

QTL Fine Mapping by Measuring and Testing for Hardy-Weinberg and Linkage Disequilibrium at a Series of Linked Marker Loci in Extreme Samples of Populations

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It has recently been demonstrated that fine-scale mapping of a susceptibility locus for a complex disease can be accomplished on the basis of deviations from Hardy-Weinberg (HW) equilibrium at closely linked marker loci among affected individuals. We extend this theory to fine-scale localization of a quantitative-trait locus (QTL) from extreme individuals in populations, by means of HW and linkage-disequilibrium (LD) analyses. QTL mapping and/or linkage analyses can establish a large genomic region (~30 cM) that contains a QTL. The QTL can be fine mapped by examination of the degree of deviation from HW and LD at a series of closely linked marker loci. The tests can be performed for samples of individuals belonging to either high or low percentiles of the phenotype distribution or for combined samples of these extreme individuals. The statistical properties (the power and the size) of the tests of this fine-mapping approach are investigated and are compared extensively, under various genetic models and parameters for the QTL and marker loci. On the basis of the results, a two-stage procedure that uses extreme samples and different tests (for HW and LD) is suggested for QTL fine mapping. This two-step procedure is economic and powerful and can accurately narrow a genomic region containing a QTL from ~30–1 cM, a range that renders physical mapping feasible for identification of the QTL. In addition, the relationship between parameterizations of complex diseases, by means of penetrance, and those of complex quantitative traits, by means of genotypic values, is outlined. This means that many statistical genetic methods developed for searching for susceptibility loci of complex diseases can be directly adopted and/or extended to QTL mapping for quantitative traits.

Introduction

For diseases and quantitative traits with complex genetic and environmental determinations, with the use of current analytical and technical tools, fruitful linkage analyses and quantitative-trait locus (QTL) mapping in humans and in experimental organisms generally can locate a disease-susceptibility locus or a QTL to a genomic region of ~30 cM. In the present study, QTLs denote loci underlying the variation of continuous quantitative traits. One central objective of linkage analyses and QTL mapping is to find individual specific genes underlying the differential susceptibilities of complex diseases and those underlying the variation of quantitative traits. However, physical mapping of such individual genes generally is not feasible, unless a genomic region containing such a gene can be reduced, through the use of fine-scale mapping, to a region of ~1 cM.

Fine mapping is relatively simple for Mendelian traits

with high penetrance, by means of haplotype analyses of recombination event(s) in extended pedigrees whose members are densely genotyped at markers at ~1-cM intervals (Boehnke 1994; Glaser et al. 1995). For more complex diseases, linkage-disequilibrium (LD) mapping (Hastbacka et al. 1992, 1994; Bennett et al. 1995) and association studies (Corder et al. 1993) have successfully been employed to locate genes for diastrophic dysplasia, insulin-dependent diabetes mellitus type 2 (IDDM2), and late-onset Alzheimer disease. Maturing under constant development in recent years, some fine-mapping techniques (e.g., Terwilliger and Ott 1992; Spielman et al. 1993; Curtis and Sham 1995; Sham and Curtis 1995*a*, 1995*b*; Kaplan et al. 1997; Martin et al. 1997; Tregouet et al. 1997; Sham 1997; Xiong and Guo 1997*a*; Boehnke and Langefeld 1998; Lazzeroni and Lange 1998; Nielsen et al. 1998; Spielman and Ewens 1998; Xiong and Jin 1999) have been proposed for complex diseases. These techniques use different types of samples from populations, such as random case patients and control individuals, nuclear families, sibs, or case patients only. Comparatively speaking, development of fine-mapping techniques for continuously distributed quantitative traits has received relatively less attention (however, see Allison 1997; Rabinowitz 1997; Tregouet et al. 1997; Xiong and Guo 1997*b*; Fulker et

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al. 1999). In particular, there are few studies of fine mapping of the QTL from unrelated samples in large random-mating populations. This may, in part, be a result of the perceived importance of the diseases within the framework of clinical diagnosis and public health. However, the clinical importance and health relevance of many quantitative traits (such as bone mass, diabetes, obesity, cholesterol level, and blood pressure) have increasingly been recognized (Fulker et al. 1999). In addition, as has repeatedly been tested by the Hardy-Weinberg (HW)–equilibrium law, random mating holds reasonably well for samples that are carefully selected from relatively homogeneous populations of the same ethnicity and/or geographic location (see Deng et al. 1998).

Development of fine-mapping techniques for complex diseases and quantitative traits has largely been done in isolation; the direct relevance and the applicability of the techniques developed for complex diseases have seldom, if ever, explicitly been given for analyses of quantitative traits or vice versa. This is largely a result of the different parameterizations of genetic diseases and quantitative traits. For example, genetic diseases are commonly modeled by population prevalence, penetrance of the underlying susceptibility loci (which is the probability that a disease will develop in an individual, given that he/she has a certain genotype), and phenotype rates. However, for quantitative traits, the central parameters are population mean and variance and the genotypic values of QTLs (Falconer 1989; Lynch and Walsh 1998). It is well known that complex diseases can be viewed as threshold traits that can be modeled by underlying, continuously distributed quantitative traits (liabilities, Falconer 1989; Lynch and Walsh 1998). Therefore, a theoretical framework that unifies and bridges the techniques developed for complex diseases and quantitative traits is needed. This theoretical framework will provide a basis for direct adoption of many methods of mapping disease loci for QTL.

A novel fine-mapping approach was recently proposed and was successfully applied to fine map a susceptibility locus for hereditary hemochromatosis to a genomic region of ~600 kb (Feder et al. 1996). In the Feder et al. study, fine mapping was accomplished by utilization of the degree and pattern of HW disequilibrium among affected individuals and of LD among affected and nonaffected individuals, for a panel of densely typed markers. Fine mapping through use of the HW test was examined and was extended theoretically (Nielsen et al. 1998) for complex genetic diseases. It has been shown, both empirically (Feder et al. 1996) and theoretically (Nielsen et al. 1998), that HW-disequilibrium tests done only with the use of affected individuals can be more powerful and accurate in the fine mapping of a disease-susceptibility locus than can LD methods

done with the use of both affected and control individuals.

In this article, we developed the methodological counterpart of the novel fine-mapping approach used by Feder et al. (1996) and Nielsen et al. (1998) for fine localization of QTL(s) for quantitative traits. We extended the method so that samples from both extremes of the quantitative-trait distribution can be combined for analyses, to substantially increase the mapping power. Under a variety of parameter space and inheritance models, we performed extensive computer simulations to investigate and to compare the statistical properties (power and size) of the new method for QTL fine mapping. On the basis of these results, we proposed and examined a two-stage procedure for fine mapping a QTL from a genomic region of ~30–1 cM, which will make physical mapping feasible for identification of individual QTLs. In addition, we present a general framework that may serve as a theoretical bridge to unify mapping techniques for complex diseases and those for quantitative traits.

Methods

Two Alleles at Both the Marker Locus and the QTL

Define a QTL locus with two alleles: A1 and A2. Allele A2 may also be regarded as all of the non-A1 alleles with similar genetic effects. Let p be the frequency of the allele A1, and let $q = 1 - p$ be the frequency of allele A2. Let a be the mean (genotypic value) for individuals with genotype A1A1, let d be the genotypic value of individuals with A1A2, and let $-a$ be the genotypic value of A2A2 individuals. This is a general model for a QTL (Falconer 1989). The d value is equal to 0, a , and $-a$, respectively, under additive, dominant, and recessive genetic effects. Under partial dominant or partial recessive genetic effects, $-a < d < a$ but $d \neq 0$. The additive genetic variance of this locus is $\sigma_A^2 = 2pq[a + (q - p)d]^2$, and the dominant genetic variance is $\sigma_D^2 = (2pqd)^2$ (Falconer 1989). The total genetic variance resulting from this QTL is $\sigma_G^2 = \sigma_A^2 + \sigma_D^2$. We assume that the variance resulting from all other QTLs and all random environmental effects is σ_E^2 . The heritability, h^2 , that results from this QTL is $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_E^2)$. The phenotypic value of an i th individual in the population is $y_i = \mu + G_i + e_i$, where μ is the mean baseline value of the quantitative trait, where G_i is the genotypic value at the QTL for the i th genotype, and where e_i represents a random variable for the combined effects of all the rest of the polymorphic QTLs and all random environmental effects. G_i is equal to a , d , and $-a$, respectively, for genotypes A1A1, A1A2, and A2A2. Without loss of generality, we can assume that $\mu = 0$. We can also assume that e_i follows a normal

distribution with a mean of 0 and variance of σ_E^2 —that is, $e_i \sim N(0, \sigma_E^2)$.

As in the original investigations of Feder et al. (1996) and Nielsen et al. (1998), random mating is assumed, and, thus, HW equilibrium holds in the population. The quantitative trait in the population then follows a mixture distribution of three normal distributions, each of which is weighted by the respective genotype frequencies in the population

$$p(x) = p^2 N(x, a, \sigma_E^2) + 2pq N(x, d, \sigma_E^2) + q^2 N(x, -a, \sigma_E^2),$$

where $N(x, \mu, \sigma^2) = (1/\sqrt{2\pi}\sigma) \exp\{-[(x-\mu)^2/2\sigma^2]\}$, which is the probability density function for a normal random variable x with mean μ and variance σ^2 .

Let us define $\phi(x) = \int_{-\infty}^x (1/\sqrt{2\pi}) e^{-w^2/2} dw$. This is a cumulative distribution function of a standard normal variable. Given a low-threshold value T , we can compute the proportion (φ_T) of the population that has values (y) of the quantitative trait $<T$, or vice versa, to compute T from a given φ_T by use of the following relationship:

$$\varphi_T = p^2 \phi\left(\frac{T-a}{\sigma_E}\right) + 2pq \phi\left(\frac{T-d}{\sigma_E}\right) + q^2 \phi\left(\frac{T+a}{\sigma_E}\right). \quad (1)$$

Similarly, given the upper-threshold value $U (>T)$, we can compute the proportion (φ_U) of the population with $y > U$, or vice versa, to compute U from a given φ_U by use of the following relationship:

$$\varphi_U = 1 - \left[p^2 \phi\left(\frac{U-a}{\sigma_E}\right) + 2pq \phi\left(\frac{U-d}{\sigma_E}\right) + q^2 \phi\left(\frac{U+a}{\sigma_E}\right) \right].$$

Let y be the value of the quantitative trait of a random individual and let $y_{A_1A_1}$ be that of a random individual of genotype A_1A_1 ; $y_{A_1A_2}$ and $y_{A_2A_2}$ are similarly defined. It can easily be seen that

$$\Pr(y < T | A_1A_1) = \Pr(y_{A_1A_1} < T) = \phi\left(\frac{T-a}{\sigma_E}\right) = \phi_{11}, \quad (2a)$$

$$\Pr(y < T | A_1A_2) = \Pr(y_{A_1A_2} < T) = \phi\left(\frac{T-d}{\sigma_E}\right) = \phi_{12}, \quad (2b)$$

and

$$\Pr(y < T | A_2A_2) = \Pr(y_{A_2A_2} < T) = \phi\left(\frac{T+a}{\sigma_E}\right) = \phi_{22}. \quad (2c)$$

Therefore,

$$\begin{aligned} \Pr(A_1A_1 | y < T) &= P_{A_1A_1|y<T} \\ &= \Pr(A_1A_1) \Pr(y_{A_1A_1} < T | A_1A_1) / \Pr(y < T) \\ &= p^2 \phi_{11} / \varphi_T \end{aligned}$$

and

$$\begin{aligned} \Pr(A_1 | y < T) &= p_{A_1|y<T} = \Pr(A_1, y < T) / \Pr(y < T) \\ &= [p \Pr(A_1A_1 | A_1) \Pr(y < T | A_1A_1) \\ &\quad + p \Pr(A_1A_2 | A_1) \Pr(y < T | A_1A_2)] / \Pr(y < T) \\ &= (p^2 \phi_{11} + pq \phi_{12}) / \varphi_T. \end{aligned}$$

Recall that random mating is assumed in the whole population.

