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Genome Screens Using Linkage Disequilibrium Tests: Optimal Marker Characteristics and Feasibility

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Summary

Linkage disequilibrium (LD) testing has become a popular and effective method of fine-scale disease-gene localization. It has been proposed that LD testing could also be used for genome screening, particularly as dense maps of diallelic markers become available and automation allows inexpensive genotyping of diallelic markers. We compare diallelic markers and multiallelic markers in terms of sample sizes required for detection of LD, by use of a single marker locus in a case-control study, for rare monophyletic diseases with Mendelian inheritance. We extrapolate from our results to discuss the feasibility of single-marker LD screening in more-complex situations. We have used a deterministic population genetic model to calculate the expected power to detect LD as a function of marker density, age of mutation, number of marker alleles, mode of inheritance of a rare disease, and sample size. Our calculations show that multiallelic markers always have more power to detect LD than do diallelic markers (under otherwise equivalent conditions) and that the ratio of the number of diallelic to the number of multiallelic markers needed for equivalent power increases with mutation age and complexity of mode of inheritance. Power equivalent to that achieved by a multiallelic screen can theoretically be achieved by use of a more dense diallelic screen, but mapping panels of the necessary resolution are not currently available and may be difficult to achieve. Genome screening that uses single-marker LD testing may therefore be feasible only for young (<20 generations), rare, monophyletic Mendelian diseases, such as may be found in rapidly growing genetic isolates.

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Introduction

Under certain assumptions about population history, linkage disequilibrium (LD) between a marker locus and a disease locus is believed to indicate that the two loci are closely linked (Briscoe et al. 1994; Plomin et al. 1994; Jorde 1995; Kaplan et al. 1995). The first instance of a disease-causing mutation must necessarily occur on a particular background haplotype. The disease allele is then associated with the alleles carried at other loci on that same haplotype. This association, or LD, is reduced over time (measured in generations) as recombinations occur between the disease locus and linked loci, and the rate of decay of LD is related to the recombination fraction between the disease locus and the locus of interest. For tightly linked loci, recombination events are rare, and LD will therefore remain strong for many generations, whereas for larger recombination fractions LD will decay more rapidly. These principles suggest the use of LD testing as a tool for disease-gene localization.

There are two distinct but related situations in which the use of LD to localize disease loci is of interest. First, there is the case in which traditional meiotic mapping techniques have mapped the gene of interest to a particular region of a chromosome. One possible approach at this point is to collect more family data to refine the location of the disease locus by meiotic methods. However, the disadvantage of this approach is the prohibitively large sample sizes needed to narrow the regions sufficiently to embark on gene identification studies (Boehnke 1994). Collection of a sufficiently large collection of families may be impossible, especially for recessive diseases. Even when large samples of appropriate pedigrees could, in principle, be obtained, the associated sampling and genotyping costs may far exceed those of a case-control study based on LD testing. A major reason for this difference in cost is that both theoretical arguments and empirical evidence suggest that strong LD with a disease locus is usually confined to an interval of <2 cM—and, often, much less (Thompson and Neel 1997; Wijsman 1997). Sample sizes necessary to detect such LD by use of a diallelic marker are generally relatively modest (Olson and Wijsman 1994). Because of these practical considerations of feasibility and cost, LD

testing following initial meiotic mapping of a gene has become a popular approach, and there are numerous examples of its success in narrowing the regions containing disease loci (e.g., see Hästbacka et al. 1992; MacDonald et al. 1992; Ellis et al. 1994; Goddard et al. 1996; Votruba et al. 1997).

A second use of LD testing is in the context of an initial genome screen for a disease locus of interest. The increasing density of markers available for mapping, coupled with the increasing automation of genotyping, suggests that genome screening that uses LD testing may soon be a feasible method of disease-gene localization in isolated populations (Houwen et al. 1994), in selected hybrid populations (Briscoe et al. 1994; Shriver et al. 1997), and perhaps even in large randomly mating populations (Risch and Merikangas 1996; Brown and Hartwell 1998). Genome screens for LD recently have been used by Hovatta et al. (1997), to find regions of interest in the search for a schizophrenia gene in a recent internal isolate of the Finnish population, and by Escamilla et al. (1996), to search for genes for bipolar disorder in a Costa Rican population.

If LD testing is to be used for disease-gene localization in either of the aforementioned situations, a method of approximating its power—and, therefore, determining the required sample size—is necessary. Sample size issues have previously been explored for single-marker LD testing both for population-based (Thompson et al. 1988) and case-control (Olson and Wijsman 1994) designs, but only for diallelic markers. Restriction of previous studies to diallelic markers is largely a result of the ease with which a single measure of disequilibrium can be defined—a single parameter allows description of the distribution of the test statistic under various levels of disequilibrium, leading to expressions that can be used for statistical power and sample size estimation.

Although sample size issues have been explored for diallelic markers, most applications of LD testing currently involve multiallelic markers. The approach most commonly implemented is to collapse alleles, effectively creating a diallelic marker, and then perform LD testing in the usual way. Unfortunately, this collapsing is often either data based or completely arbitrary and can result in both type I and type II errors (Weir and Cockerham 1978; Goddard et al. 1996). Sham and Curtis (1995) considered Monte Carlo association tests that either combined alleles or considered each allele in turn against the rest and found that the power to detect association was reduced when there were many marker alleles. In fact, it is not necessary to reduce the problem to one involving a diallelic marker. For an m-allele marker, one can test for LD by using either a χ^2 test, with $m - 1$ df, or, when data are sparse, by Fisher's exact test. Although there is no single measure of disequilibrium in this case, there does exist a single measure of association (Kaplan et al. 1997) that can be used to estimate statistical power to detect association (Ott and Rabinowitz 1997).

