Am. J. Hum. Genet. 64:1194–1205, 1999

Comparison of Linkage-Disequilibrium Methods for Localization of Genes Influencing Quantitative Traits in Humans

Grier P. Page and Christopher I. Amos

Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston

Summary

Linkage disequilibrium has been used to help in the identification of genes predisposing to certain qualitative diseases. Although several linkage-disequilibrium tests have been developed for localization of genes influencing quantitative traits, these tests have not been thoroughly compared with one another. In this report we compare, under a variety of conditions, several different linkage-disequilibrium tests for identification of loci affecting quantitative traits. These tests use either single individuals or parent-child trios. When we compared tests with equal samples, we found that the truncated measured allele (TMA) test was the most powerful. The trait allele frequencies, the stringency of sample ascertainment, the number of marker alleles, and the linked genetic variance affected the power, but the presence of polygenes did not. When there were more than two trait alleles at a locus in the population, power to detect disequilibrium was greatly diminished. The presence of unlinked disequilibrium (D'^*) increased the false-positive error rates of disequilibrium tests involving single individuals but did not affect the error rates of tests using family trios. The increase in error rates was affected by the stringency of selection, the trait allele frequency, and the linked genetic variance but not by polygenic factors. In an equilibrium population, the TMA test is most powerful, but, when adjusted for the presence of admixture, Allison test 3 becomes the most powerful whenever $D'^* > .15$.

Introduction

Linkage-equilibrium methods such as the LOD-score method (Ott 1991) and the sib-pair method have been used to locate genes whose variation causes qualitative, Mendelian diseases. Linkage disequilibrium has been an essential key to the fine-scale localization and positional cloning of some causative genes (Hastbacka et al. 1994). Variance-component and Haseman-Elston methods have been developed for localization of genes that influence quantitative traits in humans; however, these methods require large samples for localization of genes with small effects in the populations. For example, 2,000–20,000 sib pairs are required to map loci that explain 10% of the total phenotypic variance (Page et al. 1998).

The use of linkage-disequilibrium methods may reduce the total sample size required for establishment of linkage to genes influencing quantitative traits in humans. Linkage-disequilibrium methods test the hypothesis that association arises because specific marker loci and trait-affecting loci are tightly linked and that alleles at the loci are not in Hardy-Weinberg equilibrium. Linkage disequilibrium arises from historical mutations, migrations, bottlenecks, or other population genetic events (Lander and Schork 1994). For example, when a new mutation enters a population as a new event in an individual, the mutation is in disequilibrium with the entire genome of the founding individual. As the mutation is passed through the generations, the alleles at loci that are more tightly linked are more likely to be cotransmitted than are the more distantly linked or unlinked loci. As a result, individuals who have a trait-affecting allele are more likely to also inherit the founder's marker allele at loci tightly linked to the trait locus, compared with the general population, and loci that more distantly linked will not exhibit any excess of the founder's alleles. Several tests based on linkage disequilibrium have been developed and studied for localization of genes that influence quantitative-trait variation. We focus on nine of these tests. These tests include two types of measured-genotype test, two types of truncated measured-genotype test, a "case-control" (CC) test, and four transmission/disequilibrium tests (TDTs) for localization of genes influencing quantitative traits (the "QTDTs") (Allison 1997).

The measured-genotype test (Boerwinkle et al. 1986, 1987) compares the mean quantitative-trait values of

Received October 16, 1998; accepted for publication February 4, 1999; electronically published March 17, 1999.

Address for correspondence and reprints: Dr. Christopher I. Amos, Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe, Box 189, Houston, TX 77030. E-mail: camos@request.mdacc.tmc.edu

0002-9297/99/6404-0032$02.00

individuals who have either specific alleles (in what is called the "MGA test") or specific genotypes (in what is called the "MGG test"). The means are compared by ANOVA, and we consider the sampling of individuals to be at random.

Risch and Zhang (1995) have observed that the number of sibling pairs that must be genotyped, but not phenotyped, in order to establish linkage could be dramatically reduced by selection of families that have children with highly discordant quantitative-trait values. Allison (1997) has applied extreme sampling to develop the QTDT tests, and extreme-sampling methods may be applied to other tests for disequilibrium.

One test that uses extreme sampling is the CC test, which compares the frequencies of the genotypes or alleles in individuals with high quantitative-trait values versus those with low quantitative-trait values. Here we consider only tests of alleles and use a χ^2 test to measure the significance of the associations.

We additionally suggest new tests, which are based on either the MGG test or the MGA test. Unlike the situation with the regular MGG test or MGA test, individuals are selected on the basis of having extreme quantitative-trait values. We call these tests the "truncated measured genotype" (TMG) test and the "truncated measured allele" (TMA) test. Analyses are performed in a manner similar to that used for the MGG test or the MGA test.

These tests may be subject to bias from population admixture and genetic drift (Spielman and Ewens 1996). Admixture arises if the population being sampled is the result of two populations that have recently begun to intermarry and have different allele frequencies at both the trait and marker loci. The differences in the allele frequencies cause a difference between the observed haplotypic frequencies and the expected ones. Disequilibrium due to admixture can even be detected if the marker and trait loci are unlinked, and it will be replicated in repeated sampling from the same population.

A series of TDTs have been developed to remove the bias due to admixture. Originally developed for qualitative traits (Spielman et al. 1993), the ideas have been extended to quantitative traits (Allison 1997; Rabinowitz 1997). These tests use parental marker data to remove disequilibrium due to admixture. Rather than testing for a difference between the means of the children's quantitative-trait values based on only the child's alleles or genotype, these methods study the transmission of marker alleles from parent to the child. The children's quantitative-trait values are then compared on the basis of the transmitted allele. In the absence of disequilibrium between a marker allele and the trait locus, the probability that any specific allele has been transmitted from a heterozygous parent is independent of the child's quantitative-trait value; thus, the means of the transmitted alleles should be equal. But, if there is disequilibrium between the marker and a trait-affecting allele, then the probability that an allele has been transmitted is dependent on the child's quantitative-trait value, and this should be statistically detectable. The first four tests proposed by Allison (1997), A1-A4, are examined here. Allison test 5 was not used, because of difficulty in the implementation of the method.