Departure from HW equilibrium at the QTL can be measured by the disequilibrium coefficient $D_{A_1A_1} = P_{A_1A_1} - p_{A_1}^2$ (Weir 1996). Among individuals with $y < T$,

$$\begin{aligned} D_{A_1A_1|y<T} &= P_{A_1A_1|y<T} - p_{A_1|y<T}^2 \\ &= p^2 q^2 (\phi_{11} \phi_{22} - \phi_{12}^2) / \varphi_T^2. \end{aligned}$$

HW disequilibrium can also be measured by F_{A_1} (Feder et al. 1996; Nielsen et al. 1998), among individuals with $y < T$:

$$\begin{aligned} F_{A_1|y<T} &= \frac{P_{A_1A_1|y<T} + P_{A_2A_2|y<T} - p_{A_1|y<T}^2 - p_{A_2|y<T}^2}{1 - p^2 - q^2} \\ &= \frac{2D_{A_1A_1|y<T}}{2pq} = \frac{pq(\phi_{11}\phi_{22} - \phi_{12}^2)}{\varphi_T^2}. \end{aligned}$$

A marker locus closely located near the QTL may be in LD with the QTL. That is, alleles at the marker locus and the QTL are associated. Denote a marker allele as M and denote the rest as m , with respective frequencies denoted as p_M and q_m . The degree of the association can be expressed by a measure of LD: $D_{A_1M} = P_{A_1M} - p_{A_1} p_M$ (Crow and Kimura 1970). If A_1 and M are in coupling disequilibrium (Crow and Kimura 1970), then $D_{A_1M} > 0$. Otherwise, if they are in repulsion disequilibrium, $D_{A_1M} < 0$. Among individuals with $y < T$,

$$\begin{aligned} \Pr(MM|y < T) &= P_{MM|y < T} \\ &= [\Pr(MM, A_1 A_1)\Pr(y < T|A_1 A_1) \\ &\quad + \Pr(MM, A_1 A_2)\Pr(y < T|A_1 A_2) \\ &\quad + \Pr(MM, A_2 A_2)\Pr(y < T|A_2 A_2)]/\varphi_T \\ &= [P_{A_1 M}^2 \phi_{11} + 2P_{A_1 M}(p_M - P_{A_1 M})\phi_{12} \\ &\quad + (p_M - P_{A_1 M})^2 \phi_{22}]/\varphi_T \end{aligned}$$

and

$$\begin{aligned} \Pr(M|y < T) &= p_{M|y < T} \\ &= \Pr(M | A_1)\Pr(A_1 | y < T) \\ &\quad + \Pr(M | A_2)\Pr(A_2 | y < T) \\ &= P_M + D_{A_1 M}[p\phi_{11} + (q - p)\phi_{12} - q\phi_{22}]/\varphi_T. \end{aligned} \tag{3}$$

Therefore, among individuals with $y < T$, the HW disequilibrium coefficient at the marker locus is

$$\begin{aligned} D_{MM|y < T} &= P_{MM|y < T} - p_{M|y < T}^2 \\ &= (\phi_{11}\phi_{22} - \phi_{12}^2)D_{A_1 M}^2/\varphi_T^2. \end{aligned} \tag{4}$$

$D_{MM|y < T}$ is nonzero only if there is LD—that is, $D_{A_1 M} \neq 0$ —and if

$$\phi_{11}\phi_{22} - \phi_{12}^2 \neq 0 \tag{5}$$

or, equivalently,

$$\phi\left(\frac{T - a}{\sigma_E}\right)\phi\left(\frac{T + a}{\sigma_E}\right) \neq \left[\phi\left(\frac{T - d}{\sigma_E}\right)\right]^2 \tag{6}$$

Equation (5) indicates that, at the QTL, for individuals with $y < T$, the product of the frequencies of the two homozygotes should not be equal to the square of the proportion of the heterozygote. It is clear, from equation (6), that, for any genetic effects a and d , a range of T values can be chosen so that Inequality (5) holds. Therefore, in practice, various T values can be chosen so that the HW disequilibrium at the marker locus solely reflects the LD in the whole population—that is, $D_{MM|y < T}$ is nonzero if and only if $D_{A_1 M} \neq 0$.

Note that this is quite different from complex genetic diseases (Nielsen et al. 1998), in which the penetrances (corresponding to ϕ 's in the present study; see equations [13a] and [13b] below) of genotypes are generally fixed and unknown. There always exists a possibility that HW equilibrium holds, despite the fact that the marker is closely linked to the QTL and despite the fact that they are in strong disequilibrium. Therefore, as pointed out by Nielsen et al. (1998), to fine map a disease-suscep-

tibility locus through use of the HW-equilibrium test, the genotypic penetrances must not be multiplicative.

The HW-disequilibrium measure of $F_{M|y < T}$ for the marker locus among individuals with $y < T$, is

$$\begin{aligned} F_{M|y < T} &= \frac{P_{MM|y < T} + P_{mm|y < T} - p_{M|y < T}^2 - p_{m|y < T}^2}{1 - p_M^2 - q_m^2} \\ &= \frac{D_{MM|y < T}}{p_M q_m} = \frac{(\phi_{11}\phi_{22} - \phi_{12}^2)D_{AM}^2}{\varphi_T^2 p_M q_m} \end{aligned} \tag{7}$$

In relation to the HW deviation at the QTL, we have

$$F_{M|y < T} = \Delta_{A_1 M}^2 F_{A_1|y < T}, \tag{8}$$

where $\Delta_{A_1 M}^2 = D_{A_1 M}^2/p_M q_m$, which corresponds to those given for affected individuals of complex diseases (Feder et al. 1996; Nielsen et al. 1998).

Equations (4) and (8) convey the essential point that, among extreme individuals (with $y < T$), HW disequilibrium at a marker locus corresponds to the whole-population LD between the marker locus and the QTL. Generally, in individuals at either end of the quantitative-trait distribution, HW disequilibrium exists if and only if there exists a whole-population LD between the marker locus and the QTL. Although it is the latter that has been of general interest in previous fine-mapping efforts, it may be both easier—in some situations—and equivalent to test for the former (Nielsen et al. 1998)—for example, when only case patients are readily available for diseases or when only individuals from one extreme end of the phenotypic distribution are readily available from clinics. Measurement of HW disequilibrium by means of F_M requires the estimation of marker-allele frequencies in the whole population, whereas measurement of HW disequilibrium by means of D_{MM} does not.

A direct measure of LD (Bengtsson and Thomson 1981; Lehesjoki et al. 1993; Feder et al. 1996; Nielsen et al. 1998) is

$$p_{\text{excess}} = \frac{p_{M|\text{affected}} - p_{M|\text{unaffected}}}{1 - p_{M|\text{unaffected}}}. \tag{9}$$

Nielsen et al. (1998) demonstrated that, for the complex genetic-disease model of Feder et al. (1996), p_{excess} is a function of $D_{A_1 M}$. In our general model for a quantitative trait,

$$\begin{aligned} p_{M|y > T} &= \frac{p_M - \Pr(y < T)p_{M|y < T}}{\Pr(y > T)} \\ &= \frac{p_M - \phi_T p_{M|y < T}}{1 - \phi_T}, \end{aligned}$$

where $p_{M|y<T}$ was given in equation (3). Therefore, in individuals with $y < T$,

$$p_{\text{excess}|y<T} = \frac{p_{M|y<T} - p_{M|y>T}}{1 - p_{M|y>T}} = \frac{\delta_1}{\phi_T(1 - \phi_T)[q_m + \delta_1/(1 - \phi_T)]}, \quad (10)$$

where $\delta_1 = D_{A_1M}[p\phi_{11} + (q - p)\phi_{12} - q\phi_{22}]$. Therefore, p_{excess} is also a function of D_{A_1M} for quantitative traits. Similarly, in individuals with $y > U$ (where $U > T$), corresponding measures for HW and LD can also be developed in a manner similar to that described above for $y < T$. It can be seen that HW disequilibrium can be tested with the use of samples from either the upper or the lower end of the phenotypic distribution, as opposed to being tested by means of case-control-type studies that use individuals with $y < T$ and those with $y > T$, which is necessary for an LD measurement such as p_{excess} .

If we sample individuals from both ends of the quantitative distribution with $y < T$ or $y > U$, then we can define a new index of LD as

$$q_{\text{excess}} = \frac{p_{M|y<T} - p_{M|y>U}}{1 - p_{M|y>U}}. \quad (11a)$$

q_{excess} compares the marker-allele frequency in the two opposite extreme ends of the phenotype distribution. It can be shown—as was shown earlier—that:

$$q_{\text{excess}} = \frac{(C_T/\varphi_T - C_U/\varphi_U)D_{A_1M}}{1 - q - C_U D_{A_1M}/\varphi_U}, \quad (11b)$$

where

$$C_T = p\phi_{11} + (1 - 2p)\phi_{12} - (1 - p)\phi_{22}, \quad (11c)$$

$$C_U = p\gamma_{11} + (1 - 2p)\gamma_{12} - (1 - p)\gamma_{22}, \quad (11d)$$

and where $\gamma_{11} = 1 - \phi[(U - a)/\sigma_E]$, $\gamma_{12} = 1 - \phi[(U - d)/\sigma_E]$, and $\gamma_{22} = 1 - \phi[(U + a)/\sigma_E]$.

Similarly, if we have sample individuals from both ends of the quantitative distribution with $y < T$ or $y > U$, we can define a new index of HW disequilibrium in the combined sample of these individuals as

$$CD = [(P_{MM|y<T} - \bar{p}_M^2) - (P_{mm|y<T} - \bar{q}_m^2)] + [(P_{mm|y>U} - \bar{q}_m^2) - (P_{MM|y>U} - \bar{p}_M^2)], \quad (12a)$$

where \bar{p}_M and \bar{q}_m are, respectively, the average frequencies of alleles M and m in individuals with $y < T$ and in

those with $y > U$. Combined disequilibrium is denoted as “CD”. It can be shown that

$$CD = 2(C_T/\varphi_T - C_U/\varphi_U)D_{A_1M}. \quad (12b)$$

Apparently, $CD = q_{\text{excess}}/2(1 - p_M - C_U/\varphi_U)D_{A_1M}$.