Given that markers with two or more alleles can be used in LD testing, it is also of interest to determine what types of markers and screening panels provide the most power to detect LD. In particular, how do multiallelic markers such as microsatellite markers (Weber and May 1989) compare with diallelic markers, such as single-nucleotide polymorphisms (Nickerson et al. 1992)? This question has become particularly timely as automated and inexpensive genotyping methods have become available for diallelic loci (see, e.g., Chee et al. 1996). The answer to this question is not immediately obvious. Although multiallelic markers will be more polymorphic—and, therefore, more informative—than diallelic markers, an increase in the number of alleles results in an increase in the df of the test statistic, which can result in a loss of power to detect association.

In the present report, we present information crucial to the design of effective studies to screen for markers demonstrating LD with a disease locus. We compare diallelic markers to multiallelic markers, in terms of sample sizes required for detection of LD, by use of single-marker tests, and find that multiallelic markers are almost always superior. We also describe relative marker spacings necessary for diallelic and multiallelic markers to achieve similar power. Finally, we describe the Mendelian genetic models and population histories under which LD can be detected by use of reasonable sample sizes and marker densities.

Methods

We consider a typical case-control study design, in which marker allele frequencies on chromosomes carried by affected individuals are compared with marker allele frequencies on chromosomes carried by unaffected individuals. We first describe an appropriate test statistic and its asymptotic distributions in the presence and absence of LD. We then discuss the parameters necessary to approximate the power, and we describe power calculations for a rare recessive disease. We generalize the results for a dominant disease, and, finally, we discuss the parameter values used in our population genetic model.

Test Statistic

Consider the design in which n cases and an equal number of controls are sampled. Olson and Wijsman (1994) showed that, for a diallelic marker, power is always maximized for a given total sample size when equal numbers of cases and controls are considered. Since this aspect of the study design is under the control of the experimenter, we do not consider other partitions of the

total sample size. For this case, the data can be described in a $2 \times m$ table, where m is the number of marker alleles. One can define a χ^2 statistic to test the null hypothesis that the marker allele frequencies in the two groups are equal, as follows:

$$X^2 = \sum_{\text{all cells}} \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$= 2n \sum_{i=1}^{m} \frac{(\hat{p}_i - \hat{q}_i)^2}{\hat{p}_i + \hat{q}_i} ,$$

where $\hat{p}$ and $\hat{q}$ represent the observed marker allele frequencies in the case individuals and control individuals, respectively, and n is the number of cases sampled.

Under the null hypothesis of equal marker allele frequencies, X^2 is asymptotically distributed as χ^2_{m-1}. An α-level test therefore rejects the null hypothesis when $X^2 \geq \chi^2_{m-1,1-\alpha}$. Under the alternative of unequal marker allele frequencies (i.e., LD), X^2 is asymptotically distributed as $\chi^2_{m-1}(\gamma)$, where $\gamma = 2n \sum_{i=1}^{m} [(p_i - q_i)^2/(p_i + q_i)] \equiv 2nG^2$. Therefore, the asymptotic power of the test at level α is given by $\Pr[\chi^2_{m-1}(\gamma) \geq \chi^2_{m-1,1-\alpha}]$, which is easily obtained from standard statistical software, given values of n, m, α, and G^2.

It is interesting to note that the noncentrality parameter is monotone increasing in G^2, which is a genetic distance measure proposed by Balakrishnan and Sanghvi (1968). LD testing is therefore equivalent to testing the hypothesis that this genetic distance is zero. G^2 can be thought of as a weighted version of the Euclidean distance between the vectors **p** and **q**, and therefore it is expected to increase as the dimensionality (i.e., m, the number of marker alleles) of vectors **p** and **q** increases. If the df of the test were to remain constant, this would result in an increase in power with an increase in the number of alleles. However, as the number of marker alleles increases, so does the df of the test. This will counteract, to some extent, the increase in G^2, and, thus, the impact of the use of multiallelic markers on power to detect LD is not immediately apparent.

Computation of G^2

The parameters of the power calculation are easily specified, with the exception of G^2, which depends on the population history. To calculate G^2, we assumed a deterministic population genetic model in which a single mutation was introduced into the population t generations ago, with a frequency of r. This could have occurred either by spontaneous mutation or by the immigration of an individual carrying the mutation (founder effect). We will refer to this time t as the age of the mutation. Initial disequilibrium was assumed to be complete—that is, all the initial mutations happened on the same chromosomal background. The configuration of haplotype frequencies at the time of initial mutation ($t = 0$) can then be described as in table 1, where the vector **s** denotes the marker allele frequencies at the time of the initial mutation and the initial mutation is assumed to occur on a background of allele i.

We further assumed nonoverlapping generations, random mating, no migration, no subsequent mutation at the disease or marker locus, no genetic drift, and that the overall population frequencies of the marker alleles and the disease mutation remained constant. These assumptions are the same as those used by Devlin and Risch (1995) and Devlin et al. (1996), except that they considered a growing population and allowed genetic drift. Our results therefore reflect the expectation for G^2 over all possible drift histories. Under these assumptions, the population frequency, at time t, of chromosomes carrying the disease mutation d and marker allele j ($\pi^{(t)}_{j,d}$, $j = 1, \ldots, m$) is given by

$$\pi^{(t)}_{j,d} = rs_j + (1 - \theta)^t(-rs_j), \; j = 1, \ldots, m, \; j \neq i$$

$$= [1 - (1 - \theta)^t]rs_j \qquad (1)$$

and

$$\pi^{(t)}_{i,d} = rs_i + (1 - \theta)^t(r - rs_i)$$

$$= [1 - (1 - \theta)^t]rs_i + (1 - \theta)^t r \; . \qquad (2)$$

Equation (1) expresses the frequency of haplotypes carrying both the disease mutation and marker allele j as the expected frequency if there were no LD, plus the initial disequilibrium, which has decayed over time. Alternatively, it can be expressed as the probability that a chromosome carrying the disease mutation has recombined with a normal chromosome carrying allele j at the marker locus. Similarly, equation (2) expresses the frequency of haplotypes carrying both the disease mutation and marker allele j as the expected frequency if there were no LD, adjusted for the remaining LD, or, alternatively, as the probability that no recombinations occurred, plus the probability that a recombination did occur but with a chromosome carrying allele i. If we let the vectors **x** and **y** represent the marker allele frequen-