Linkage-disequilibrium tests have been compared elsewhere, but quantitative-trait tests have not been extensively compared. Spielman and Ewens (1996) have addressed the increase in error-rates that is associated with non–TDT-based tests of qualitative traits in the presence of admixture. Kaplan et al. (1997) have examined the power of qualitative TDT tests with multiple alleles. Ott and Rabinowitz (1997) have observed that the power of disequilibrium tests increases with increasing marker heterozygosity. In describing new quantitative TDT methods, Allison (1997) has compared the power of the tests that he developed but has not compared his novel tests versus existing disequilibrium tests for localization of quantitative traits. The goal of the present study is to compare disequilibrium tests for localization of genes that influence interindividual variation in quantitative traits, by examination of the performance of the MGA, MGG, TMA, TMG, CC, and A1–A4 tests under a variety of conditions. The conditions that were varied include the linked genetic variance, the polygenic variance, the trait allele frequency, the number of marker alleles, the sample-ascertainment scheme, and disequilibrium due to the admixture. We also applied these different analytical methods to the analysis of data on apolipoprotein E (apo E) genotypes and apo E's relationship to LDL-cholesterol levels.

Methods

Tests of Disequilibrium

We compared nine linkage-disequilibrium methods for localization of quantitative traits. The tests include the measured-genotype approach, which can be implemented in two ways: on the basis of the alleles (i.e., the MGA test) and on the basis of the genotypes (i.e., the MGG test). For these tests, unrelated individuals are genotyped and phenotyped. The phenotypic values are binned into groups, on the basis of the individuals' alleles or genotype. For a two-allele system, there are two bins on the basis of alleles and three bins on the basis of genotypes. As implemented, the MGA test uses an individual's quantitative-trait value twice. If an individual is homozygous, his or her trait value is put into the same bin twice. The means of the bins are then compared. For all analyses, we used simple ANOVA to compare the groups. Because, in small samples, we reuse individ-

ual's phenotypic values twice, the variation in the homozygous groups may be less than that in heterozygotes, but we assumed homoscedasticity of variances.

We have chosen to use extreme sampling for several of the disequilibrium tests. Families or individuals were considered eligible for use if the child's quantitative-trait value was in either the upper or lower n deciles. Tn includes families in which the child is in the upper n deciles, whereas Bn includes families in which the child is in the lower n deciles. For example, T3B3 sampling involves selection of individuals from the upper and lower 3 deciles only. For the CC test and the TMG test, three sampling schemes—T1B1, T1B3, and T3B3—were used. For TDT sampling, nine types of analysis were performed, with all combinations of T2–T4 and B2–B4.

The TMG test and the TMA test are based on the MGG test and the MGA test, but they use extreme sampling in the collection of samples. Individuals are selected only from the upper and lower ends of the phenotypic distribution. The TMA test and the TMG test bin trait values on the basis of alleles or genotypes. ANOVA methods are then applied, under the assumption that sample sizes are large enough for the central-limit theorem to provide accurate significance levels. We have checked this assumption for the samples sizes that we have studied as a part of the simulation studies and have found it to be valid (data not shown).

The CC test selects individuals with either high or low quantitative-trait values as determined by discordant sampling. The allele frequencies in individuals from the "high" group versus those in individuals from the "low" group are compared by a χ^2 test.

Allison (1997) has developed five TDT-based tests for localization of quantitative-trait loci; we have chosen to investigate the first four of them, A1–A4. Nuclear families with two parents and one child have been used. Only families in which, at a marker locus, one parent is a homozygote and the other parent is a heterozygote can be used for analysis. The child's phenotypic value is binned on the basis of which allele is transmitted to the child. These tests have been formulated for use with diallelic marker loci. The sample for A1 is collected without regard to the child's phenotypic value. The bins are then compared by a t-test. A2 involves selection of children with extreme phenotypes. The frequencies of the transmitted alleles in the children with high quantitative-trait values versus those in children with low quantitative-trait values are compared by a χ^2 test. Children with extreme phenotypes were also selected for A3. The means of children who received the different alleles are compared by a t-test. A4 selects individuals with an extreme phenotype. The proportion of children with a high quantitative-trait value who received a specific allele is compared with the proportion of children with a low quantitative-trait value who received the same allele from their heterozygous parent. The differences are compared by a Z-test. A more detailed description of the tests can be found in the report by Allison (1997).

The Genetic Model and Simulation Method

The quantitative-trait value, X_i, of the ith individual is assumed to result from the effect of a single gene, g_i; residual polygenic effects, pg_i; and random environmental effects, e_i. Therefore, $X_i = \mu + g_i + pg_i + e_i$, where, μ is a fixed effect and pg_i and e_i are random effects with mean 0 and variances σ^2_{pg} and σ^2_e, respectively. Amos (1994) has described the genetic model for a two-allele system. If g, pg, and e are assumed to be independent and to act additively, then the total variance of the trait is $\sigma^2_t = \sigma^2_a + \sigma^2_{pg} + \sigma^2_e$, with the total genetic variance being $\sigma^2_g = \sigma^2_a + \sigma^2_{pg}$.

We simulated nuclear families with two parents and a single child. An individual's quantitative-trait value was considered to be the result of contributions from linked genetic, unlinked genetic, and random effects. The contribution from the linked genetic effect was considered to be dependent on the genotype at the trait locus, as described further in the following subsection. The genotypic mean trait values were specified on the basis of the trait allele frequency and the proportion of linked genetic variance. The polygenic and environmental contributions to the parents' quantitative-trait values were determined by generation of random numbers: $N(0,\sigma^2_{pg})$ and $N(0,\sigma^2_e)$, respectively. These values were added to the linked genetic effect, to derive the parental quantitative-trait value. The contribution of nongenetic factors to the child's quantitative-trait value was also determined by a random-number generator, $N(0,\sigma^2_e)$. The polygenic contribution was generated by determination of the mean of the child's parents' polygenic values, μ_{pg}, and subsequent generation of a random number, $N(\mu_{pg},\frac{1}{2}\sigma^2_{pg})$. These terms were then summed with the child's major genotypically specified contribution to the trait, to obtain the child's quantitative-trait value.

Individuals were simulated with 16 marker loci, grouped into eight pairs of loci. The first marker in each pair had 2 alleles and the second had 10. All the alleles at each marker locus had the same frequency in the general population. The frequencies of the alleles in the general population were always set to be $1/n$ (where n is the no. of alleles at the marker locus). The distance from the marker to the trait loci could be specified; normally, the first seven loci pairs were linked at various recombination fraction (θ) values, and the final one was unlinked at $\theta = .5$.

For each simulation study, we assumed that a mutation had occurred on a founding haplotype. For the diallelic trait locus applications, we use "A" to denote the allele associated with a founding haplotype A. The foun-

der haplotype consisted of a single trait allele, A, with frequency p, and alleles at 16 marker loci were assigned on the basis of the marker-allele frequency in the general population. We allowed this founder haplotype to decay as a function of generations (t) and θ. If it is assumed that the trait haplotype crossed only with nontrait haplotype chromosomes, that no double-recombinational events occurred, and that the population is infinite, then the alleles at the marker loci were in equilibrium in the general population when founders were excluded. The expected proportion of alleles at a specific locus that will still be in disequilibrium with a particular allele is given by $E[(1-\theta)^t]$ (Li 1976). By the variation of t and θ, any amount of disequilibrium (D') could be specified.