It can easily be shown that the disequilibrium measures $D_{MM|y<T}$, F_M , p_{excess} , q_{excess} , and CD are all monotonic functions of the degree of LD in the whole population (D_{A_1M}). Therefore, testing for LD through testing for HW disequilibrium can be performed by use of marker-genotype or allele frequencies of one extreme end and of those in the other extreme end of the phenotype distribution. It is noted that some disequilibrium measures can be negative (e.g., $D_{MM|y<T}$ and F_M in equations [4] and [7]). Therefore, the absolute values of these measures should be used in QTL fine mapping, via the peaks of these measures.

Multiple Alleles at Both the Marker Locus and the QTL

If there are multiple alleles at both the marker locus and the QTL, then the previously developed theory can still be applied, by classification of one allele either as A_1 at the QTL locus or as M at the marker locus and by classification of the rest of the alleles either as A_2 or as m at the QTL and the marker locus, respectively. However, it would be of interest to present a more rigorously defined general theory for QTL fine mapping—one that corresponds to those theories given elsewhere (Nielsen et al. 1998) for disease-susceptibility-gene fine mapping. We define the genotypic value for genotype $A_r A_s$ at the QTL as G_{rs} , where A_r and A_s denote the r th and s th alleles, respectively, at the QTL locus. With various notations defined as they were earlier, then for a threshold value T that corresponds to $\varphi_T = \Pr(y < T)$, we define

$$\phi_{rs} = \Pr(y < T | A_r A_s) = \Pr(y_{A_r A_s} < T) = \Phi\left(\frac{T - G_{rs}}{\sigma_E}\right) \quad (13a)$$

and

$$\varphi_T = \sum P_{A_r A_s} \phi_{rs}. \quad (13b)$$

φ_T and ϕ_{rs} correspond, respectively, to population prevalence and genotype-specific penetrances (or phenocopy rates) for diseases, as in the study by Nielsen et al. (1998). $P_{A_r A_s}$ is the frequency of the genotype $A_r A_s$. Since the rest of the theory of QTL fine mapping for quantitative traits in the instance where $y < T$ can closely follow those theories developed, for diseases, by Nielsen et al. (1998), they will not be elaborated. For individuals with $y > U$ or for the combined sample of those with $y < T$ and those with $y > U$, the mapping theory for quantitative traits follows naturally from the framework

and from the theory developed in the Two Alleles at Both the Marker Locus and the QTL section; therefore, it will not be elaborated here. In fact, the framework, as reflected by equations (13a) and (13b), relates, in a general manner, the parameterizations for complex diseases—in terms of population prevalence, penetrance, and phenocopy rates—with those for quantitative traits, in terms of genotypic values and variance. Thus, this framework can serve as a bridge between the methods developed for gene mapping for continuous quantitative traits and those developed for dichotomous complex diseases. This framework may provide a basis for direct adoption and/or extension of many methods developed for gene mapping of complex diseases to map QTLs by specification of appropriate thresholds.

Statistical Tests

We presented four types of measures that reflect marker/ QTL association resulting from linkage and LD. The first type, p_{excess} (equations [9] and [10]), characterizes LD from random samples with $y < T$. The second type includes HW-disequilibrium measures $D_{MM|y < T}$ (equation [4]) and F_M (equation [7]), in random samples with $y < T$. The third and fourth types, are, respectively, q_{excess} (equations [11a] and [11b]) and CD (equations [12a] and [12b]), both of which use samples with $y < T$ and those with $y > U$ ($U > T$).

The first two types of measures correspond to those used elsewhere (Feder et al. 1996; Nielsen et al. 1998) for fine mapping the disease-susceptibility locus, whereas the last two are new and unique to QTL fine mapping. The last two measures take advantage of our ability to sample individuals with $y < T$ and those with $y > U$. However, this is not feasible for complex diseases with unknown, continuously distributed liabilities that are manifested only through individuals being affected or unaffected, presumably through underlying thresholds. These four types of measures correspond to different practical approaches, in terms of study-subject sampling and data analyses. To compare these approaches, we consider the power of the corresponding test statistics.

The χ^2 test can be used—and is popular—for testing for association on the basis of random samples of case patients and control individuals; this is the design that corresponds to measures 1 and 3. The df is $k - 1$, where k is the number of alleles at the marker locus being tested. Let the tilde (\sim) denote an estimated value from the sample, for the type 1 measure

$$\chi_1^2 = 2n \sum_{i=1}^k \frac{(\tilde{p}_{i|y < T} - \tilde{p}_{i|y > T})^2}{\tilde{p}_{i|y < T} + \tilde{p}_{i|y > T}} \tag{14}$$

For the type 3 measure,

$$\chi_3^2 = 2n \sum_{i=1}^k \frac{(\tilde{p}_{i|y > U} - \tilde{p}_{i|y < T})^2}{\tilde{p}_{i|y > U} + \tilde{p}_{i|y < T}},$$

where $2n$ denotes the total sample sizes of individuals with $y < T$ (case patients) and those with $y > T$ (control individuals). The χ_1^2 test corresponds to the χ_{cc}^2 test used elsewhere (Nielsen et al. 1998). Under the alternative hypothesis of LD, the noncentrality parameters of χ_1^2 and χ_3^2 statistics (Meng and Chapman 1966) are, respectively,

$$\begin{aligned} \lambda_1 &= 2n \sum_{i=1}^k (p_{i|y < T} - p_{i|y > T})^2 / p_{i|y < T} + p_{i|y > T} \\ &= 2n \sum_{i=1}^k \delta_i^2 / \{\varphi_T(1 - \varphi_T)[2\varphi_T(1 - \varphi_T)p_i | y < T \\ &\quad + (1 - 2\varphi_T)\delta_i]\}, \end{aligned} \tag{15a}$$

where

$$\delta_i = [p(\phi_{11} - \phi_{12}) + (1 - p)(\phi_{12} - \phi_{22})]D_{A_{1i}}, \tag{15b}$$

and

$$\begin{aligned} \lambda_3 &= 2n \sum_{i=1}^k \frac{(p_{i|y < T} - p_{i|y > U})^2}{p_{i|y < T} + p_{i|y > U}} \\ &= 2n \sum_{i=1}^k \frac{(C_T/\varphi_T - C_U/\varphi_U)^2 D_{A_{1i}}^2}{2q_i + (C_T/\varphi_T + C_U/\varphi_U)D_{A_{1i}}}, \end{aligned} \tag{16}$$

where $D_{A_{1i}}$ is the LD measure for allele A1 and the i th marker allele. C_T and C_U are defined in equations (11c) and (11d). It should be noted that the coefficient on the left-hand side of equation (14) should be “2” rather than “4” (as was seen—most likely as the result of an error—in Nielsen et al. [1998]).

The χ^2 -test statistics for HW disequilibrium that correspond to the type 2 and type 4 measures (Weir 1996) are as follows:

$$\begin{aligned} \chi_2^2 &= 2n \sum_{i=1}^k \frac{(\tilde{P}_{ii|y < T} - \tilde{P}_{ii|y < T}^2)^2}{\tilde{P}_{ii|y < T}^2} \\ &\quad + 2n \sum_i^k \sum_{j < i}^k \frac{(\tilde{P}_{ij|y < T} - 2\tilde{p}_{i|y < T}\tilde{p}_{j|y < T})^2}{2\tilde{p}_{i|y < T}\tilde{P}_{j|y < T}}, \end{aligned} \tag{17}$$

where $2n$ is the number of individuals with $y < T$. The χ_2^2 test can also be performed in individuals with $y > U$. In this study, for the χ_2^2 test, we examined only those individuals with $y < T$. The χ_2^2 test corresponds to the χ_{HW}^2 test used elsewhere (Nielsen et al. 1998).

For the type 4 measure, the corresponding test statistic is

$$\chi_4^2 = 2n \sum_{i=1}^k \frac{(\tilde{P}_{ij|y<T} - \tilde{p}_i^2)^2 + (\tilde{P}_{ij|y>U} - \tilde{p}_i^2)^2}{\tilde{p}_i^2} + 2n \sum_i^k \sum_{j<i}^k \frac{(\tilde{P}_{ij|y<T} - 2\tilde{p}_i\tilde{p}_j)^2 + (\tilde{P}_{ij|y<U} - 2\tilde{p}_i\tilde{p}_j)^2}{2\tilde{p}_i\tilde{p}_j},$$

where \tilde{p}_i is the estimated average frequency of the i th marker allele in the combined sample of individuals with $y < T$ and those with $y > T$. The total sample size is $2n$. n individuals are from the bottom end of the phenotype distribution, and the other n individuals are from the top end. χ_2^2 has $[k(k - 1)/2]$ df, and χ_4^2 has $[k(k + 1) - 3]$ df. Equation (17) corresponds to equation (5) in the study by Nielsen et al. (1998). It should be noted that, in equation (5) in the study by Nielsen et al. (1998), the coefficient of the first term should be 2 rather than 1 (again, this is presumably the result of an error). Under the alternative hypothesis of linkage and LD between the marker locus and the QTL, the noncentrality parameters of the χ_2^2 and χ_4^2 statistics are, respectively,

$$\lambda_2 = 2n \sum_i^k \sum_j^k \frac{(\varphi_T \delta_{ij} - \delta_i \delta_j)^2}{\varphi_T^2 (\varphi_T p_i + \delta_i) (\varphi_T p_j + \delta_j)}, \quad (18)$$

where p_i is the population frequency of the i th marker allele. In equation (18), $\delta_{ij} = \sum_r \sum_s \phi_{rs} D_{ri} D_{sj}$. D_{ri} measures the LD between the i th marker allele (with frequency p_i) and the r th QTL allele (with frequency q_r). $D_{ri} = P_{ri} - p_i q_r$, where P_{ri} is the population frequency of the haplotype $A_r M_r$. When summed over all the alleles at either the marker or the QTL locus, $\sum_i D_{ri} = \sum_r D_{ri} = 0$. δ_{ij} measures genotypic disequilibrium at the marker loci (for details, see Nielsen et al. [1998]). δ_i and δ_j are defined in the same manner as in equation (15b).

$$\lambda_4 = 2n \sum_{i=1}^k \frac{(P_{ij|y<T} - \tilde{p}_i^2)^2 + (P_{ij|y>U} - \tilde{p}_i^2)^2}{\tilde{p}_i^2} + 2n \sum_i^k \sum_{j<i}^k \frac{(P_{ij|y<T} - 2\tilde{p}_i\tilde{p}_j)^2 + (P_{ij|y<U} - 2\tilde{p}_i\tilde{p}_j)^2}{2\tilde{p}_i\tilde{p}_j}.$$

The analytical relationship between λ_4 and D_{ri} cannot be easily obtained, especially for the multiple-allele situation. The analytical relationship is not important for our investigation or for the practical applicability of our methods. Our simulation shows that there is a positive and monotonic relationship between λ_4 and D_{ri} .