Table 1

Configuration of Haplotype Frequencies at the Time of Initial Mutation ($t = 0$)

	Disease Locus		
Marker Locus	Mutation	Normal	Total
Allele i	r	$s_i - r$	s_i
Allele j ($j = 1, \ldots, m; j \neq i$)	0	s_j	s_j
Total	r	$1 - r$	1

cies in the disease mutation–carrying chromosomes and the normal chromosomes, respectively, we can write

$$x_j^{(t)} = s_j[1 - (1 - \theta)^t],$$

$$y_j^{(t)} = s_j\left[1 + \frac{r}{1 - r}(1 - \theta)^t\right] ,$$

$$j = 1, \ldots, m,\ j \neq i$$

and

$$x_i^{(t)} = s_i + (1 - s_i)(1 - \theta)^t,$$

$$y_i^{(t)} = s_i - \frac{r}{1 - r}(1 - s_i)(1 - \theta)^t .$$

To calculate the quantity of interest $G_i^2(t)$—that is, the expected value of $G^2(t)$, given that the mutation occurred on a background of allele i—we must obtain **p** and **q**, the marker allele frequencies in affected and unaffected individuals, respectively. Each of these quantities will be a weighted average of **x** and **y**, with the weights determined by the mode of inheritance of the disease. For a rare recessive disease, **p** is equal to **x** and **q** is approximately equal to **y**. The quantity $G_i^2(t)$ is easily calculated from **p** and **q**. If we assume that marker polymorphism preceded the disease mutation, the probability that the mutation happened on a background of allele i is simply s_i. The expected value of $G^2(t)$ is then a weighted average of the conditional expected values: $G^2(t) = \Sigma_{i=1}^m s_i G_i^2(t)$.

Dominant versus Recessive Diseases

For a fully penetrant rare dominant disease, **p** is approximately the average of **x** and **y**, because most cases will be heterozygous for the mutation, and **q** is equal to **y**. Thus G^2 is easily calculated for rare dominant diseases, too. It is also instructive to consider the following relationship between recessive and dominant diseases: for a rare mutation, where $r/(1 - r) \cong 0$, $\mathbf{y}^{(t)} \cong \mathbf{s}$. If the disease is caused by a recessive mutation t_r generations old,

$$\mathbf{p}^{(t_r)} = \mathbf{x}^{(t_r)} \text{ and } \mathbf{q}^{(t_r)} \cong \mathbf{s} \ ; \qquad (3)$$

for a rare dominant disease that is t_d generations old,

$$\mathbf{p}^{(t_d)} \cong \frac{1}{2}\mathbf{x}^{(t_d)} + \frac{1}{2}\mathbf{s} \text{ and } \mathbf{q}^{(t_d)} \cong \mathbf{s} \ . \qquad (4)$$

Now, if $\mathbf{p}^{(t_d)} = \mathbf{p}^{(t_r)}$, the value of G^2 for the two different situations would be the same. By setting $\mathbf{p}^{(t_d)} = \mathbf{p}^{(t_r)}$ and solving for t_r, we find that $t_r = [\ln(.5)/\ln(1 - \theta)] + t_d$. This means that a dominant mutation that is t_d generations old will result in approximately the same marker allele frequencies in the cases and controls as are seen for a recessive mutation that is older by $\ln(.5)/[\ln(1 - \theta)]$ generations. For a tight ($\theta = .005$) screen, $t_r = 138 + t_d$. For a looser ($\theta = .025$) screen, $t_r = 27 + t_d$. In practical terms, this means that a dominant mutation of a specified age will exhibit the LD characteristics of an older recessive mutation, and this will be reflected by increased sample size requirements for detection of LD for dominant diseases.

Parameter Values Considered

To determine the test's power on the basis of X^2, one must specify the age, in generations (t), of the mutation, the recombination fraction (θ) between the marker locus and the disease locus, the disease-mutation frequency (r), the marker allele frequencies (**s**), the number of cases sampled (n), and the mode of inheritance of the disease. Of these parameters, only θ, **s**, and n can be controlled by the investigators, so we focus on the effect that these quantities have on the power to detect LD. Throughout, we assume that an equal number of cases and controls are sampled.

In the results presented here, we have assumed a rare-mutation frequency of $r = .0025$. This corresponds to a mutation on a single chromosome in an initial population of 200 people. Because r affects the vectors **x** and **y** only through the term $r/(1 - r)$, small changes in r do not change the results noticeably. We considered fully penetrant recessive disease and dominant disease with no phenocopies, recombination fractions consistent with 1-, 2-, 5-, and 10-cM genome screens (i.e., $\theta = .005$, .01, .025, and .05), and markers with $\leqslant$10 alleles.

Results

Optimal Allele Frequency Configuration

Under the assumption that $r/(1 - r) \cong 0$ (i.e., the mutation is rare), one can show, for a recessive disease, that

$$G^2(t) = c + \sum_{i=1}^{m} \frac{s_i - a^2}{a + s_i} \ ,$$

where $a = (1 - \theta)^t/[2 - (1 - \theta)^t]$ and $c = a(1 - \theta)^t(1 + a)(ma - 1)$. Maximizing $G^2(t)$ with respect to s, subject to the constraint that $\sum s_i = 1$, and holding θ, t, and m constant yields $s_i = s_j\ \forall i,j$. Thus, for a recessive disease and a fixed number of alleles, markers whose alleles are equifrequent will be most powerful for detection of LD. Since a dominant disease behaves like an older recessive disease, this also applies to dominant diseases. For this reason, our investigations focused primarily on comparison of multiallelic and diallelic markers with equifrequent alleles.

