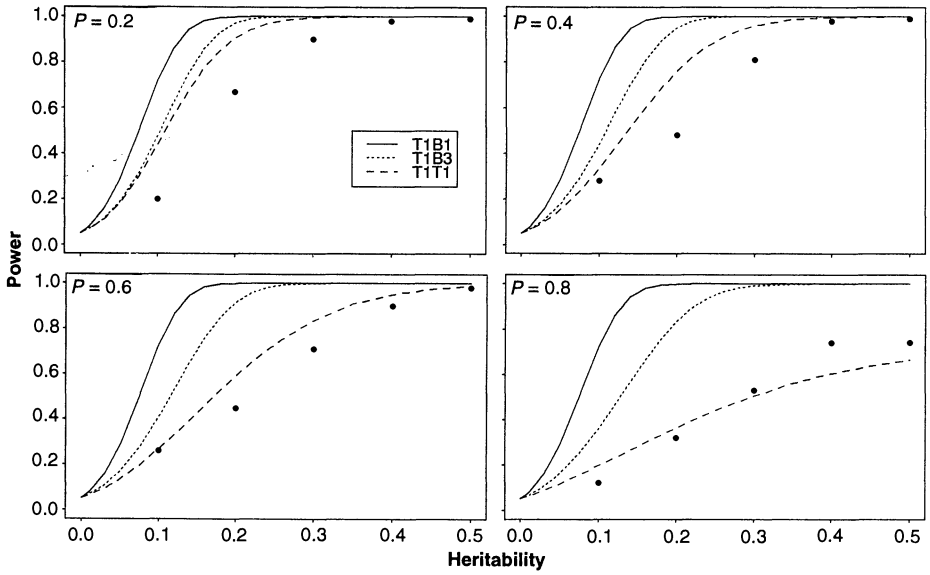

Fig. 1. Comparison of power for 240 single proband sib pairs (bullets) versus double proband sib pairs for three double selection strategies in an additive model. Symbols for all panels are as shown for $P = 0.2$.

Table 4. Number of DPSPs giving the same power as 240 SPSPs.

H	Additive model, where p =				Dominant model, where p =				Recessive model, where p =			
	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
					Top 10% and bottom 10%							
0.1	33	55	49	12	39	14	15	14	152	55	38	20
0.2	48	28	25	17	36	19	12	7	186	80	44	18
0.3	38	27	21	15	33	19	10	11	233	75	38	17
0.4	32	27	18	14	34	20	10	12	265	57	37	19
					Top 10% and bottom 30%							
0.1	63	121	121	34	78	32	47	86	214	89	75	50
0.2	85	59	62	51	64	46	42	54	259	113	79	44
0.3	62	55	51	46	52	43	39	126	328	95	58	40
0.4	48	55	44	43	45	41	39	141	375	65	49	40

Sampling Considerations

We have used formula (4) to determine sample sizes required to detect linkage with the different DPSP strategies for a range of gene frequencies, heritabilities, and residual correlations (*14*). We find that sample sizes are generally within experimental limits provided the heritability is greater than 0.1. However, an important question remains as to how many sib pairs need to be screened to obtain the desired doubly selected samples. If we assume that only one tail of the distribution is of primary clinical interest, then it may be possible to access a large number of individuals in the extreme 10th percentile of that tail from clinical or other sources. Then, the question is how many sibs of these individuals need to be screened to obtain the requisite sample size. We have also calculated these numbers (*14*). Generally, we find that when the heritability due to the locus of interest is high (≥0.5) the number of sibs requiring screening is in the low hundreds; for a low heritability of 0.1, the number is in the low to middle thousands.

The feasibility of screening large numbers of individuals will depend on whether information on the trait is readily available (such as for weight or height), or whether expensive or invasive testing is required. If the limiting factor is the expense of genotyping, but the sibling material is easily obtained, a T1B1 strategy is appropriate; on the other hand, if the sibling material is the limiting resource and the genotyping is inexpensive, the T1B3 and/or T1T1 strategy is preferred. Perhaps a reasonable trade-off is to collect all the T1B3 and T1T1 sibships, first type the T1B1 pairs and then confirm the initial positive findings with the remaining pairs.