With $k = 2$ under various parameters, the expected power of the χ_1^2 , χ_2^2 , and χ_3^2 tests can be compared directly, by comparison of the noncentrality parameters λ_1 , λ_2 , and λ_3 , since they all have $df = 1$. The detailed statistical properties (power and size) of these tests were extensively investigated by means of computer simulations. It can be shown analytically that, for the two-

allele model, the statistics (χ_1^2 , χ_2^2 , χ_3^2 , and χ_4^2) are monotonic functions of $D_{A_1 M}$, as was substantiated in the computer simulations of the present study. Therefore, they can also serve as disequilibrium measures in QTL fine mapping, since they have the advantage that they are always positive.

Computer Simulations

To substantiate our theoretical results and to illustrate and compare the statistical properties of the various tests outlined above, we performed extensive computer simulations. The computer simulations were performed under a range of parameters and population-sampling strategies (one-side extreme versus two-side extremes, and various percentiles selected, etc.). As seen elsewhere (Nielsen et al. 1998), evolving populations segregating for a biallelic QTL and biallelic markers were simulated under random mating. The simulation parameters were as follows: frequency (p) of allele A1 at the QTL and that (p_M) of allele M at a marker locus, the ratio d/a , thresholds T and U and associated φ_T and φ_U , b^2 resulting from the QTL, and sample size ($2n$), etc. Without loss of generality, σ_E^2 was set to 1.0 throughout, in the simulations used in the present study. The ratio d/a represents the genetic models simulated. $d/a = 1, \frac{1}{2}, 0, -\frac{1}{2}$, and -1 represents, respectively, dominant, partial dominant, additive, partial recessive, and recessive genetic models at the QTL. Unless otherwise specified, we considered a set of dense marker loci that are positioned at 0.25-cM intervals and that span 0–2 cM on both sides of the QTL. In simulations, recombinations between the QTL and the marker locus were independent—that is, there was no interference. The recombination rate was obtained from the physical distance between the QTL and the marker locus, by use of Haldane’s map function (Ott 1991).

Under a specific genetic model, the population started at generation G_0 , with complete association between allele A1 at the QTL and a marker allele M. Then the population evolved for 50 generations, under random mating and genetic drift. To facilitate comparison, as in the study by Nielsen et al. (1998), we retained the first 100 populations for which, after 50 generations, the difference in p ’s at the start and at the end of evolution do not differ by more than 5% of that at the G_0 generation. Unless otherwise specified, the effective population size is 15,000. The genetic drift under such a population size (N_e) is extremely small. On average, the heterozygosity decreases by $\sim(1 - \frac{1}{2N_e})$ per generation (Crow and Kimura 1970), as a result of random genetic drift. Thus, the heterozygosity at the end of evolution should be, on average, $>99.8\%$ of that at generation G_0 . For such a large population size and for minor effects resulting from genetic drift, as seen elsewhere (Nielsen

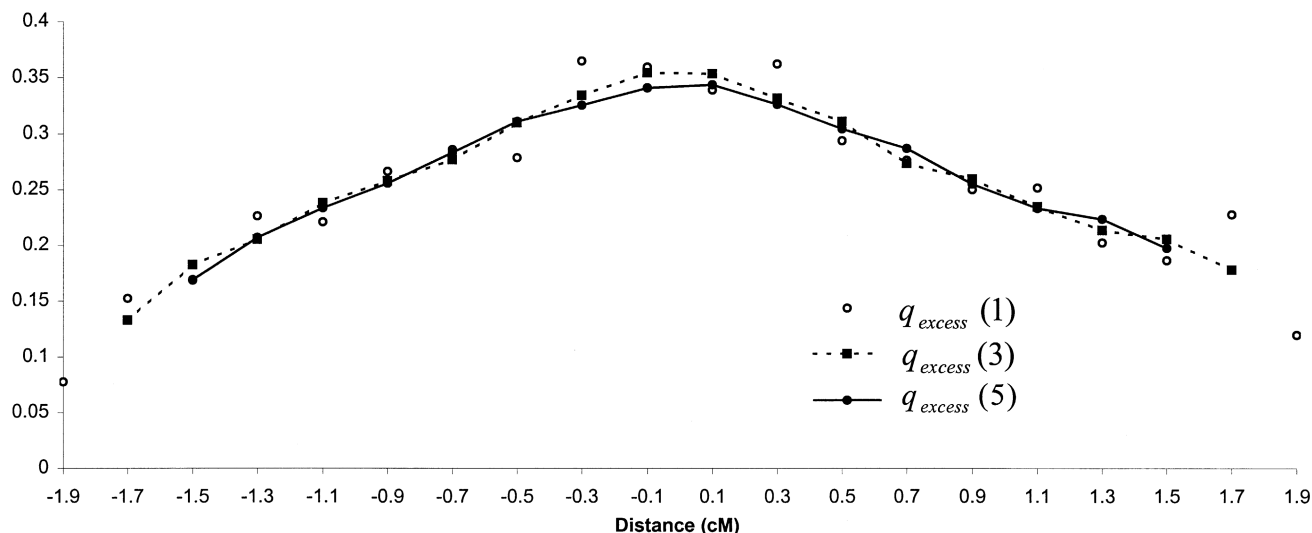


Figure 1 Illustration of the three- and five-point moving-average methods for QTL fine mapping done by use of the measure q_{excess} . If there are L markers genotyped, then there are L raw-data points of the point-wise disequilibrium measures. As is apparent from the figure, from these L raw-data points, there will be $L-2$ and $L-4$ data points, respectively, generated from the moving three- and five-point averages. The peaks of these three- or five-point averages indicate that the QTL is located nearby. The true location of the QTL is 0 on the X-axis. The data were obtained from one simulation, with the use of the following parameters: $p = .1$, $p_M = .2$, $2n = 200$, $b^2 = .20$; 100 extreme individuals were sampled from the bottom 10%, and 100 were selected from the top 10% of the phenotypic distribution, for computation of raw q_{excess} values (equation [11a]). The measures $q_{\text{excess}}(1)$, $q_{\text{excess}}(3)$, $q_{\text{excess}}(5)$ indicate the data for the raw q_{excess} value and for the three- and five-point moving averages of q_{excess} , respectively.

et al. 1998), we did not adjust for genetic drift at the marker loci.

In simulations, we first compared three quantitative genetic models that, according to equations (1) and (2a), (2b), and (2c), are essentially equivalent to the first three complex disease models in table 1 of a study by Nielsen et al. (1998). Note that the multiplicative model used by Nielsen et al. (1998) was not investigated, since the multiplicative model for a QTL can easily be transformed into an additive model by means of a simple log transformation of the quantitative data. This maneuverability of quantitative trait and a range of selectable thresholds render that the HW disequilibrium exists in extreme samples if and only if the LD between the QTL and marker loci exists in the whole population.

We compared statistical properties (power and size) for different tests, under six different genetic models with $d/a = 1, \frac{1}{2}, 0, -\frac{1}{2},$ and -1 . We then compared all these tests under a range of plausible parameter space of p , p_M , φ_T , b^2 , and $2n$. Since changing these parameters has similar effects on different tests, we often presented results for only one or two tests, which should be sufficient to demonstrate our conclusions. Under each parameter set and genetic model, we drew 5,000 appropriate samples (sampling with replacement)—each with $2n$ individuals—from each of the 100 simulated populations, to perform various statistical tests. The percentage of times that the null hypothesis of no disequilibrium was rejected was recorded for each test. This percentage is

the statistical power of a test when the populations were simulated under the alternative hypotheses of LD, and it is the size (type I error) of a test when the simulations were performed under the null hypothesis of no LD. In simulations, we investigated sampling for (1) individuals with $y < T$ (χ_2^2 test), (2) individuals with $y < T$ and $y > T$ (χ_1^2 test), and (3) individuals with $y < T$ and those with $y > U$ (χ_3^2 and χ_4^2 tests).

In practice, the position of a QTL or a disease-susceptibility locus is fine mapped by the peaks of the disequilibrium measures and/or by the test statistics that reflect the degrees of disequilibrium. To indicate the likelihood of success of QTL fine mapping from these peaks, we explored the probability that the peaks fall within a certain distance from the QTL position (the power of QTL fine mapping). To guard against noisy distributions of the disequilibrium measures or test statistics, as has occurred elsewhere (Feder et al. 1996), we located the peaks by means of the j -point moving-average method (see figure 1 in the present study and figure 2 in the study by Feder et al. [1996]). We also tested, in simulations, a series of problems that are of practical significance. For example, we investigated the power of QTL fine mapping within a certain distance of the peaks, by (1) comparing the performance of various disequilibrium measures and test statistics with the performance of the j -point moving-average method with various j 's (fig. 2) and (2) by comparing the best and worst constellation of QTL and marker positions for QTL fine mapping (fig.

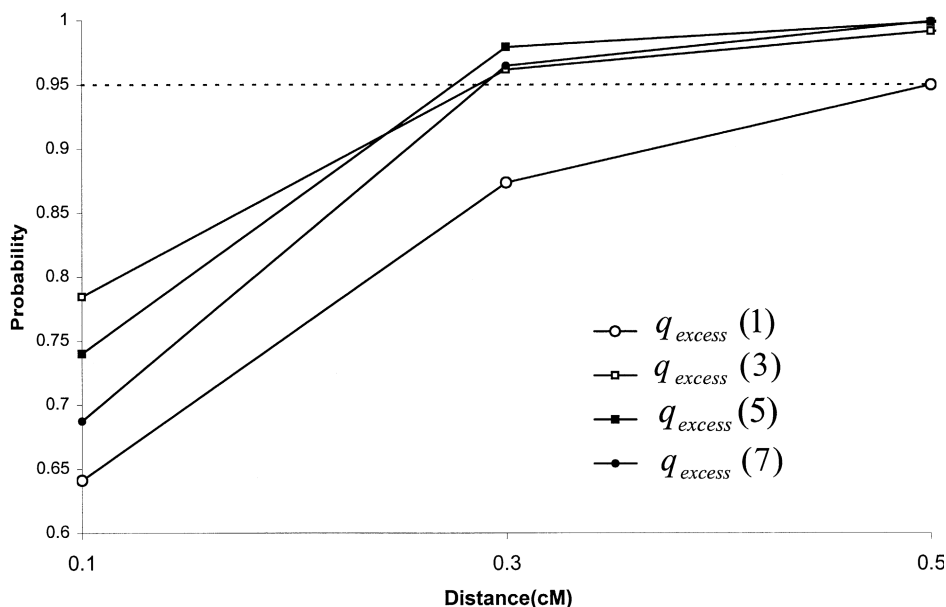


Figure 2 Comparison of QTL fine mapping by use of different average analyses (three-, five-, and seven-point moving averages) and by use of the raw measures themselves. The q_{excess} was used for illustration. In simulations, $p = .1$, $p_M = .2$, $2n = 200$, and $b^2 = .20$, and extreme samples from the bottom 10% and the top 10% of the population were used.