Sample Size Requirements

For both recessive disease and dominant disease models, the use of multiallelic markers allows a substantial drop in sample size, relative to that needed when diallelic markers are used. Tables 2 and 3 show the number of cases needed to achieve 80% power with a type I error rate of 0.1%, for a fully penetrant recessive disease and a fully penetrant dominant disease, respectively. We assumed that all markers had equifrequent alleles. Figure 1 shows the required sample size for a multiallelic marker, as a percentage of the sample size required for a diallelic marker, for selected values of t and different marker spacings. In the recessive disease case, most of the drop in sample size of multiallelic markers relative to diallelic markers is achieved by markers with six or more alleles, and the sample sizes then required are 40%–60% of the diallelic sample sizes. In the dominant disease case, most of the benefit is achieved by markers with eight or more alleles, and the sample sizes required are 30%–45% of the diallelic sample sizes. It is interesting to note that the effect of the use of multiallelic markers is more pronounced when the association is weaker—that is, for older mutations or looser marker screens. This effect is demonstrated in figure 2, for the recessive disease example with a six-allele marker. In light of this observation, it is not surprising that the effect of multiallelic markers is more pronounced in applications to a dominant disease than in those to a recessive disease, since a dominant mutation can be likened to an older recessive mutation. These results clearly show that there is a substantial benefit to the use of multiallelic markers, rather than diallelic markers, in the search for LD.

The sample sizes required for detection of LD in more-complex situations can be approximated by consideration of the proportion of case chromosomes expected to carry the mutation, a quantity that depends on the genetic model. In the dominant disease example, only one-half of the case chromosomes would be expected to carry the mutation (see eq. [4]), and this results in sample sizes that are increased relative to those required by the recessive disease case. Figure 3 shows the sample size required in order to achieve 80% power when $\alpha = .001$ (for a mutation age of $t = 20$ and a screen density of 5 cM) with six-allele markers, as a function of the fraction of case chromosomes expected to carry the mutation. For example, when the mixture fraction is equal to 1, all of the case chromosomes are expected to carry the mutation. This is equivalent to a recessive disease, and the corresponding sample size is 24, as shown in table 2. The situation in which the mixture fraction is equal to $\frac{1}{2}$ corresponds to a dominant disease, and the corresponding sample size is 80 (see table 3). Other mixture fractions can be observed as a result of more-complex models of inheritance. Consider the dominant disease case, with the added complication that there is a second locus, unlinked to the first, that can cause the same phenotype. The resulting mixture fraction depends on the population prevalence of disease caused by the second mutation, relative to the prevalence of disease caused by the dominant mutation, which is linked to our marker. If the two types of disease are equally prevalent, then the probability that a sampled case has disease caused by the mutation linked to our marker is $\frac{1}{2}$, and, even then, only one of the case's two chromosomes will be carrying the mutation. Therefore, the mixture fraction in this example is $\sim \frac{1}{4}$, and the corresponding required sample size is 262. It should also be noted that this calculation assumes that the sample of control chromosomes remains pure (see eqs. [3] and [4]). This assumption will hold as long as the disease is rare. This example demonstrates that, for complex diseases, sample sizes required for detection of LD may be unrealistically large, because the mixture fraction will be quite low.

Relative Spacings Required in Order to Achieve Equivalent Power

The use of diallelic markers requires much denser marker spacing—and, therefore, more markers—to achieve the same power as is seen in a screen that uses multiallelic markers. Figure 4 shows the marker spacing required in order to achieve 80% power when $\alpha = .001$ for a single test, as a function of the available sample size and the number of marker alleles, for a moderately old mutation ($t = 40$). For example, when 100 cases are available, the disease is recessively inherited, and diallelic markers are to be used, marker spacing must be $\leqslant$4.48 cM to achieve adequate power; if 6-allele markers were to be used, spacing of $\leqslant$6.53 cM would be adequate. In this example, use of diallelic markers requires 46% more markers than is required by use of 6-allele markers; if 10-allele markers were used, spacings $\leqslant$6.975 cM would have adequate power. The same pattern is apparent for a dominantly inherited disease, and the power difference between the use of diallelic markers and the use of multiallelic markers is even more pronounced. When 100 cases are available, the required spacings are 1.065 cM, 3.44 cM, and 3.61 cM, for 2-, 8-, and 10-allele markers, respectively. The use of diallelic markers, rather than 8-allele markers, for a dominant disease requires 223% more markers to achieve the same power. For both models of inheritance, most of the benefit of the use of multiallelic markers is achieved by use of markers with a moderate number of alleles (i.e., $m = 6$ or $m = 8$). The use of markers with many alleles ($m = 10$) offers relatively little additional improvement in either case.

Table 2

No. of Cases Required in Order to Achieve 80% Power, When α = .001, for a Rare Recessive Disease

	No. of Cases Required, for Marker Spacing of															
	1 cM					2 cM					5 cM			10 cM		
m	t = 10	t = 20	t = 40	t = 100	t = 150	t = 10	t = 20	t = 40	t = 100	t = 150	t = 10	t = 20	t = 40	t = 10	t = 20	t = 40
2	15	17	22	43	73	17	22	34	123	343	24	43	125	44	128	1,025
3	12	13	16	30	50	13	16	25	82	217	18	32	83	31	85	626
4	11	12	15	26	42	12	15	22	68	173	16	27	69	27	71	483
5	10	11	14	25	39	11	14	20	61	151	15	25	62	25	63	410
6	10	11	14	24	37	11	14	20	57	138	15	24	58	24	59	365
7	10	11	13	23	36	11	13	20	55	129	15	23	56	24	57	335
8	10	11	13	23	35	11	13	19	53	123	15	23	54	24	55	313
9	10	11	14	23	35	11	14	19	52	119	15	23	53	23	54	297
10	10	11	14	23	35	11	14	19	52	116	15	23	52	23	54	285

The number of markers required is an important part of the cost of conducting a study. Figure 5 shows the number of diallelic markers required in order to achieve 80% power when α = .001, divided by the number of multiallelic markers required, as a function of the number of cases available, for the example in which t = 40. For the recessive disease case, the multiallelic marker has six alleles; for the dominant disease case, the multiallelic marker has eight alleles. The use of diallelic markers requires substantially more genotyping to achieve the same power as is given by the multiallelic markers, particularly for dominant diseases and small sample sizes. Note that, as the sample size n increases, the ratio of the number of diallelic to the number of multiallelic markers appears to reach an asymptote; this occurs relatively quickly for a recessive disease ($n \cong$ 75, ratio $\cong$ 1.5) but more slowly for a dominant disease, or, analogously, for an older recessive disease.