One measure of disequilibrium was used in the study: D', which is defined as δ_{ij}/X, where $\delta_{ij} = p(p_i)p(q_j) - p(pq_{ij})$. If δ_{ij} is positive, then X is the minimum of $[p_iq_j,(1-p_i)(1-q_j)]$, and, if δ_{ij} is negative, then X is the minimum of $[p_i(1-q_j),(1-p_i)q_j]$, where p_i and q_j are the frequency of the ith and jth alleles at loci p and q, respectively, and pq_{ij} is the proportion of haploptypes having the ith and jth alleles at loci p and q. For clarity, disequilibrium due to admixture is denoted as "D'^*," to distinguish it from linkage disequilibrium.

To simulate families, a uniform random-number generator over the interval [0,1] determined which trait alleles were received by a parent. Whenever the random number generated was less than the frequency of the trait allele A in the general population, the trait allele A with a founding haplotype was assigned to that parent. If the parent did not receive the trait allele A, then all of the marker alleles were assigned at random, on the basis of their frequencies in the general population. If a trait allele A was assigned to one or more of the parental chromosomes, then a uniform random number was generated over the interval [0,1]. This number was compared with the probability that the founder allele at the specified locus would still be in disequilibrium, given the θ and t values. If the random number was greater, then a recombination was assumed to have occurred between the locus and the trait alleles, and all the alleles more distantly linked were assigned at random, on the basis of the marker-allele frequencies in the general population. However, if the random number was less than the probability of the marker allele remaining in disequilibrium, then no recombination occurred and the alleles from the founder haplotype were passed to this parent.

The child's two haplotypes were generated by determination of which of the parental trait alleles were passed to the child. A random number, U[0,1] was generated to determine whether a recombination event may have occurred between the parent's chromosomes. At all points beyond the crossover, the alleles were replaced by the alleles from the parent's other haplotype. No double-recombination events were allowed.

Simulation of Unlinked Disequilibrium

To simulate D'^*, the population being sampled was modeled as containing two equally frequent founder populations. The two founder populations differed in both the frequency of the trait allele with a founder haplotype and the frequency of the unlinked diallelic marker locus. The trait allele with a founder haplotype was modeled to have entered from only one of the founder populations. The mutation was simulated to be in equilibrium with the marker alleles at the unlinked locus in the subpopulation. Each parent was then simulated as having originated from one of the two subpopulations, and, with regard to parental mating, there was no reference to the subpopulation whence either parent came. Families were then simulated in a fashion similar to that used for nonadmixed populations. The amount of disequilibrium due to admixture was varied by changing the difference between the frequencies of the marker and trait alleles in two subpopulations.

Multiple Trait Alleles

The simulations described above involved only a single two-allele trait. However, evidence from qualitative and quantitative genetics suggests that most genes will have many alleles (Mohrenweiser and Jones 1998; Nickerson et al. 1998). The effect that three trait-affecting alleles have on the power of linkage-disequilibrium tests was examined by simulation of a population with three alleles at the trait locus. Two of the trait alleles were simulated with founder haplotypes called "A" and "B." The trait alleles were assumed to have equal and opposite effects. Each was independent and selected in the same fashion as were the haplotypes in a population with one founder haplotype. The third trait allele was considered to be in equilibrium with the founder population. Alleles were assigned to parents in the same way as was used for all other simulations, except that there were now two possible founder haplotypes instead of one. Each haplotype was modeled to have entered the population at the same time.

The contributions from the linked genetic effects were assigned slightly differently from what had been used in the evaluation of the diallelic trait locus. The mean quantitative-trait value was $\mu = \Sigma_{\text{geno}} p_{\text{geno}} \mu_{\text{geno}}$, where p_{geno} is the frequency of each genotype and μ_{geno} is the mean effect of each genotype. The population mean quantitative-trait value was then calculated as $\sigma_a^2 = \Sigma_{\text{geno}} p_{\text{geno}} (\mu_{\text{geno}} - \mu)$. For the purposes of simulation, p_{geno} and μ_{geno} were always set so that μ was always 0. The simulation was set so that the linked genetic variances were comparable in bi- and triallelic trait loci (Weiss 1993).

Table 1

Power as a Function of σ_a^2, with Diallelic Markers and Either T3B3 Sampling or Random Sampling: α = .001, σ_{pg}^2 = 0%, p = .5, D' = .99

σ_a^2 (%)	Power of Test								
	MGA	MGG	TMA	TMG	CC	A1	A2	A3	A4
1%	.078	.052	.154	.098	.019	.032	.052	.074	.058
2%	.229	.155	.488	.369	.069	.120	.176	.248	.203
5%	.784	.681	.973	.948	.304	.517	.693	.831	.723
10%	.997	.986	1.000	1.000	.820	.921	.986	.994	.986

Analysis

In each replication, 6,000 families were simulated. The first 1,000 families that met both the genotypic and the phenotypic requirements for each individual test at each marker were selected for analysis. Each set of 1,000 families was analyzed by the statistical test appropriate for the disequilibrium test.

A total of 1,000 replications were performed. The number of times that an iteration achieved a test statistic exceeding critical values corresponding to significances of .05, .01, .001, and .0001 was divided by 1,000 and was reported as the power for a given significance. Similar results were achieved for the error rates, by a counting of the number of times that unlinked markers achieved a result exceeding the criterion for significances of .05, .01, .001, and .0001.

To compare the power of the linkage-disequilibrium tests in the presence of admixture, the values corresponding to specific significance levels were recalculated. The test statistics for the unlinked diallelic marker were ranked for a given set of simulation parameters. The 950th of the 1,000 iterations was taken as the upper-5% critical value. The newly calculated empirical 5% critical values for each test were then used to calculate the significances of linked markers in the presence of admixture.

Analysis of apo E Data

The genotyping and phenotyping of the data have been described elsewhere (Wang et al. 1998). The sample that we used consisted of 2,001 individuals in 287 families, each with three or more available children, that were recruited via newspapers, churches, and health centers, and of 717 individuals in 118 families that were ascertained via individuals who had symptomatic premature coronary-artery disease. A total of 1,954 individuals in 307 families had information on both apo E genotype and LDL-cholesterol levels. Thus, there were a maximum of 307 independent observations when only the children were used for analysis.