3). The best constellation occurs when one of a series of markers is located at the QTL. The worst constellation occurs when the QTL is located in the middle of two of a series of markers. We also compared the power of QTL fine mapping under various sample sizes ($2n$), sample-selection criteria (5th, 10th, 15th, bottom, and top percentiles of the populations) for the study, and various b^2 's (fig. 4).

In all previous simulations, markers are densely genotyped at intervals of ~ 0.25 – 0.20 cM, as is specified on the respective figures. In practice, to fine map a QTL on the basis of genomic regions of ~ 30 cM, it is expensive to genotype the ~ 30 -cM regions with a marker at intervals of ~ 0.20 cM. To reduce the cost, we propose a two-stage procedure for QTL fine mapping. In the first stage, we genotype the ~ 30 -cM region with markers at 1-cM intervals and locate the peaks of the disequilibrium measures or test statistics. In the second stage, we genotype a marker at 0.20-cM intervals, to saturate the genomic region of ~ 3 cM on both sides of the peak located in stage 1. The performance of such a two-stage genotyping procedure was investigated in simulation (fig. 3).

Results

Statistical Properties of the Two Tests (χ_1^2 and χ_3^2) for the Three Quantitative Genetic Models Comparable to Those of Nielsen et al. (1998) for Complex Diseases (fig. 5)

The power results for the three quantitative genetic models closely agree with the corresponding results of

Nielsen et al. (see fig. 1 of Nielsen et al. [1998]) for complex genetic diseases. This demonstrates the generality of quantitative genetic models for modeling complex genetic diseases. It also substantiates the notion—reflected by the framework of equations (13a) and (13b)—that, by selection of a threshold, genetic models of quantitative traits and complex genetic diseases are interchangeable. Thus, the analytical methods developed for mapping complex disease-susceptibility locus can often be directly adopted for QTL mapping by selection of appropriate threshold values.

In agreement with the findings of Nielsen et al. (1998), the power of the test for HW disequilibrium in the extreme bottom samples with $y < T$ is generally higher than that for the direct test for LD, by use of samples with $y < T$ (case patients) and $y > T$ (control individuals) under recessive genetic models, and it is generally lower under the additive model. Under the additive model, if the marker is extremely close to the QTL or is at the QTL (within 0.25 cM), then the HW test has the same power as the LD test. Under recessive model 1, when the marker is $> \sim 1.3$ cM away from the QTL, the test for LD is slightly more powerful than the test for HW. This result is a little different from the results of the study by Nielsen et al. (1998), in which the HW test was found to always be more powerful than the LD test under the recessive model 1. However, comparison of noncentrality parameters computed, by use of equations (15a) and (16), from samples (estimated) with those from the whole population (theoretical) revealed the same pattern that corresponds to the power for the re-

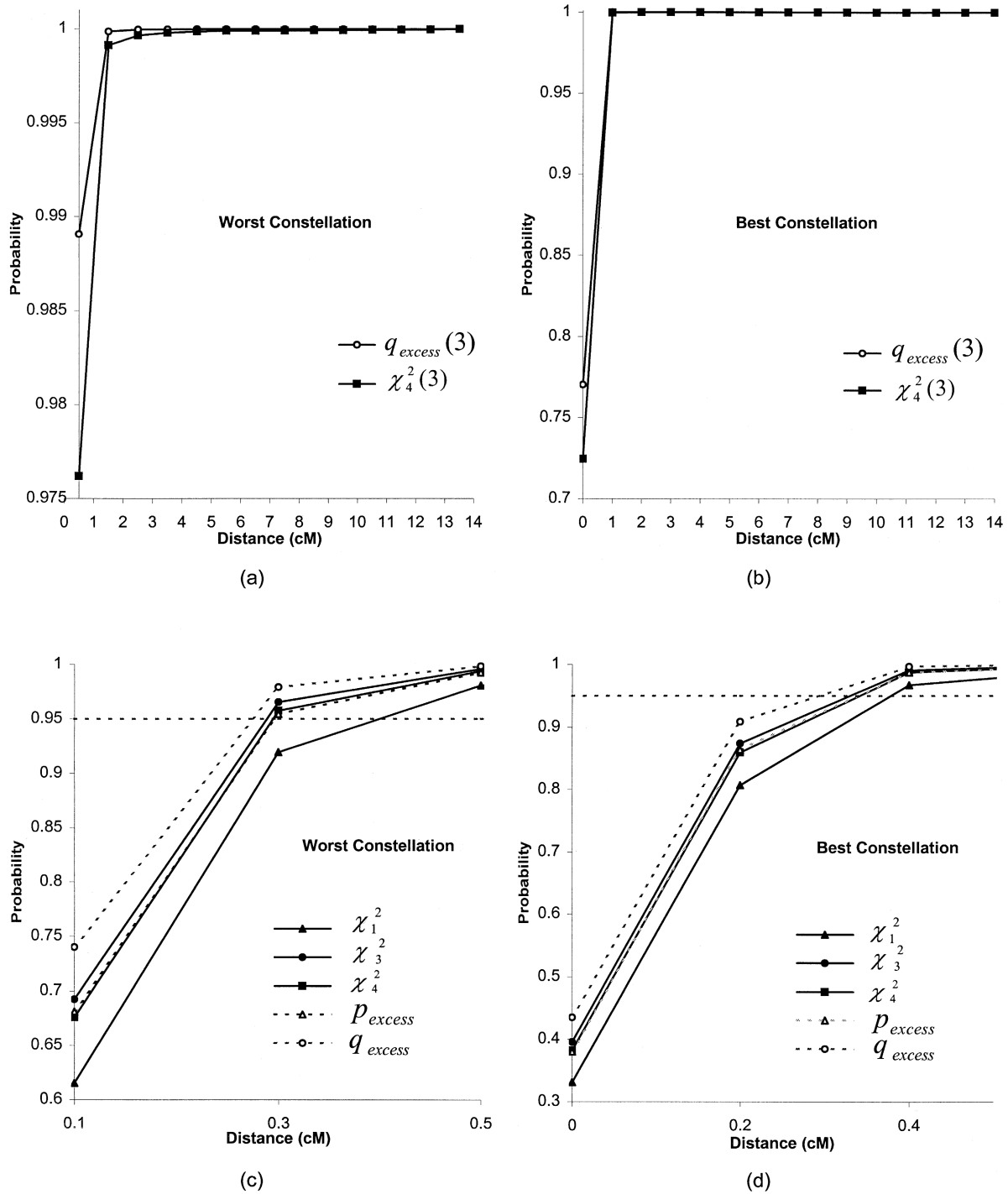


Figure 3 Two-stage QTL fine mapping and comparison of the power of QTL fine mapping under the best (panels *a* and *c*) and worst (panels *b* and *d*) constellations of the QTL and the markers and with various disequilibrium measures or test statistics. The “best” and “worst” constellations refer to instances when the QTL position is the same as one marker and when the QTL is in the middle of two markers, respectively. In simulations, $p = .1$, $p_M = .2$, $2n = 200$, and $b^2 = .20$. The three- and five-point moving-average methods were used, respectively, in the first stage (panels *a* and *b*) and in the second stage (panels *c* and *d*) of QTL fine mapping. In the first stage, χ^2_4 -test statistics and the q_{excess} measure were used for HW and LD, with the use of 100 individuals from the bottom 10th percentile and 100 individuals from the top 10th percentile of the population. In the second stage, χ^2_1 , χ^2_2 , χ^2_3 , and χ^2_4 statistics and D_{MM} , F_M , p_{excess} , and q_{excess} measures were used and compared. Since the powers of χ^2_2 , D_{MM} , and F_M are much smaller than those of the other measures or statistics, they are not presented. Panels *a* and *b* demonstrate the power of the first stage of QTL fine mapping, with genotyping of the genomic region at 1-cM intervals. Panels *c* and *d* demonstrate the power of QTL fine mapping, with genotyping of the genomic region at 0.2-cM intervals around the peaks obtained in the first stage. The genetic effect of the QTL is partial recessive for simulations in this figure and in figures 2 and 4.

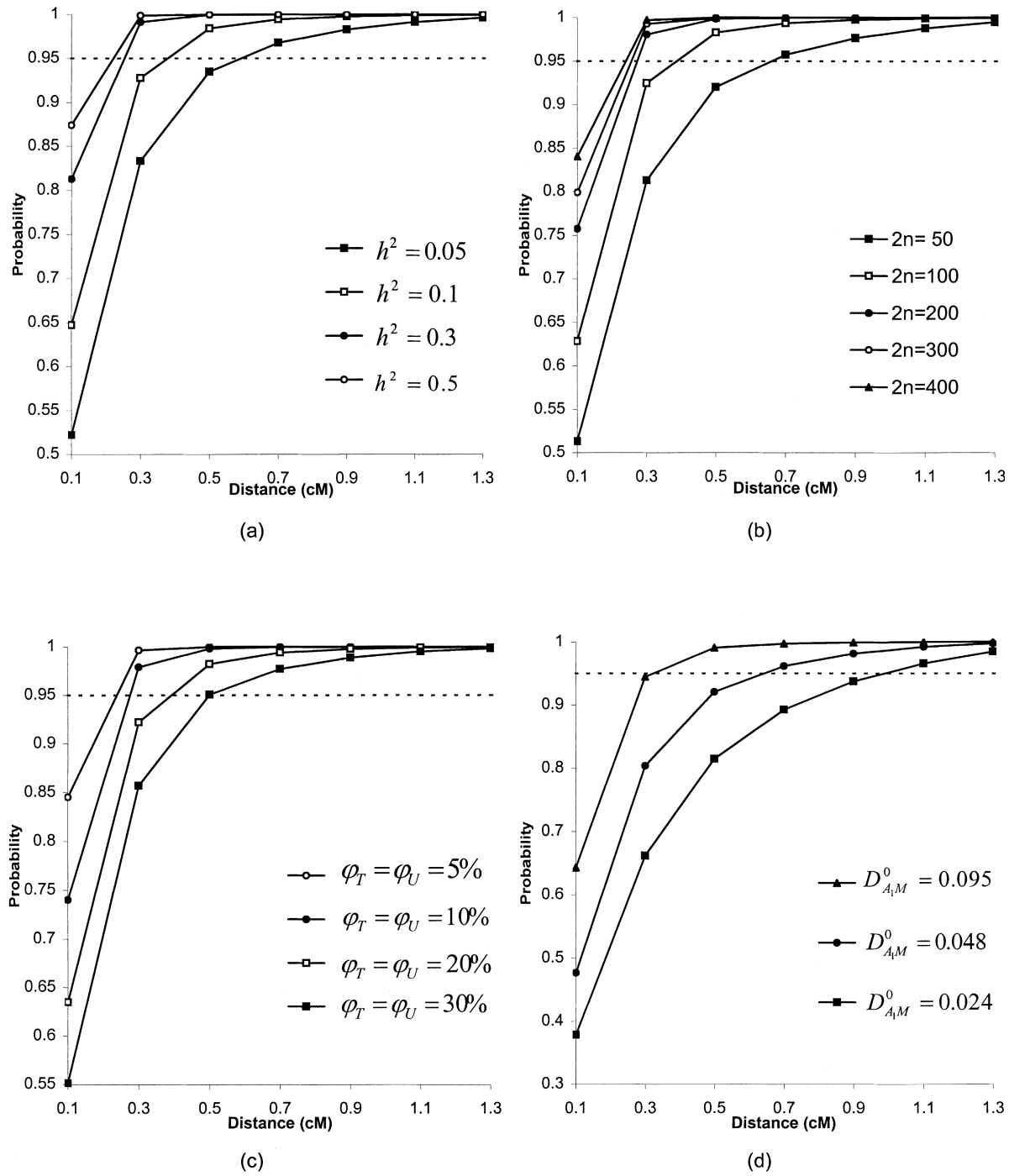


Figure 4 Performance of QTL fine mapping under (a) various h^2 , (b) various sample sizes ($2n$), (c) various selection criteria of the samples (5th, 10th, and 20th percentiles are respectively selected from the top and bottom distributions of the population), and (d) various degrees of LD, as measured by $D_{A_1M}^0$ at the G_0 generation. χ_3^2 and χ_4^2 tests for HW and LD, with the use of samples from the bottom 10th percentile (100 individuals) and top 10th percentile (100 individuals) of the population, are illustrated. In simulations, unless otherwise specified, $p = .1$, $p_M = .2$, $2n = 200$, and $h^2 = .20$; $D_{A_1M}^0$ is the maximum amount of LD simulated at the G_0 generation. After 50 generations of evolution, the expected LD is $D_{A_1M}^{50} = (1 - c)^{50} D_{A_1M}^0$.