Feasibility of LD Testing

The results graphed in figure 6 are useful for determining when LD testing is a reasonable approach to disease-gene localization, for a recessive disease. Required sample size is shown for young (t = 10 or 20), moderately old (t = 40), and old (t = 100) mutations, for the four screening densities considered, for 80% power, and a testwise α = .001. For the tighter screens (1 and 2 cM), the sample sizes required for detection of LD with a recessive disease are reasonable for all four ages of mutation, for both diallelic and six-allele markers. However, the use of six-allele markers always allows a reduction in sample size, compared with that required when diallelic markers are used. For example, when the disease mutation is old (t = 100) and a 2-cM screen is used, use of six-allele markers reduces the sample size from n = 123 to n = 57, a reduction that is of practical use for a rare recessive disease, for which sampling of cases can be difficult. For a 5-cM screen, diallelic markers can detect LD by use of reasonable sample sizes for only the two younger mutations. For a moderately old mutation (t = 40), the required diallelic sample size of n = 125 is reduced to n = 58 when six-allele markers are used, again demonstrating that the use of multiallelic markers is quite beneficial in this context. For the oldest mutation, LD is not detectable with a reasonable sample size, regardless of the number of marker alleles used.

Table 3

No. of Cases Required in Order to Achieve 80% Power, When α = .001, for a Rare Dominant Disease

	No. of Cases Required, for Marker Spacing of															
	1 cM					2 cM					5 cM			10 cM		
m	t = 10	t = 20	t = 40	t = 100	t = 150	t = 10	t = 20	t = 40	t = 100	t = 150	t = 10	t = 20	t = 40	t = 10	t = 20	t=40
2	71	79	98	181	302	79	98	148	503	1,382	109	183	511	186	525	4,111
3	48	54	66	118	192	54	66	98	314	837	73	120	319	121	327	2,440
4	42	46	55	97	154	46	55	80	247	642	61	97	251	99	257	1,836
5	38	42	50	86	135	42	50	72	213	541	55	86	216	88	221	1,520
6	36	40	47	79	123	40	47	67	193	479	52	80	195	81	200	1,324
7	35	38	45	75	116	38	45	64	179	437	50	76	181	77	186	1,190
8	35	38	44	73	111	38	44	62	170	407	48	73	172	74	176	1,093
9	34	37	44	71	107	37	44	61	163	385	48	72	165	72	168	1,019
10	34	37	43	70	104	37	43	60	158	367	47	70	161	71	163	961

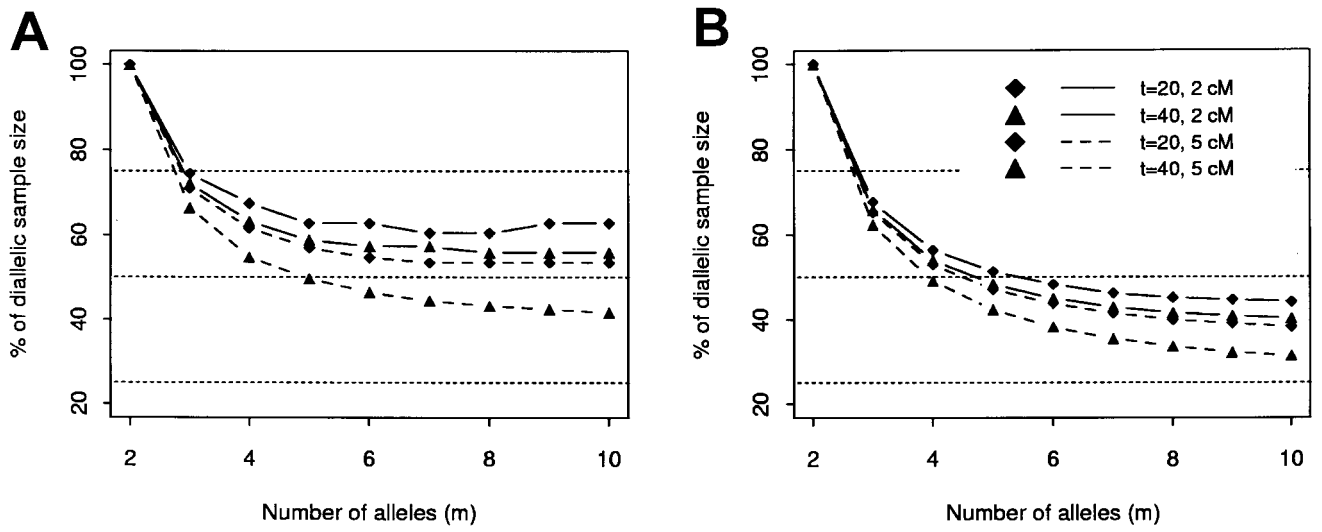

Figure 1 Sample size required in order to achieve 80% power when $\alpha = .001$, as a percentage of sample size needed in order to achieve the same power for a diallelic marker. Sample sizes are shown for two screening densities (2 cM and 5 cM) and two mutation ages ($t = 20$ and $t = 40$). *A*, Recessive disease. *B*, Dominant disease.

Finally, for the least-dense screen (10 cM), LD is detectable with reasonable sample sizes only for the younger mutations, and sample sizes are substantially reduced by use of multiallelic markers (e.g., $t = 20$, $n = 128$ for diallelic markers vs. $n = 59$ for six-allele markers).