We used all the tests to analyze the apo E and LDL-cholesterol data. For the CC test, in addition to the usual allele-based analysis, genotype-based analysis using contingency tables also was performed. For the MGA, MGG, and A1 tests, the oldest child in each family with apo E genotypes and LDL-cholesterol levels was used. For the TMA, TMG, CC, and A2–A4 tests, a child in each family with the most extreme LDL-cholesterol level who was in either the T1B1 sample or the T3B3 sample and who also had an apo E genotype was used for analysis. If two individuals in a family had equal LDL-cholesterol levels, the older child was used for analysis. LDL-cholesterol levels for the samples were as follows: T1, 159 mg/dl; T3, 126 mg/dl; B1, 72 mg/dl; and B3, 92 mg/dl. The cutoffs were determined on the basis of the distribution of phenotypes in the entire sample of children; no adjustment was made for age or sex.

The apo E genotype was considered in several ways. First, as a single triallellic marker, on the basis of alleles (apo E_a) and genotypes (apo E_g), and, second, as two diallelic markers, on the basis of alleles ($Cys112Arg_a$ and $Arg158Cys_a$) and genotypes ($Cys112Arg_g$ and $Arg158Cys_g$). The Arg variant at position 112 creates the apo e4 allele, and the Cys variant at position 158 creates the apo e2 allele.

Results

Power

Tables 1 and 2 show the power of the disequilibrium tests when diallelic marker loci (table 1) and decallelic marker loci (table 2) are used. Shown is the power that a sample of 1,000 individuals has for detection of a locus accounting for various amounts of the phenotypic variance, with a significance of α = .001. In both tables, the powers for the MGA, MGG, and A1 tests are shown for an unselected sample, whereas the powers for the other tests were selected on the basis of T3B3 sampling. The disequilibrium in both tables is D' = .99. On the basis of 1,000 analyzed individuals, the TMA test is the most powerful, with 100% power to detect a 10% effect, but power decreases to 15% for a locus accounting for 1% of the phenotypic variance. The TMG test is the next most powerful, followed by the A3, MGA, A4, A2, MGG, A1, and CC tests. The CC test had 82% power

Table 2

Power as a Function of σ_a^2, with Decallelic Markers and Either T3B3 Sampling or Random Sampling: α = .001, σ_{pg}^2 = 0%, p = .5, D' = .99

σ_a^2 (%)	Power of Test				
	MGA	MGG	TMA	TMG	CC
1%	.065	.015	.198	.026	.010
2%	.344	.054	.711	.148	.049
5%	.954	.514	.999	.904	.505
10%	1.000	.984	1.000	1.000	.972

Table 3

Power of the TMA Test under Three Sampling Schemes, as a Function of σ_a^2, Sampling Scheme, and Number of Markers: α = .001, σ_{pg}^2 = 0%, p = .5, D' = .99

	Power of Sampling					
	T1B1		T3B3		T1B3	
σ_a^2 (%)	2 Markers	10 Markers	2 Markers	10 Markers	2 Markers	10 Markers
1%	.518	.713	.154	.198	.201	.238
2%	.922	.997	.488	.711	.580	.802
5%	1.000	1.000	.973	.999	.987	1.000
10%	1.000	1.000	1.000	1.000	1.000	1.000

to detect a 10% effect but had <2% to detect a 1% effect.

When decallelic markers are considered (table 2), the TMA test is still the most powerful, having 99.9% power to detect a 5% effect, but the MGA is the next most powerful, with 95% power, followed by the TMG, MGG, and CC tests, which have 90%, 51%, and 50% power, respectively. The Allison tests cannot use more than two-allele marker loci and were not considered in the comparison.

Table 3 (TMA test) and table 4 (A3 test) show the power for detection of disequilibrium, for various numbers of marker alleles, sampling schemes, and linked genetic variance. Both tables show the power to detect a trait allele acting additively with a frequency of $p = .5$ and a significance of $\alpha = .001$. The disequilibrium between the marker loci and the trait locus is $D' = .99$. The power shown in table 3 is for the TMA test using both di- and decallelic marker loci. In general, the decallelic marker loci have more power than the diallelic loci, under the same sampling condition. Using diallelic loci, the TMA test has 92% power to detect a 2% effect when T1B1 sampling is used but has only 58% when T1B3 sampling is used and only 49% when T3B3 sampling is used. The more stringent the selection, the greater the power of a given sample size, when decallelic loci are used. T1B1 sampling has 98% power to detect a 2% effect, but T3B3 sampling has only 71% power to detect a locus of similar effect. Table 4 gives the power of the A3 test; again, the more stringent the sampling, the greater the power of a given sample size. Selection strategies with similar stringency had similar power. T2B4 sampling, T3B3 sampling, and T4B2 sampling all select 60% of individuals for analysis. These selection strategies have, respectively, 23.6%, 24.8%, and 22.5% power to detect a 2% effect with 0.1% significance.

Tables 5 (diallelic markers) and 6 (decallelic markers) show how holding the linked genetic variance constant ($\sigma_a^2 = 2\%$) and varying the trait-allele frequency affects the power that the tests have for detection of disequilibrium, with 5% significance. Maximal power is obtained when the trait-affecting locus has two equally frequent alleles and decreases as the alleles become more disparate. The power of the TMA test varies from 91% at $p = .5$ to ~24% at $p = .05$. The relationship between the power of the different tests is not constant across trait-allele frequencies. When $p = .5$ the A3 test is more powerful than either the MGA test or the MGG test, but when $p = .1$ the A3 test has less power than these tests. The relative power of the other three Allison tests also decreases relative to that of the tests based on isolated individuals. The tests that can use decallelic loci (table 6) appear to keep the same relationship to one another, across the range of trait-allele frequencies.

Table 7 shows the power of both the TMG test and the A1 test, as a function of polygenic variance, with different numbers of marker alleles and different trait-allele frequencies: the conditions used were $\sigma_{pg}^2 = 0\%$, 10%, and 30% of the total phenotypic variance, when $\alpha = .05$, $t = 100$, $\theta = .0001$, and $\sigma_a^2 = 2\%$. The power to detect disequilibrium is not affected by either the presence of polygenes or the number of markers alleles, the type of test, or the trait-allele frequency. The results are similar for all disequilibrium tests (data not shown).