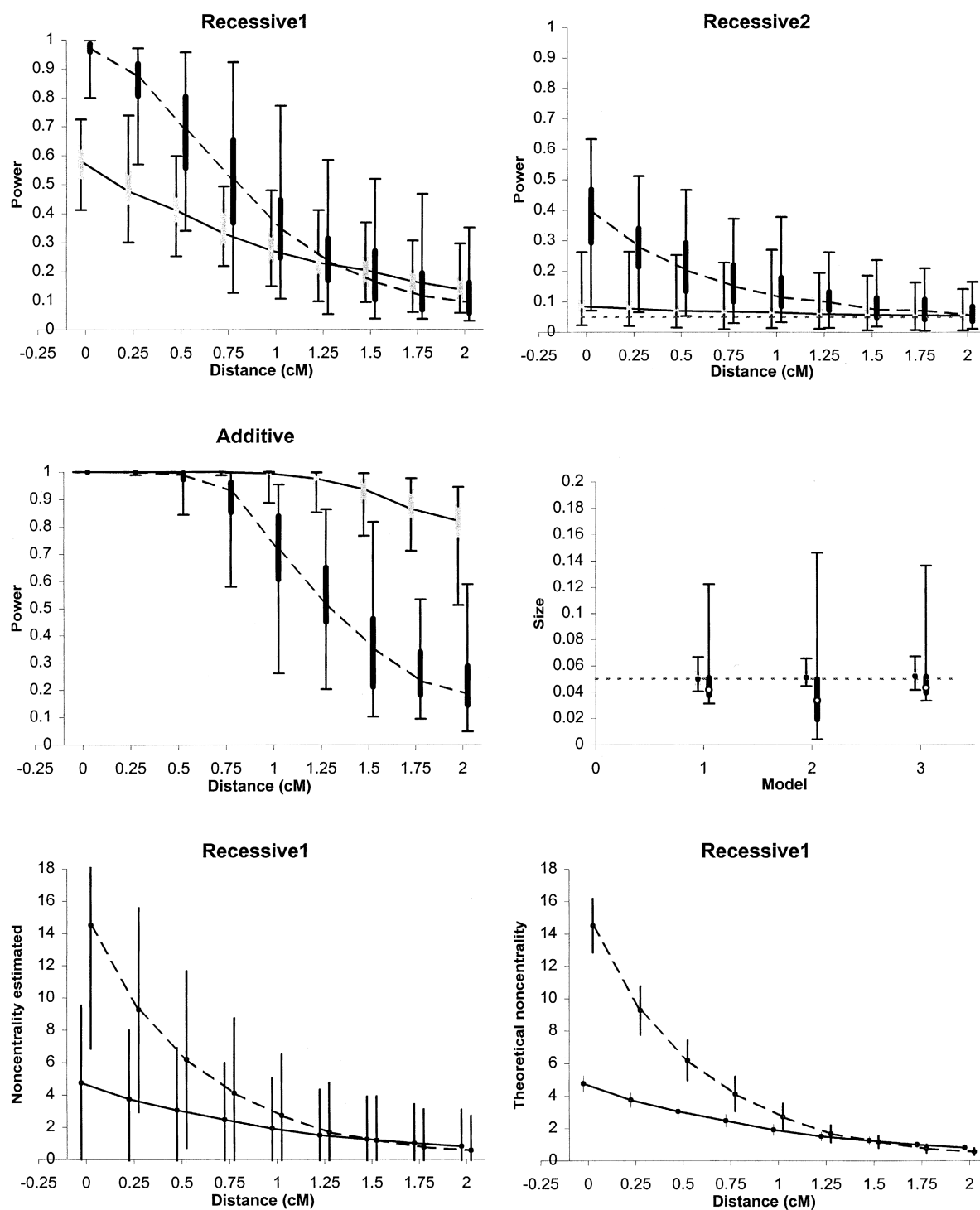


Figure 5 Statistical properties of the χ^2_1 test (gray-shaded box) and χ^2_3 test (blackened box). In simulations, $p = .1$ and $p_M = .2$, and, in the initial generation, $P_{A_{1M}} = .1$ and $D_{A_{1M}} = .08$. Corresponding to model 1 used by Nielsen et al. (see table 1 in Nielsen et al. [1998]), the genetic effects for the QTL (recessive 1) are: $a = -50$ and $d = 50$; corresponding to model 2 (recessive 2), $a = -99.62$ and $d = 99.62$; and corresponding to model 3 (additive), $a = -23.45$ and $d = 0$. In these quantitative-trait models, $h^2 = .99$. Sample sizes are $2n = 200$. In the recessive 1, recessive 2, and additive models, the bottom 6%, 10%, and 10% of the populations were defined as “affected,” corresponding to T values of ~ 50 , 99, and 24, respectively. It can be easily verified, from equations (2a), (2b), and (2c), that, for the quantitative-trait model, ϕ_{11} , ϕ_{12} , and ϕ_{12} are exactly the same as those in the three models used by Nielsen et al. (1998) for complex diseases. The symbols for power in the three plots represent the range of the proportions of times that the null hypothesis of no disequilibrium was rejected for the 100 simulated populations. The points joined by the connecting line are the medians, and the bottom and top edges of the boxes represent the sample 25th and 75th percentiles; the whiskers extend the range of the results. For the size plot, the symbols represent the proportion of times a true null hypothesis was rejected. The last two plots give the estimated and theoretical noncentrality parameters for the recessive 1 model.

cessive 1 model. In addition, the sizes of the two tests for the three models are all close to the expected theoretical level of .05. In Nielsen et al. (1998), the size of the tests for LD, for the first three models, was always lower than the expected 0.05 level, signaling potential deviation from theoretical expectations in their simulations. Therefore, our results should be robust. The size of the HW test has larger variation than does the LD test, which is consistent with the findings of Nielsen et al. (1998).

Comparison of Statistical Properties of the Tests under Various Genetic Models (fig. 6).

The relative powers of the four tests vary according to different genetic models. In the recessive model, the rank of the powers of different tests is $\chi_2^2 > \chi_4^2 > \chi_3^2 > \chi_1^2$. In simulations, χ_1^2 is the LD test applied to samples from the bottom 10% and the top 90% of the populations. χ_2^2 is the HW test applied to samples from the bottom 10% of the population. χ_3^2 and χ_4^2 are, respectively, LD and HW tests, both of which are applied to samples from the bottom 10% and top 10% of the populations. The rank of the powers of different tests is, respectively, $\chi_3^2 > \chi_4^2 > \chi_1^2 > \chi_2^2$ in the rest of the four models. The χ_3^2 and χ_4^2 tests differ little from each other in these four models, particularly when the marker is within 1 cM of the QTL. However, in the recessive model, the χ_4^2 test is significantly better than the χ_3^2 test, especially when the marker locus is within 1 cM of the QTL. The power of the χ_2^2 test, which is equivalent to the χ_{HW}^2 test used by Nielsen et al. (1998), is very unstable. In the recessive model, the χ_2^2 test has the highest power, whereas in the rest of the models, it has the lowest power. In particular, for the partial recessive model, its power is $\sim .05$, which is equivalent to the type 1 error rate. Therefore, the χ_3^2 and χ_4^2 tests that are applied to the samples from two extreme ends of the phenotypic distribution generally are the most powerful tests, and the power of the χ_4^2 test is the most robust one across different models. The sizes of the four tests all centered around the expected .05 level for all the models tested, thereby substantiating our simulation results. While the χ_2^2 test has the largest variation in size, the variations in size of the other three tests are relatively small and are essentially the same.

Comparison of Statistical Power of the Tests under Various Parameters with Partial Recessive Genetic Effects (fig. 7)

In simulations, all the tests were examined. Since the effects of changing various parameters are generally the same for different tests, we presented the results for only one or two tests (fig. 7). With increasing h^2 of the QTL, the power to detect disequilibrium at the marker locus

increases. Although the power of the χ_4^2 test is almost uniformly more powerful than that of the χ_2^2 test, the increase of power with increasing h^2 was more dramatic for the χ_2^2 test than it was for the χ_4^2 test. Furthermore, the increase is larger for markers that are close to the QTL compared with those that are far away. With increasing sample sizes ($2n$), the power to detect disequilibrium increases. The increase is much larger for markers that are far away from the QTL than it is for those close by the QTL. This conclusion is also true for the effect of increasing stringency of selection criteria of the samples (from the 20th percentile, to the 10th percentile, to the 5th percentile of the population, respectively). With an increasing degree of LD between markers and the QTL, the power increases. Although we only presented results for the effects of various degrees of LD through changing p while fixing p_M , our simulation results (not presented here) show that the effects are the same for various degrees of LD by changing p_M while fixing p in simulations.