Figure 7 shows similar results for a dominant disease, comparing diallelic markers to eight-allele markers. For a 1-cM screen, LD is detectable for all four ages of mutation, and sample sizes are considerably smaller for the multiallelic markers. For a 2-cM screen, LD is detectable for all four ages of mutation with eight-allele markers, but with diallelic markers and the oldest mutation, the required sample size is prohibitively large ($n = 503$), even for this dense a screen. When a 5-cM screen is used, detection of LD by use of diallelic markers is feasible only for younger mutations. The use of eight-allele markers makes detection possible for the moderately old mutation ($t = 40$), but the sample size remains unrealistically large for the oldest mutation. For the loose screen (10 cM) and with diallelic markers, LD is detectable only for the very youngest mutation, and, even here, sample sizes are quite large ($n = 186$). Use of eight-allele markers makes detection of LD possible with the second youngest mutation, but sample sizes remain prohibitively large for the two older mutations.

Tables 4 and 5 show the above information in a different way. In table 4 for recessive disease, a minus sign ("−") denotes a situation in which sample sizes were <100 for both diallelic and six-allele markers, and a plus sign ("+") denotes a situation in which both sample sizes were greater than 300. Other cells contain the diallelic sample size, followed by the six-allele sample size. Those cells with numeric entries represent situations in which the use of multiallelic markers may make LD testing possible. Similar information for a dominant disease is displayed in table 5. In this case, diallelic markers are being compared to eight-allele markers, and a minus sign ("−") denotes sample sizes less than 150 and a plus sign ("+") denotes sample sizes greater than 300. Numeric entries represent situations in which the use of multiallelic markers makes LD testing possible.

Table 4

No. of Cases Required in Order to Achieve 80% Power to Detect LD, When $\alpha = .001$, for Recessive Disease, for $m = 2$ and $m = 6$

	No. of Cases Required (M = 2/M = 6), for Marker Spacing of[a]			
t	1 cM	2 cM	5 cM	10 cM
10	−	−	−	−
20	−	−	−	128/59
40	−	−	125/58	+
100	−	123/56	+	+

[a] A minus sign (−) denotes that, for both values of *m*, <100 cases were required; a plus sign (+) denotes that, for both values of *m*, >300 cases were required.

Markers with Nonequifrequent Alleles

All of the above results are for markers with equifrequent alleles. In order to investigate the performance of this test when used with markers that are more typically available, we considered the markers used in the GAW10 simulated data set (MacCluer et al. 1997), which were

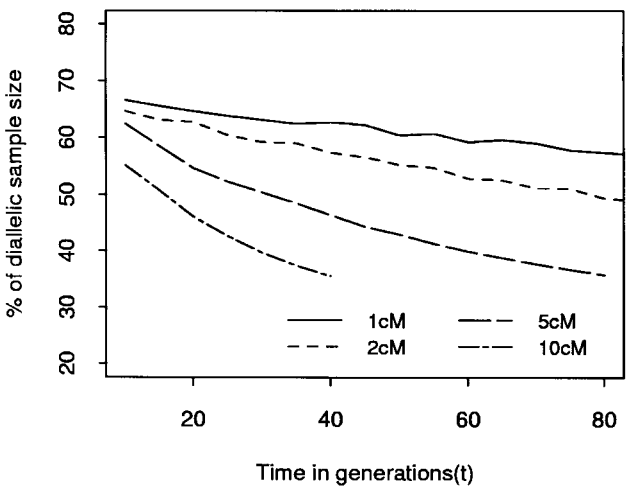

Figure 2 Sample size required in order to achieve 80% power to detect LD with a recessive disease, when $\alpha = .001$ and a six-allele marker is used, as a percentage of sample size needed in order to achieve the same power for a diallelic marker, as a function of time and marker-screening density.

a random sample from the Research Genetics (Cooperative Human Linkage Consortium) mapping panels. Figure 8 shows a histogram of the sample size required in order to achieve 80% power when $\alpha = .001$, for a recessive mutation with $t = 40$, when using a 5-cM screen density. The markers selected are the 104 six-allele markers in the GAW10 data set, which have a mean heterozygosity of 0.75. The vertical lines denote the required sample sizes with use of the ideal equifrequent six-allele marker, and the ideal equifrequent diallelic marker. Because the markers sampled from a real mapping panel do not have equifrequent alleles, none of them achieve the minimal sample size required by the ideal six-allele marker. On average, these markers require a sample size of about 70, a 17% increase above the minimal sample size of 59. While the real markers require larger sample sizes than the ideal considered in this paper, it is important to note that even these more realistic sample sizes are much smaller than that required by the best diallelic marker. The results for mutations of different ages and markers with different numbers of alleles were very similar and are not presented here.

The heterozygosity of a marker is closely related to the value of $G^2(0)$ and therefore to the power of the test based on that marker. Examination of the GAW10 markers indicates that, for a fixed number of marker alleles, the relationship between heterozygosity and $G^2(0)$ is approximately linear ($\rho = .992$ for the six-allele markers, recessive disease) and increasing, and it is therefore not unreasonable to use heterozygosity to chose between markers with the same number of alleles. Choosing between markers with different numbers of alleles is not as simple, because the number of alleles affects the df of the test statistic. For example, consider a four-allele marker with equifrequent alleles and a six-allele marker with four equifrequent alleles and two very rare alleles. The two have similar heterozygosities and values of $G^2(0)$, but the test based on the former has 3 df, whereas the test based on the latter has five. Clearly, the former marker will yield a more powerful test. When choosing between markers with the same heterozygosity and different numbers of alleles, the one with fewer alleles will be more powerful.

In determining the sample size required for a genome screen, several approaches are possible. One is to calculate the sample size needed for the desired power for each marker in the panel, and then take the maximum of these as the target sample size. Any mapping panel will contain some suboptimal markers, and therefore this approach might yield quite large sample sizes and expensive studies. An alternative is to choose the sample size so that the average power over all markers in the panel is greater than or equal to the desired power.