Table 4

Power of A3: α = .001, σ_{pg}^2 = 0%, p = .5, D' = .99, as a Function of σ_a^2 and Sampling Scheme for Diallelic Marker Loci

	Power of Sampling								
σ_a^2 (%)	T2B4	T2B3	T2B2	T3B4	T3B3	T3B2	T4B4	T4B3	T4B2
1%	.069	.088	.125	.059	.074	.084	.044	.057	.066
2%	.236	.324	.390	.202	.248	.324	.149	.199	.225
5%	.808	.887	.954	.746	.831	.873	.653	.733	.772
10%	.994	.998	1.000	.991	.994	.998	.975	.987	.994

Table 5

Power of Tests, as a Function of Trait-Allele Frequency, with Either T3B3 Sampling or Random Sampling and with Diallelic Marker Loci: σ_a^2 = 2%, σ_{pg}^2 = 0%, p = .5, D' = .99, α = .0

Trait-Allele Frequency	Power of Test								
	MGA	MGG	TMA	TMG	CC	A1	A2	A3	A4
.05	.162	.137	.238	.172	.088	.104	.109	.143	.126
.1	.293	.232	.432	.328	.159	.145	.154	.200	.182
.5	.718	.623	.910	.849	.416	.545	.665	.755	.696

Figure 1 shows the effect on the power of the TMA test when there are three trait alleles. Two of the alleles are equally frequent and have equal and opposite effects and randomly assigned founder haplotypes and entered the population 100 generations ago; the third allele has no founding haplotype and an effect intermediate between those of the two alleles with founding haplotypes. For both di- and decallelic marker loci, the power maximizes at <100%. The power of the TMA test is ~90%, for both σ_a^2 = 5% and σ_a^2 = 10%, when $\theta < .001$ ($D' > .99$), whereas table 1 indicates that power is nearly 100% when there are only two trait alleles. Additional information will not allow an increase in the power. For a diallelic locus, the power maximizes at slightly >50%.

Error Rates

The effect that disequilibrium due to admixture has on the error rates is shown in table 8, which compares the error rate for all nine disequilibrium tests when σ_a^2 = 10%, σ_{pg}^2 = 0%, and p = .5, for a nominal α = .05 as a function of D'^* when diallelic marker loci are used. In the absence of disequilibrium due to admixture, the observed significance levels of all the tests conformed well with the nominal levels for the tested sample size of either 1,000 individuals or 1,000 TDT trios. In general, the more powerful the test when there is not disequilibrium due to admixture, the greater the error rate in the presence of disequilibrium due to admixture. The TMA test, the most powerful, had the greatest excess error rate, only 15% when D'^* = .2 but 94% when D'^* = .8, when T3B3 sampling was used. The error rates for the Allison test were unaffected by the presence of disequilibrium due to admixture. In addition, the greater the linked genetic variance, the greater the increase in error rate for tests involving isolated individuals. Other factors that increased the error rate were the stringency of selection, the trait-allele frequency, and the amount of disequilibrium due to admixture. Error rates were not affected by the presence of polygenes (data not shown).

Power in the Presence of Disequilibrium Due to Admixture

Figure 2 shows the power that the nine disequilibrium tests have for detection of a 10% linked genetic effect when p = .1 and there are no polygenes, after adjustment the critical values have been adjusted for the presence of disequilibrium due to admixture. When the disequilibrium due to admixture is low, D'^* = .05, the decrease in the power of the tests using isolated individuals is small. However, when D'^* = .2, the A3 test using T3B3 sampling became the most powerful test. The power of the A3 test is 73%, and the TMA test has 70% power for a sample of 1,000 eligible individuals. As D'^* becomes larger, the Allison tests perform progressively better than the tests based on isolated individuals. By the time $D'^* > .5$, all the Allison tests are more powerful than any of the non–TDT-based tests.

Analysis of apo E Data

Table 9 presents the P values for each of the tests, as well as the sample size for each of the apo E analyses. In the population that we studied, the frequencies of the alleles were as follows: e2, .076; e3, .772; and e4, .152. A total of 307 families had at least one child with both LDL-cholesterol levels and apo E genotypes. For similar sample sizes, the MGA test had results that were more significant than those of the MGG test, for all the models tested, but the Arg112Cys locus was nonsignificant for both the MGA test and the MGG test. The TMA test and the TMG test, when T3B3 sampling was used, had sample sizes similar to those of the MGA test and the MGG test (307 vs. 301). However, the TMA test's and the TMG test's association of the Cys112Arg locus was highly significant for LDL-cholesterol levels. When T1B1 sampling was used, only slightly more than half the families had a usable individual. As a result of smaller sample size, the T1B1-sampling results are less significant than

Table 6

Power of All Tests, as a Function of Trait-Allele Frequency, with Decallelic Markers and Either T3B3 Sampling or Random Sampling: σ_a^2 = 2%, σ_{pg}^2 = 0%, p = .5, D' = .99, α = .05

Trait-Allele Frequency	Power of Test				
	MGA	MGG	TMA	TMG	CC
.05	.358	.132	.524	.218	.171
.1	.523	.194	.761	.346	.252
.5	.817	.404	.959	.666	.435

Table 7

Power of the TMG Test and the A1 Test, as a Function of Polygenic Variance, Trait-Allele Frequency, and Number of Marker Alleles: σ_a^2 = 2%, p = .5, D' = .99, α = .05

	Power of Test								
	TMG								
	2 Markers			10 Markers			A1, 2 Markers		
σ_{pg}^2 (%)	.5	.1	.05	.5	.1	.05	.5	.1	.05
0%	1.000	.625	.266	1.000	.998	.882	.997	.587	.333
10%	1.000	.602	.270	1.000	.992	.911	.999	.572	.340
30%	1.000	.609	.249	1.000	.993	.908	.998	.569	.335

the T3B3-sampling results. Interestingly, the CC tests, under both selection schemes, provides power comparable to that of the TMA test and the TMG test.

The sample sizes for the Allison tests were smaller than those involving random sampling. At locus Cys112Arg, only 122 families met the inclusion criterion; at locus Arg158Cys, only 76 did so. Despite the smaller sample size, the Arg158Cys locus was more significantly associated with differences in LDL-cholesterol levels than was the Cys112 Arg locus. The A2 test performed quite well compared with the A3 test.

Discussion

The methods that we have called the "truncated measured genotype" tests had not previously been given a name, but the tests have been previously used (Surgochov et al. 1996). The premise behind the test is that, as Lander and Botstein (1989) pointed out, the extreme observations contain most of the information for genetic-linkage studies; thus, when only individuals with extreme phenotypic values are genotyped, less genotyping effort need be expended to find a locus of given size. Extreme sampling is used for several other tests considered here, including the CC test and the A2–A4 tests. The TMA test and the TMG test are performed in a manner similar to that used for the MGA test and the MGG test, with ANOVA being used for determination of the significance levels of any differences between the means of the bins. Use of extreme sampling violates the ANOVA test's assumption that the underlying distribution is continuous and normally distributed; if a sufficiently large sample is taken, the central-limit theorem assumes that the significance levels generated by ANOVA will be correct. The results of the simulations that have been performed in the present study suggest that this is a valid assumption (data not shown).