The Power of QTL Fine Mapping

Recall that QTL fine mapping is achieved through the peaks of the disequilibrium measures or test statistics. The power of QTL fine mapping is defined as the probability that the peaks fall within a certain distance from the QTL. For the two-stage fine-mapping procedure (fig. 3), in the first stage, with the measures q_{excess} and χ_2^2 , the power of QTL mapping is $>99\%$ for a physical distance of ~ 1 cM, when $h^2 = .2$. If $h^2 = .05$, the data not shown indicate that the power is generally $>99\%$ for a distance of 3 cM. In the second stage, the power is generally $>97\%$ for a distance of 0.5 cM. The above conclusion is robust for different constellations of the QTL and markers. Under the partial recessive model presented, the power is low for D_{MM} and χ_2^2 that measure the HW disequilibrium in samples with $y < T$ (data not shown), corresponding to the pattern in figure 2. However, the power of the other disequilibrium measures and test statistics is quite high (fig. 3c and d). The rank of the power of these disequilibrium measures and test statistics is $q_{\text{excess}} > \chi_3^2 > \chi_4^2 > p_{\text{excess}} > \chi_1^2$. Generally speaking, with the measures of disequilibrium by q_{excess} , χ_3^2 , χ_4^2 , and p_{excess} , the QTL can be located within 0.5 cM of the peaks with $>95\%$ probability and within 1 cM of the peaks with $>99.5\%$ probability. For the measures q_{excess} and χ_4^2 , the power to locate the QTL to a region within 0.5 cM of the peaks is $\sim 99\%$.

Use of the moving-average method greatly improved the power for QTL fine mapping over the raw disequilibrium measures. For a distance >0.3 cM from the QTL, the three-, five-, and seven-point moving averages generally have roughly the same power (fig. 2). The power of the QTL fine mapping increases rapidly, with an in-

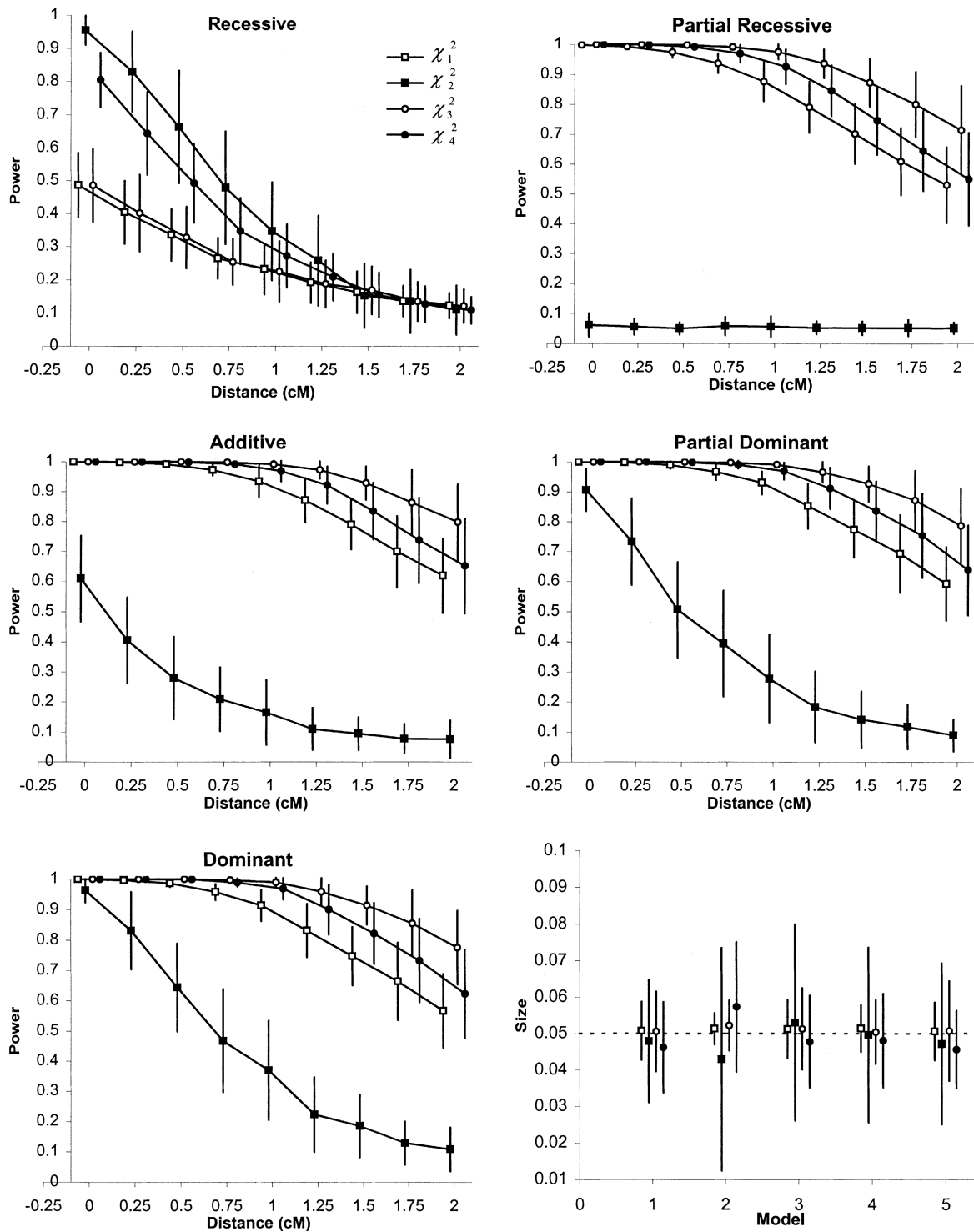


Figure 6 Comparison of statistical properties of different tests under various genetic models. Different symbols were used to differentiate the four tests, as is indicated on the first plot. On each plot, the data are the mean and SD at each marker (for the power plots) or for each model (the size plot), over 100 simulated populations, with each population sampled 5,000 times. Models 1–5 on the size plot correspond, respectively, to recessive (1), partial recessive (2), additive (3), partial dominant (4), and dominant (5) models. For models 1–5, the genetic parameters (a and d) are, respectively, $(-2.51, 2.51)$, $(-1.85, 0.93)$, $(-1.18, 0)$, $(-0.83, -0.42)$, and $(-0.64, -0.64)$. For all the simulations in this figure, $b^2 = .20$, $p = .10$, $p_M = .2$, and $2n = 200$.

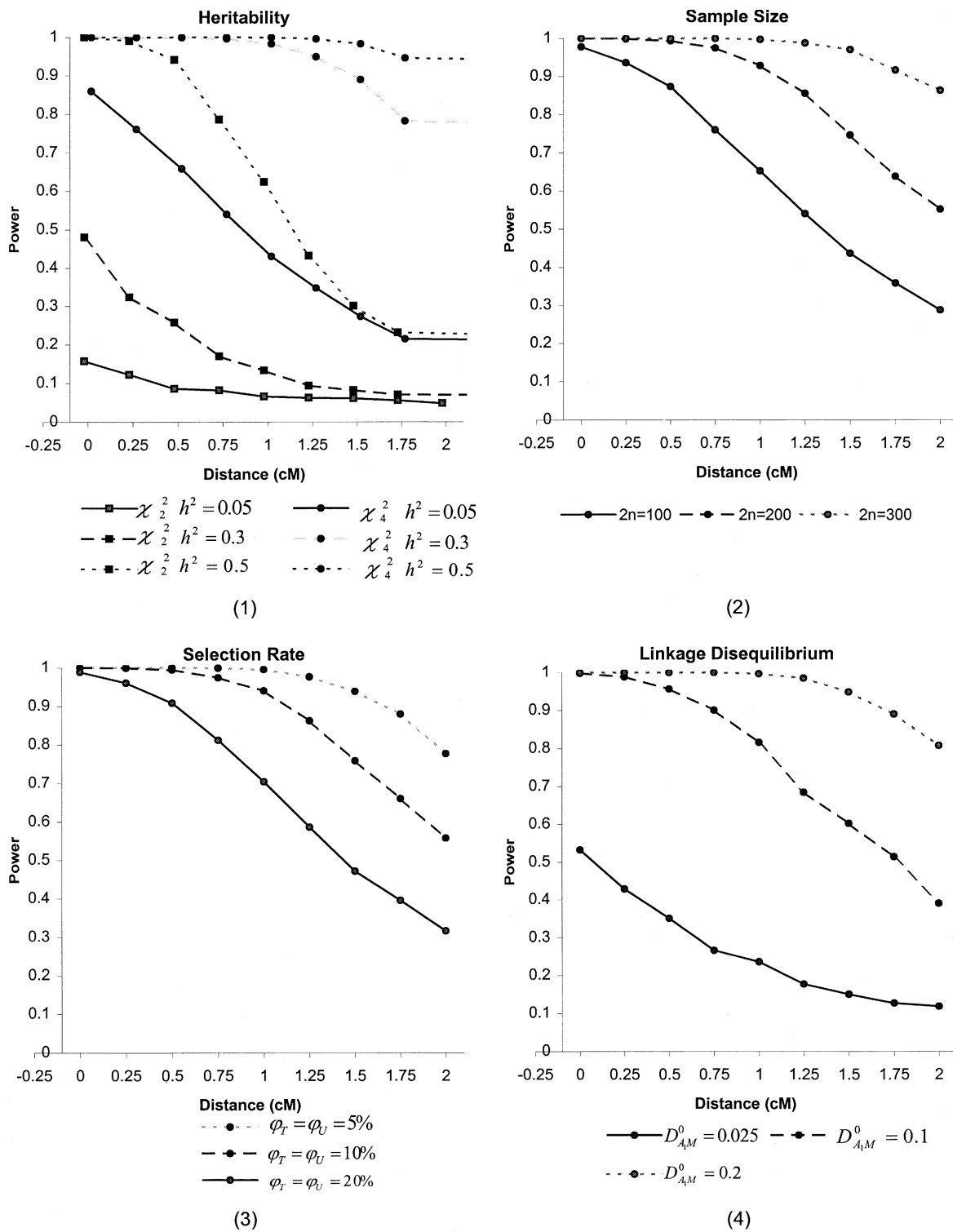


Figure 7 Comparison of statistical power under various parameters, with partial recessive genetic effects. In panel (1), the results for χ^2_2 and χ^2_4 tests are presented. In the remaining three panels (2-4), only the results for the χ^2_4 test are presented. Unless otherwise specified in the panels, $p = .1$, $p_M = .2$, sample size $2n = 200$, and $h^2 = .20$, and extreme samples from the bottom 10% (for the χ^2_2 test) or those from the bottom 10% and the top 10% of the population (the sample selection) were used for testing. Different levels of h^2 in panel (1), $2n$ in panel (2), sample selection in panel (3), and D_{A1M} in the initial population at the G_0 generation in panel (4) were indicated in the respective panels. $D_{A1M} = P_{A1M} - p p_M \cdot p_M = .5$ in panel (4), and various levels of D_{A1M} were achieved by varying p for allele A1, which is in complete LD, in the G_0 , with the marker allele M .

creasing h^2 of the QTL (plot a of fig. 4). For a QTL with $h^2 > .3$, the power for a distance of 0.3 cM from the peaks is generally $>99\%$. Even for a QTL with $h^2 = .05$, the power is $\sim 97\%$ for a distance of 0.7 cM from the peaks. By contrast, a QTL with $h^2 = .05$ is usually beyond the practical power to be detected by almost all of the other current linkage analyses and/or QTL-mapping methods. The power of QTL fine mapping increases rapidly with increasing sample sizes ($2n$) (fig. 4b). While $2n = 200$ is simulated in all other simulations and is of high power—even if $2n = 50$ —the power to detect the QTL is quite decent and is $>95\%$ for a distance of 0.7 cM from the peaks. However, as can be seen, to accurately locate the QTL, the sample sizes should be as large as possible. For example, if $2n = 400$, the probability that the peak is the QTL per se is $\sim 84\%$ and is $>99\%$ for a distance of 0.3 cM from the QTL. The power of QTL mapping increases with the stringency of the sample selection (fig. 4c). If the samples are selected from the top and the bottom 5th percentiles of the population, then the peaks will be on the QTL with 85% probability and will be within 0.3 cM of the QTL with $>99\%$ probability. Even if samples are selected from the top and bottom 30% of the population, the peaks have a 55% probability of being the QTL and a 95% probability of being within 0.5 cM of the QTL. The power of QTL fine mapping increases with an increasing disequilibrium between markers and the QTL (fig. 4d)—a critical determinant of the success of the QTL fine-mapping approach.