Conclusions

Our data clearly demonstrate that the only design that is uniformly powerful in all genetic situations is the extreme discordant sib pairs. This is because these pairs are unlikely to share alleles ibd for any genetic model. Sib pairs concordant for extreme values can also be useful, but which tail provides the power varies with the conditions. As a general rule, it is the tail corresponding to the lower allele frequency that is useful; however, the degree to which this is the case depends on dominance. For an additive locus, both tails are useful when the allele frequencies are equal, but when one allele drops below 0.3 in frequency, only that tail should be sampled. For a locus dominant for high values, sampling the upper tail is useful up to an allele frequency of about 0.3; otherwise, the lower tail should be sampled. Similarly, for a locus that is recessive for high values, sampling the upper tail is useful

up to an allele frequency of around 0.7.

When studying a trait where only one tail is of clinical significance (such as blood pressure or weight), it is tempting to sample sib pairs concordant for that tail. This will be particularly so when the distribution shows skewness toward the end with clinical significance. Skewness can be an indicator of a major gene with an allele of low frequency. If such is the case, sampling sib pairs concordant for that tail will be a practical strategy. However, if the skewness is due to other reasons (such as nongenetic reasons) this strategy could fail. Furthermore, this strategy would not be powerful to identify other contributing loci that do not produce skewness.

Historically, investigators have usually searched for rare alleles in the population. For many traits, there will be multiple genes underlying variation. These genes may well have high allele frequencies and the strategies used for rare genes cannot be applied. In general, the only design with power to detect all these genes, irrespective of allele frequencies and degree of dominance, is the discordant pairs.

A question that often arises in sib pair analysis is what to do with additional sibs beyond the pair. Given the severity of the selection criterion, we would not expect many families to have multiple siblings meeting the criteria. However, we did examine the case of sib trios, where one sib was in the top 10% and the other two sibs in the bottom 10% or bottom 30%, respectively. We examined identity by descent for the two discordant pairs, which are statistically independent. We found (*14*) that the power was essentially the same as for two unrelated sib pairs; in other words, the expected deviation of number of alleles ibd from the null hypothesis for each of the two pairs was the same as for two single pair sibships. Thus, any families with multiple sibs of a proband meeting criteria should be included for analysis; all qualifying pairs that include the proband can be included as independent pairs.

Another question is whether this approach can be extended to qualitative traits or disease states in which an individual is either affected or not affected. The key is to define individuals who lie at the opposite end of the putative distribution underlying the disease. It is not sufficient to simply use affected/unaffected pairs, because there will be a range of values classified as unaffected. However, if it is possible to define a continuous trait associated with disease, then it may be possible to define individuals at the opposite end of this continuum.

The power to detect linkage with discordant sib pairs increases in the presence of a residual sib correlation, especially at low heritability for the tested locus, whereas concordant pairs show a decrease in power. (In simpler terms, if siblings are discordant in the presence of other genes that cause them to be similar, then they are more likely to be discordant at any contributing locus.) The two major determinants of power to detect linkage for a locus contributing to a quantitative trait are the heritability due to that locus and the residual correlation. Because the power to detect a weak locus increases with residual correlation, using discordant pairs should make it possible to detect such loci provided the total heritability (due to the tested locus and the residual correlation) is high enough. Thus, it appears that total heritability may be a more important factor than the number of loci contributing to that total. Our calculations suggest that, when the total heritability is in the range of 30%, there will be sufficient power to detect linkage if the genetic variation is primarily attributable to three or fewer loci; at 50% total heritability, five or fewer loci would be detectable.

Finally, we note that the extreme discordant sib pairs represent a powerful design for association studies of candidate genes (*14*). Thus, the same sample can be used for linkage and association studies.

The recent interest in identifying genes for quantitative traits necessitates the development of powerful methods for tackling this task. We have shown that judicious sample selection, in particular the use of extreme discordant sib pairs, should facilitate this endeavor. This approach may enable investigators to identify genes involved in complex traits such as hypertension, obesity, or behavior that would not have been possible with other study designs.

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