Although many linkage-disequilibrium tests exist for the localization of genes influencing quantitative traits, a thorough comparison of all the methods has not been performed. Allison (1997) has compared the performance of the tests that he has developed but has not compared his method with other tests of association for quantitative traits. Our goal has been to provide this comparison.

Tables 1 and 2 compare the performance of several disequilibrium tests. All the tests compared are for a sample of 1,000 people. For both di- and decallelic markers, the TMA test was the most powerful per person analyzed. The MGA test had less power per person analyzed, but the increased power of the TMA test was due to selected sampling. The TMA test requires that 1,738 individuals be genotyped to establish disequilibrium to a locus accounting for 10% of the total phenotypic variance, with 90% power and α = .0001, for T3B3 sampling and diallelic marker loci, when p = .5 and D' = .6, so a total of 2,897 (1,738/.6) individuals need to be phenotyped to perform that TMA test; but the MGA test requires that only 2,682 individuals be phenotyped. Similar observation are seen for the other parallel tests—the TMG/MGG tests and the A1/A3 tests (data not shown)—indicating that the selection is enriching with respect to the most informative individuals.

As did Allison, we found that the A3 test is generally the most powerful of the TDT-based tests, but it is less powerful than either the TMA test or the TMG test. The CC test was the least powerful for diallelic loci, and the MGG test and the CC test were equally least powerful for decallelic loci. The CC test was least powerful because much of the information contained in the continuous distribution is lost when the phenotype is reclassified as discrete.

The number of marker alleles affects both the power to detect disequilibrium and the relationship between the powers of the tests. For the TMA test the decalleic markers had more information, but for the TMG test the diallelic markers had more information. This is a function of df when the tests are compared: 1 df for the

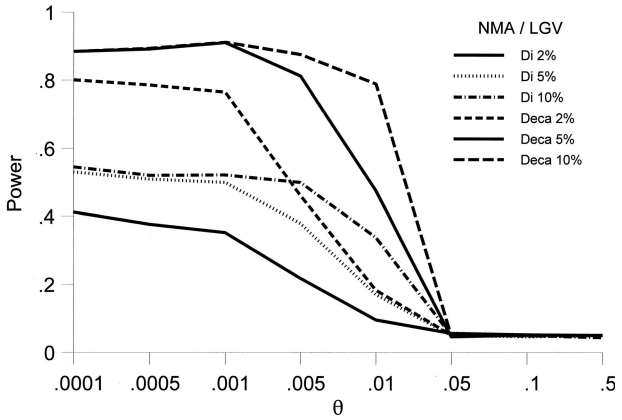

Figure 1 Power of the TMA test when there are three trait alleles, as a function of θ, number of marker alleles (NMA), and linked genetic variance (LGV): σ_{pg}^2 = 0%, t = 100, p = .1, .1, and .8.

Table 8

Error Rates for Tests, with Diallelic Marker as a Function of (D'^*), with Either Random or T3B3 Sampling: $\sigma^2_a = 10\%$, $\sigma^2_{pg} = 0\%$, $\theta = .5$, $p = .5$, Nominal $\alpha = .05$

	Power of Test								
D'^*	MGA	MGG	TMA	TMG	CC	A1	A2	A3	A4
.0	.05	.05	.05	.05	.05	.05	.05	.05	.05
.2	.098	.088	.067	.138	.126	.045	.042	.054	.055
.4	.246	.198	.126	.391	.306	.047	.038	.046	.050
.6	.543	.424	.244	.747	.634	.046	.033	.050	.045
.8	.782	.678	.389	.935	.833	.033	.032	.040	.038

TMA test and 2 df for the TMG test, when diallelic marker loci are used. When decallelic loci are used, the TMA test has 9, or $n - 1$, df, and the TMG has 54, or $n(n + 1)/2 - 1$, df. This difference in df contributes to the decrease in power of both the MGG test and the TMG test when decallelic, rather than diallelic markers, are used. The simulations used here assumed that the trait allele acts additively. However, if there is dominance, effects of heterosis may be lost when either the TMA test or the MGA test is used.

Tables 3 and 4 show linkage-disequilibrium tests under a variety of sampling schemes. As would be expected, the more stringent the sampling, the greater the power of a given sample size. Although an increase in power was observed for selected sampling, the large increase observed by Risch and Zhang (1995) was not seen. The sample size required for detection of disequilibrium when the MGA test is used is 2,682 but is only 950 when the TMA test and T1B1 sampling are used; this is a decrease of only slightly less than threefold. We did not expect that there would be a sample-size reduction as large as that found by Risch and Zhang (1995). Since only a single individual was selected per family, there was no way to account for the effect of sib-sib correlation and/or shared genetic factors, the key to the sample-size reduction observed by Risch and Zhang (1995). These observations may only hold in the case of a single "major" gene, since there is evidence that extreme sampling on an oligogenic model may not increase power (Allison et al. 1998).

Tables 5 and 6 show that the power to detect disequilibrium decreases with decreasing trait-allele frequency. This observation is different from that of Allison (1997), who reported that power increased with decreasing trait-allele frequency, but is similar to that of Abel and Müller-Myhsok (1998). The difference can be attributed to different simulation methods. Allison (1997) assumed total disequilibrium between a marker and trait allele—that is, the specific marker allele is found only with the trait allele, and vice versa. When the trait-allele frequency is varied, then, to get a constant linked genetic variance, the effect of the trait alleles must be increased. When one can separate the trait-carrying and non–trait-carrying chromosomes, as one can do with Allison's tests, the test is dominated by the differences in the trait-allele effects. The simulations reported here did not follow those conditions. Disequilibrium could decay, and all copies of a marker allele were never in complete disequilibrium with a trait allele. Under the assumption that the marker alleles have equal frequencies in the general population, that the marker locus is diallelic, and that the trait-allele frequency is .5, a maximum of two-thirds of the specific marker allele would be in disequilibrium with a trait allele: $\frac{1}{2}/[\frac{1}{2} + (\frac{1}{2} * \frac{1}{2})]$. All of the chromosomes with the trait allele will have the same specific marker allele, but, also, 50% of the chromosomes without a trait allele will also have the same specific marker allele. If the trait allele has a frequency of .1, then only 10/55 of the specific marker alleles with be in disequilibrium with the trait allele: $\frac{1}{10}/(\frac{1}{10} + \frac{9}{10} * \frac{1}{2})$. Since the specific marker allele becomes less informative when the trait-allele frequency decreases, the power to detect disequilibrium decreases.