Discussion

In this paper, we consider the utility of single-marker LD testing that uses a case-control study design with either diallelic or multiallelic markers. We focus primarily on an initial genome screen for a disease locus of interest, although the issues raised are also relevant to localization of a previously mapped gene. We use a deterministic population genetic model, ignoring genetic drift, to calculate the expected marker allele frequencies in the cases and controls, as a function of the marker

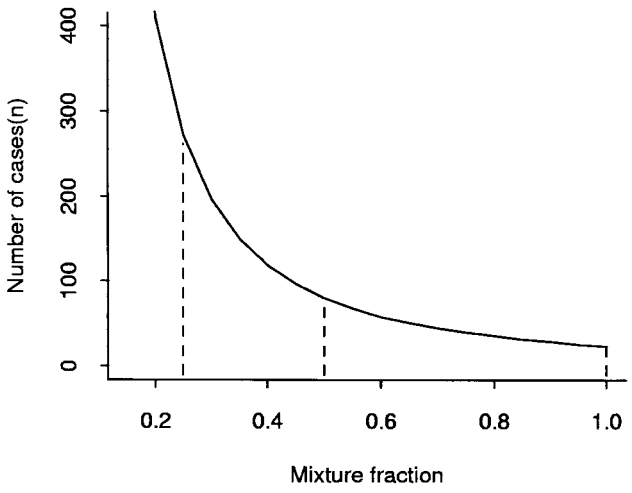

Figure 3 Sample size required in order to achieve 80% power to detect LD , when $\alpha = .001$, for screen density 5 cM, $t = 20$, and six-allele markers, as a function of the fraction of case chromosomes expected to carry the linked mutation.

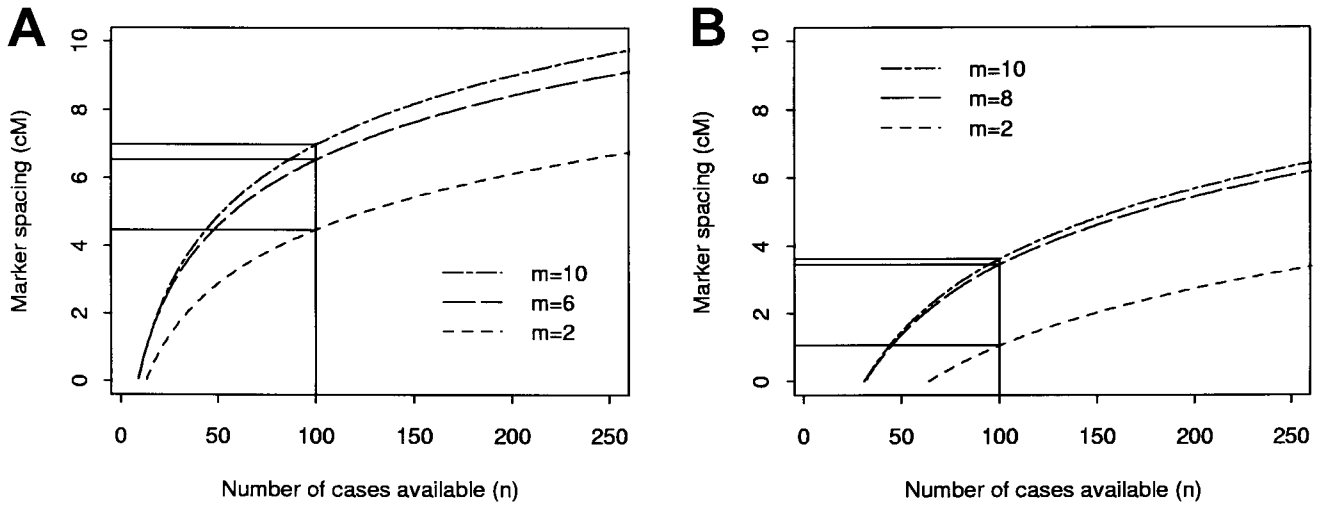

Figure 4 Maximum possible marker spacing to achieve 80% power to detect LD with a mutation of age $t = 40$ when $\alpha = .001$, as a function of sample size and number of marker alleles. *A*, Recessive disease. *B*, Dominant disease.

density, the age of the mutation, the number of marker alleles, and the mode of inheritance of a rare disease of monophyletic origin. This allows us to compare diallelic markers to multiallelic markers in terms of sample sizes required for detection of LD, by use of single-marker tests, and to describe the relative marker spacings necessary for diallelic markers to achieve the same power as is given by multiallelic markers. Finally, we describe the genetic models and population histories under which LD can be detected by use of a single marker, with reasonable sample sizes and marker densities.

In the situations considered here, multiallelic markers always offer an improvement over diallelic markers under otherwise equivalent conditions, in terms of power to detect LD. This improvement in power is achieved by moderately polymorphic markers (six to eight alleles), suggesting that it is not necessary to go to great lengths to obtain extremely polymorphic markers. It is important to note that all the results presented here used a Type I error level of $\alpha = .001$ for each test in a genome screen. The overall Type I error rate for the genome screen is related to the number of tests performed and therefore depends on the screen density used. Our results show that use of diallelic markers rather than multiallelic markers requires a denser screen to achieve the same power with a fixed sample size and a specific testwise Type I error. Since more significance tests are performed in the denser screen, the overall Type I error of that screen is larger than for the less dense screen that is possible with multiallelic markers. One could correct the testwise Type I error to make the genome-wide Type I errors comparable. This would further decrease the power of tests that use diallelic markers for a given screening density, or it would require an even denser map of diallelic markers to retain the same power. These considerations show conclusively that the use of diallelic markers requires much denser screens and therefore more genotyping to achieve the same power as is given by a less-dense screen with use of multiallelic markers.