Discussion

Stimulated by the work of Feder et al. (1996) and Nielsen et al. (1998), we have developed and investigated several methods with which to accurately localize a QTL from extreme population samples. These are based on a large established genomic region (~ 30 cM) that contains the QTL. The QTL can be fine mapped by examination of HW disequilibrium and LD at a series of closely linked marker loci that cover the genomic position for the QTL. The test can be performed for samples of individuals belonging to either high or low percentiles of the phenotype distribution or for combined samples of these extreme individuals. The statistical properties (the power and the size) of the tests of this approach were investigated and were compared extensively under various genetic models and parameters for the QTL and the marker locus. Based on the results, a two-stage procedure that uses extreme samples and different tests (for HW and LD) is suggested for QTL fine mapping. This procedure is economic, efficient, and powerful and can generally narrow (with $>95\%$ probability) a genomic region containing QTL from ~ 30 –1

cM, a range that renders physical mapping feasible to clone the QTL.

Although numerous approaches of association, linkage, and/or LD mapping have been and continue to be developed for complex diseases, their direct relevance to QTL mapping of quantitative traits has seldom, if ever, been explicitly or correctly given. The development of mapping methods for qualitative and quantitative traits has largely been done in isolation. Nielsen et al. (1998) correctly pointed out that much of their theory could be applied to the study of quantitative traits. In fact, as can be seen from equations (13a) and (13b), to bridge the mapping methods from complex diseases and quantitative traits, the penetrances are functions of the genotypic values, the threshold, and the environmental variance. Equations (13a) and (13b), although simple, may actually serve as a bridge that organically joins mapping methods for complex diseases and for quantitative traits in an integrated framework. Therefore, many methods that are developed for qualitative disease traits (see Spielman et al. 1993; Sham and Curtis 1995a, 1995b; Kaplan et al. 1997; Xiong and Guo 1997a; Spielman and Ewens 1998) can be directly adopted for quantitative-trait mapping by specification of a threshold in the quantitative-trait distribution. In many situations, the methods for disease traits not only can be directly adopted but also can be extended to improve the mapping power for QTLs. This can be achieved by the ability (demonstrated in the present study) to employ samples from the discontinuous top and bottom percentiles of the population (e.g., with $y < T$ and $y > U$) for the quantitative traits. However, for complex traits with continuously distributed liabilities, samples can only be contrasted in terms of affected and non-affected individuals. On the scale of an unmeasurable underlying liability, affected individuals and non-affected individuals reflect the continuously neighboring samples with $y < T$ and $y > T$. To test LD, samples of the discontinuous bottom and top percentiles (those having individuals with $y < T$ and $y > U$) are usually much more powerful and robust than are the neighboring bottom and top samples (those having individuals with $y < T$ and $y > T$). For example, the χ^2_3 test that employs samples from the bottom 20 and top 20 percentiles is more powerful and robust than is the χ^2_1 test that employs samples from the bottom 20 and top 80 percentiles. Generally speaking, the measures and statistics for HW and LD in samples from the two discontinuous ends of the phenotypic distribution have the largest power and are most robust across various genetic models. This is understandable, since the contrast of allele or genotype frequencies (generated as a result of population LD) in the samples of discontinuous top and bottom percentiles is much larger than that in the samples of continuous top and bottom percentiles. There-

fore, the ability, in the present study, to sample discordant extreme individuals for quantitative traits usually may confer higher power to locate QTLs than to locate equivalent disease-susceptibility loci, as defined by equations (13a) and (13b).

For our new QTL-mapping approach, various measures and statistics for HW and LD were examined. Their relative powers largely depend on specific genetic effects. The absolute powers of various measures and test statistics for QTL fine mapping depend on a number of factors, such as the b^2 resulting from the QTL, the degree of LD, sample size, criteria for sample selection, etc. Generally speaking, as is revealed by the extensive computer simulations used in the present study, with 100 individuals from the top 10th percentile and 100 from the bottom 10th percentile of the population, the approach used in this study has extremely high power and accuracy for locating a QTL. For a specific sample selection, if multiple disequilibrium measures and test statistics are applicable, they should all be applied respectively in analyses. Various measures and test statistics not only may have different power for different specific genetic effects of the QTL (which is unknown), but they also can provide a mechanism with which to examine the consistency of each other. This is actually the approach adopted by Feder et al. (1999).

LD is a complex phenomenon that can result from random genetic drift, selection, mutation, and population history (such as population expansion, population admixture, and population bottlenecks, etc. [Hartl and Clark 1989]). The degree of LD is determined by a number of parameters associated with the aforementioned processes that generate LD, all of which are generally unknown, in practice, for real populations. Regardless of the detailed process and mechanism that generate the LD, what is essential and relevant to LD fine-mapping methods are the amount and pattern of LD between the QTL (or the disease locus) and the markers closely located in the genome. Therefore, in the present study, the LD generated in the simulation in the final generation should be of general significance to the LD generated by other processes, with regard to gene fine mapping by means of the LD methods.

It is well known that, if there is population stratification, spurious association may result between a particular marker locus and diseases or quantitative traits in association studies. However, it should be noted that, in the present study, the QTL fine-mapping approach used is based on a series of markers, instead of one or two. Despite extensive molecular studies for population subdivision, it has never been found that populations (especially human populations) are differentiated at a series of closely linked loci. Therefore, population stratification is unlikely to exist for a series of closely linked loci, even in a mixed sample of different populations.

Therefore, even if population stratification exists at one or two marker loci, their effects are likely to be diminished with use of the moving-average method in the QTL fine-mapping approach used in this study. This is because the peak is located by the averages of the neighboring points. Importantly, the current mapping approach is for QTL fine mapping based on established genomic regions that contain a QTL established from previous linkage and/or QTL mapping analyses that should be robust to population stratification. Therefore, spurious results from our fine-mapping approach seem unlikely. This is especially true if we select samples carefully, with regard to the ethnic and geographic origins of the study subjects, so that they are from homogeneous populations with minimum population subdivision. It is noted that, although the qualitative effects of population stratification have long been recognized, the detailed quantitative effects of various degrees of population stratification on various relevant mapping methods have seldom, if ever, been investigated. The effects of various degrees of population subdivision on our fine-mapping approach and on other approaches will have to come from extensive simulation studies that have yet to be performed. Results of a recent investigation (Deng et al. unpublished data) suggest that the LD methods used by Feder et al. (1996) and Nielsen et al. (1998), as well as those developed in the present study, that employ extreme samples are fairly robust, compared with customary case-control studies, to a range of various degrees of population admixture, in which HW equilibrium is violated. However, in the presence of apparent population substratification in the sample, the methods developed, in this study, for randomly mating populations should be treated with caution in application. Methods, such as TDT (Allison 1997; Rabinowitz 1997), for quantitative-trait analyses can be adopted. Research is being conducted (Deng et al. unpublished data) to develop fine-mapping techniques that are similar to the ones developed in this study but that employ TDT that should be robust to the confounding of population admixture.

It is known that if there are multiple QTLs closely linked in the genome, current linkage analyses and/or QTL-mapping methods generally yield biased and inaccurate positions for these QTLs, even if linkage can be established in this genomic region (Lynch and Walsh 1998). The high power and extraordinary accuracy of this study's fine-mapping approach, which can pinpoint a single QTL to a genomic region from ~ 30 – 1 cM, may offer an opportunity to separate QTLs that are closely linked in the genome—a hypothesis that needs to be examined in our future studies. It is also known that QTL mapping and linkage analyses (especially those for the whole genome) usually suffer from inflated type 1 errors. That is, many of the identified QTLs may be

spurious. Confirmation of the identified QTL genomic regions, by means of regular QTL mapping and linkage analyses, is notoriously difficult and expensive, and few replications have been achieved as a result of the lower statistical power. For example, the extensive studies of body-mass index or obesity (Chagnon et al. 1998) demonstrated this difficulty. The ability of our approach to exclude a spurious QTL genomic region is not known but is likely to be high, as is suggested by its high power to fine map a QTL. We will extensively investigate this issue in future studies, by devising appropriate statistical measures and tests for exclusion analyses of QTLs. In the present investigation, random genetic drift is ignored, which is reasonable for QTL fine mapping in large populations. However, future studies that account for genetic drift (e.g., see Devlin et al. 1996) will be pursued for the current approach. As a direct extension of the work by Feder et al. (1996) and Nielsen et al. (1998) for disease-locus fine mapping, as in their development and successful application of the method, our investigation of QTL fine mapping was conducted under ideal conditions of ignoring new mutations at the marker locus and the QTL. LD fine mapping that incorporates mutations (especially at the marker loci) is of interest and represents the direction that we are going to pursue to fine-tune the work of both Feder et al. (1996) and Nielsen et al. (1998) and the work in the present study. The approach for such future work can be pursued by means similar to those used by, for example, Xiong and Guo (1997a).

There are a few advantages in QTL fine mapping, compared with the fine mapping of disease-susceptibility loci with use of the HW-testing approach. As pointed out by Nielsen et al. (1998), for disease-susceptibility loci, if the genotypic penetrances are multiplicative, then the HW approach is not supposed to be of any power. For a quantitative trait, if the locus genetic effects are multiplicative, then the simple log transformation of the quantitative-trait values can yield additive within-locus genetic effects; thus, the HW-testing approach can still be powerful. In contrast, an equivalent simple data transformation is not available for complex binary-disease traits to render the HW test powerful. As was correctly pointed out by Nielsen et al. (1998), an absence of HW disequilibrium does not necessarily imply that a marker locus and a QTL are not in LD. Underlying all the measures and test statistics of the current approach is the LD between marker loci and the QTL. The LD is captured and magnified in extreme samples, where the QTL genotypes and alleles are disproportionately represented. The disequilibrium is the highest at the QTL locus, since it underlies the sample selections of extremes. The phenotypically neutral markers will also be disproportionately represented in the extreme samples if they are linked to and are in LD with the

QTL. As the degree of linkage and LD decreases, the disproportional representation of the marker alleles and genotypes will also decrease. It is this correlation between disequilibrium (HW and/or LD) and the physical distance between a panel of linked marker loci and a QTL that provides a basis for QTL fine mapping with use of the peaks of the disequilibrium measures and/or test statistics.

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