Our simulations allow for the trait-allele frequency and linked genetic variance to be specified and known and that there to be alleles that are found on the founder haplotype but not on a haplotype with trait allele A. However, the marker-allele frequencies in the simulated population are not the same as those in the general population. When an allele at a locus is in disequilibrium with the trait allele, the expected total count of that marker allele is increased by $2N(1 - \theta)^t$ over the number expected in the general population, and the other alleles decrease proportionally, across all alleles. The expression $(1 - \theta)^t$ denotes the expected proportion, of the total number of trait haplotypes, that continues to carry the original allele at the marker locus.

Unlike linkage tests that use identity by descent (Risch and Zhang 1995; Page et al. 1998), polygenic factors do not increase the ability to detect the effect of linked loci. This is to be expected, since only a single individual

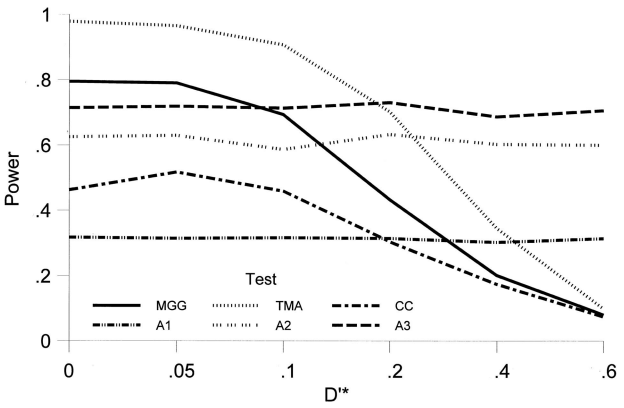

Figure 2 Adjusted power rates for all tests, as a function of D'^*: $\sigma^2_a = 10\%$, $\sigma^2_{pg} = 0\%$, adjusted to $\alpha = .05$, and $p = .1$.

Table 9

Significance, at Various Sample Sizes, of Disequilibrium Analysis for Effect of Variation, at apo E Locus, on Variation in LDL-Cholesterol

Test and Sampling	Power (Sample Size) for[a]					
	Cys112Arg$_a$	Cys112Arg$_g$	Arg112Cys$_a$	Arg158Cys$_g$	apo E$_a$	apo E$_g$
MGA:						
Random	.1211 (307)	NA	.0031 (307)	NA	.0018 (307)	NA
MGG:						
Random	NA	.2814 (307)	NA	.0027 (301)	NA	.0210 (307)
TMA:						
T3B3	.0050 (301)	NA	<.0001 (301)	NA	<.0001 (301)	NA
T1B1	.0175 (173)	NA	<.0001 (173)	NA	<.0001 (173)	NA
TMG:						
T3B3	NA	.0154 (301)	NA	<.0001 (301)	NA	<.0001 (301)
T1B1	NA	.0555 (173)	NA	.0003 (173)	NA	.0004 (173)
CC:						
T3B3	.0040 (301)	.0129 (301)	.0002 (301)	.0007 (301)	<.0001 (301)	.0007 (301)
T1B1	.0150 (173)	.0460 (173)	<.0001 (173)	.0002 (173)	<.0001 (173)	.0006 (173)
A1:						
Random+TDT	.4782 (122)	NA	.1110 (76)	NA	NA	NA
A2:						
T3B3+TDT	.1210 (119)	NA	.1170 (75)	NA	NA	NA
T1B1+TDT	.0620 (70)	NA	.0290 (42)	NA	NA	NA
A3:						
T3B3+TDT	.1255 (119)	NA	.0841 (75)	NA	NA	NA
T1B1+TDT	.1680 (70)	NA	.0576 (42)	NA	NA	NA
A4:						
T3B3+TDT	.1305 (119)	NA	.1245 (75)	NA	NA	NA
T1B1+TDT	.1750 (70)	NA	.0912 (42)	NA	NA	NA

[a] NA = not applicable.

was used per family, and there is no way to account for the similarity between relatives that is due to shared polygenes.

Simulations involving trait loci with three trait alleles, two of which have equal and opposite effects and arose on independent founder haplotypes, also were performed. All the tests have the correct error rate under the null hypothesis, for di- and decallelic marker loci; however, under the alternative hypotheses the power never reached 100%. When independent haplotypes are formed for two trait alleles, both haplotypes may have the same allele at a particular locus, and the probability of this event is 1 − heterozygosity of the marker locus, where heterozygosity is $(1 - \Sigma p_i^2)$ and p_i is the frequency of the ith allele. The nonuniqueness of founding haplotypes has been noted in previous studies; in Greek populations, for example, the LDL receptor has at least six independent mutations causing familial hypercholesterolemia, each with its own founding haplotype (Traeger-Synodinos et al. 1998); at two separate marker loci, two different disease-causing mutations are in disequilibrium with the same allele. We suspect that most genes that affect quantitative variation will have more than two alleles; for example, apo E has two diallelic polymorphisms (Funke et al. 1986), and Lp(a) (Boerwinkle et al. 1989) has many.

Although linkage-disequilibrium methods tend to require sample sizes smaller than those required by linkage-equilibrium methods, disequilibrium methods are subject to increases in error rates, because of population admixture and stratification. Another factor blocking the acceptance of linkage-disequilibrium methods is that the degree of increase in error rates that is due to admixture has not been rigorously studied. Spielman and Ewens (1996) have given limited estimates of the increase in error rates for non–TDT-based family-based tests of qualitative disequilibrium. Our analyses provide results for comparison of the error rates of different linkage tests in the presence of disequilibrium due to admixture

As has been predicted, the error rate of disequilibrium tests based on isolated samples—that is, non-QTDT—is affected by the presence of disequilibrium due to admixture, and the nominal error rates of the Allison test, QTDT, were not affected by disequilibrium due to admixture. Note that all tests had the correct error rates when there was no disequilibrium due to admixture. In general, the more powerful a non-QTDT test was, the more liable it was to errors resulting from admixture. Factors that influence the power of a linkage-disequilibrium test also influence error rates. Selection increases power, but it also increases the error rate in admixed populations. Decreasing the trait-allele frequency decreases both power and error rate. Because disequilibrium due to admixture actually does exist, association tests that have more power to detect any disequilibrium

will also be more powerful for detection of undesired disequilibrium. Replicate sampling from the same population may have the same amount of disequilibrium due to admixture. As Allison (1997) and Rabinowitz (1997) have predicted, TDT-based tests are not affected by the presence of disequilibrium due to admixture.

The use of non–TDT-based tests in an admixed population will cause an increase in the false-positive error rates of the test. However, the number of false-positive results will be dependent on the amount of disequilibrium due to admixture present in the sampled population. Although the amount of disequilibrium that would result from admixture of specific populations has been considered (Dean et al. 1994), the actual amount of disequilibrium due to admixture has not ever been well characterized, and thus we cannot make inferences about the absolute number of false-positive results that may result from the use of non–TDT-based tests.

The presence of unlinked disequilibrium leads to a substantial increase in error rates, but even in the presence of unlinked disequilibrium there may still be disequilibrium due to linkage. Figure 2 has been provided in order to show the effect that unlinked disequilibrium will have on the power to detect an effect of given size after an adjustment is made to the critical values so that the proper level of significance in achieved even in the presence of admixture. The presence of unlinked disequilibrium, D'^{*}, strongly decreases the power to detect an effect of given size, for tests not based on TDT sampling. The power of the Allison (1997) tests are not affected by the presence of disequilibrium due to admixture. For $D'^{*} > .5$, all the Allison tests are more powerful than tests using isolated individuals.

Analysis of the apo E data by the disequilibrium tests is quite informative. As expected, variation in the apo E gene was found to affect interindividual variation in LDL-cholesterol levels. Tests using both alleles and genotypes found valid results. The use of selected sampling increased the power to detect the effect of apo E, until the selection criterion began to reduce the available sample size. In view of both the sample size that was available ($\leqslant$307 individuals) and the size of the effect of apo E (~8% of the total phenotypic variance in LDL-cholesterol levels), several of the tests found very significant results for the apo E locus. Several of the tests (A1–A4) did not find significant results, primarily because of the diminished available sample size.

When a study is developed or disequilibrium tests are applied to an existing data set, it is essential to bear in mind that, unless the study is carefully designed to collect only random individuals or TDT trios, much of the data collected cannot be validly analyzed without allowance for familial correlations.

In summary, the TMA test is the most powerful test of disequilibrium, under a variety of conditions. The linked genetic variance, the trait-allele frequency, and the stringency of selection affect both all tests' power to detect disequilibrium and the error rates of non–QTDT-based tests. The presence of polygenes did not affect either the disequilibrium tests' power to detect disequilibrium or their error rate. Allison's (1997) TDT-based tests were not affected by the presence of disequilibrium due to admixture, but all the other tests were.

Acknowledgments

We would like to thank Drs. Jonathan C. Cohen and Jingpin Wang for graciously providing the apo E genotypes and LDL-cholesterol levels. We would like thank Drs. Maureen Goode and David Allison and two anonymous reviewers for their comments on this work. This research was supported by grants GM-52607 and ES-09912 (to C.I.A.). The apo E data were collected under grant HL-53917 (to Jonathon C. Cohen).

References

Abel L, Müller-Myhsok B (1998) Maximum-likelihood expression of the transmission/disequilibrium test and power considerations. Am J Hum Genet 63:664–667

Allison DB (1997) Transmission-disequilibrium tests for quantitative traits. Am J Hum Genet 60:676–690

Allison DB, Heo NM, Schork, NJ, Wong S-L, Elston RC (1998) Extreme selection strategies in gene mapping studies of oligogenic quantitative trait do not always increase power. Hum Hered 48:97–107

Amos CI (1994) Robust variance-components approach for assessing genetic linkage in pedigrees. Am J Hum Genet 54: 535–543

Boerwinkle E, Chakraborty R, Sing CF (1986) The use of measured genotype information in the analysis of quantitative phenotypes in man. Ann Hum Genet 50:181–194

Boerwinkle E, Menzel HJ, Kraft HG, Utermann G (1989) Genetics of the quantitative Lp(a) lipoprotein trait. III. Contributions of Lp(a) glycoprotein phenotypes to normal lipid variation. Hum Genet 82:73–78

Boerwinkle E, Viscikis S, Welsh D, Steinmetz J, Hamash SM, Sing CF (1987) The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphisms in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglycerides in a sample of unrelated individuals. Am J Med Genet 27:567–582

Dean M, Stephens JC, Winkler C, Lomb DA, Ramsburg M, Boaze R, Stewart C, et al (1994) Polymorphic admixture typing in human ethnic populations. Am J Hum Genet 55: 788–808

Funke H, Rust S, Assman G (1986) Detection of apolipoprotein E variants by an oligonucleotide "melting" procedure. Clin Chem 32:1285–1289

Hastbacka J, de la Chapelle A, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, et al (1994) The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 78:1073–1087

Kaplan NL, Martin ER, Weir BS (1997) Power studies for the transmission/disequilibrium tests with multiple alleles. Am J Hum Genet 60:691–702

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Schork NJ (1994) Genetic dissection of complex traits. Science 265:2037–2048

Li CC (1976) First course in population genetics. Boxwood Press, Pacific Grove, CA

Mohrenweiser HW, Jones IM (1998) Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation. Mutat Res 400:15–24

Nickerson DA, Taylor SI, Weiss KM, Clark AG, Hutchinson RG, Stengard J, Meyers RH, et al (1998) DNA sequence diversity in a 9.7 kb region of the human lipoprotein lipase gene. Nat Genet 19:233–240

Ott J (1991) Analysis of human genetic linkage, rev ed. Johns Hopkins University Press, Baltimore

Ott J, Rabinowitz D (1997) The effect of marker heterozygosity on the power to detect linkage disequilibrium. Genetics 147:927–930

Page GP, Amos CI, Boerwinkle E (1998) The quantitative LOD score: test statistic and sample size for exclusion and linkage of quantitative traits in human sibships. Am J Hum Genet 62:962–968

Rabinowitz D (1997) A transmission disequilibrium test for quantitative trait loci. Hum Hered 47:342–350

Risch N, Zhang H (1995) Extreme discordant sib pairs for mapping quantitative trait loci in humans. Science 268: 1584–1589

Spielman RS, Ewens WJ (1996) The TDT and other family-based tests for linkage disequilibrium and association. Am J Hum Genet 59:983–989

Spielman RS, McGinnis RE, Ewens WJ (1993) Transmission test for linkage disequilibrium: the insulin region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506–516

Surguchov AP, Page GP, Smith L, Patsch W, Boerwinkle E (1996) Polymorphic markers in apolipoprotein C-III gene flanking regions and hypertriglyceridemia. Arterioscler Thromb Vasc Biol 16:941–947

Trager-Syndinos J, Mavroidis N, Kanavakis E, Drogari E, Humphries SE, Day IN, Kattamis C, et al (1998) Analysis of low density lipoprotein receptor gene mutations and microsatellite haplotypes in Greek FH heterozygous children: six independent ancestors account for 60% of probands. Hum Genet 102:343–347

Wang J, Freeman DJ, Grundy SM, Levine DM, Guerra R, Cohen JC (1998) Linkage between cholesterol 7α-hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. J Clin Invest 101:1283–1291

Weiss KM (1993) Genetic variation and human disease. Cambridge University Press, Cambridge