Recent technological developments (Chee et al. 1996) suggest that it may soon be possible to type diallelic markers quickly and inexpensively, making it more cost effective to use very dense screens of diallelic markers,

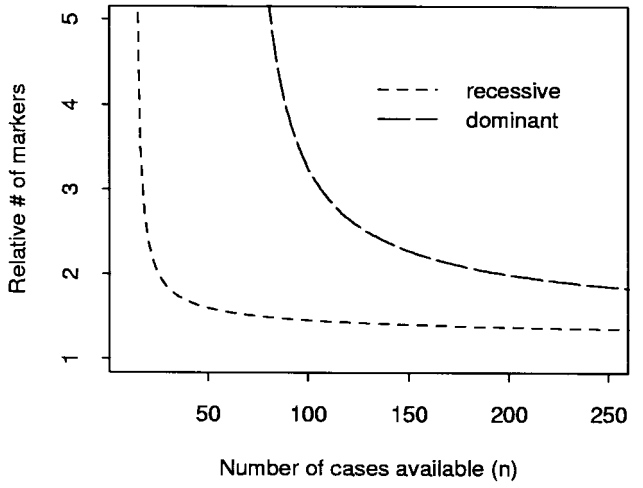

Figure 5 Number of diallelic markers required for a genome screen (80% power and $\alpha = .001$), relative to number of multiallelic markers required, as a function of available sample size. In the recessive disease case the multiallelic marker has six alleles, whereas in the dominant disease case it has eight alleles.

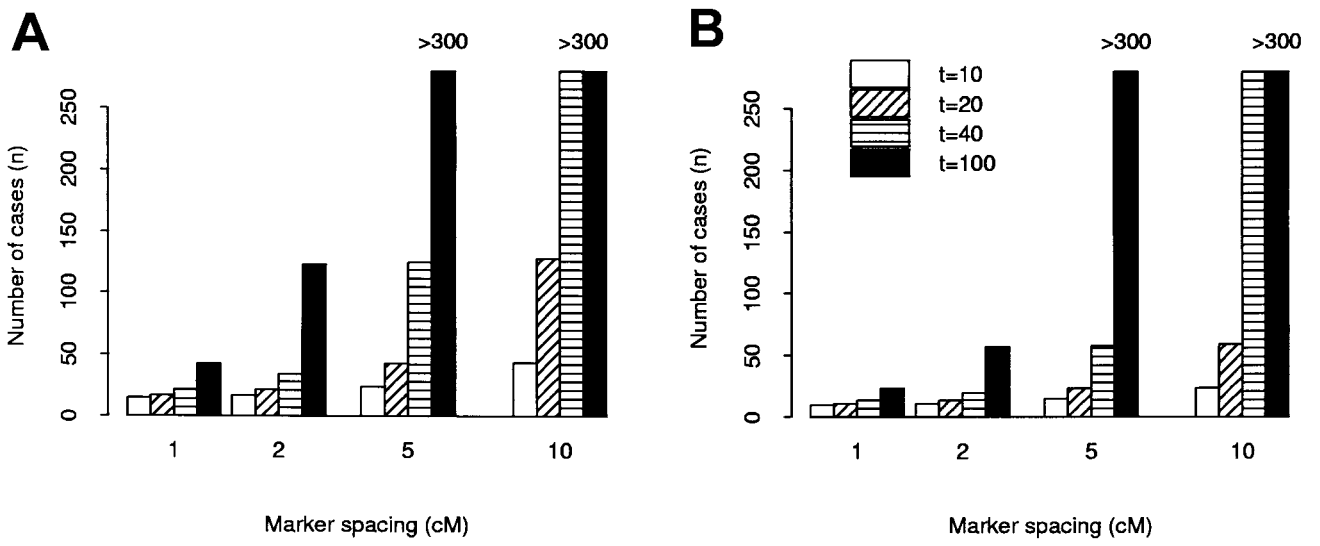

Figure 6 Sample size required in order to achieve 80% power to detect LD with a recessive mutation, when $\alpha = .001$, as a function of screen density and age of mutation, for diallelic (*A*) and six-allele (*B*) markers.

rather than more widely spaced screens of multiallelic markers. We can speculate on the conditions under which a screen with diallelic markers may be more cost effective than a screen with multiallelic markers. Our results suggest that, for detection of LD, diallelic markers may become useful sooner with recessive diseases than with dominant or complex diseases. This is because the difference in required marker density, for diallelic markers versus multiallelic markers, is not as great for a recessive disease as it is for a dominant disease. To achieve equivalent overall cost for a sample of $n = 100$ cases and a mutation age of $t = 40$, the per-genotype cost for a diallelic marker would need to be 64% of the per-genotype cost for a 10-allele marker, when a recessive disease is being analyzed; for a dominant disease, the per-genotype cost for a diallelic marker would have to be 29% of the per-genotype cost for a 10-allele multiallelic marker; for a more complex disease, for which the proportion of the disease chromosomes in the cases is <50%, which corresponds to a dominant disease, the relative cost of the diallelic marker would have to be even lower. Regardless of the cost of genotyping, at this point in time, accurate maps are available only to a resolution of 10 cM (Yuan et al. 1997). Improved resolution requires that either more families must be typed for the current mapping panels or methods such as sperm typing (Arnheim 1991) must be used to increase the number of sampled meioses. With the mapping panels currently available, 10-cM screens with multiallelic markers are the only option. More-dense screens with diallelic markers must wait for an improvement in map resolution.

We have shown that, even in the ideal case of a fully penetrant recessive disease caused by a single mutation, a 10-cM genome screen does not have reasonable power to detect LD unless the mutation under consideration is very recent—that is, $t = 10$ or 20. Even when the mutation is this recent, LD with a dominant mutation is detectable in a 10-cM screen with reasonable sample sizes only if multiallelic markers are used. If a 5-cM screen is used, LD with a recessive mutation is detectable by use of reasonable sample sizes even when a mutation is moderately old ($t = 40$), with either type of marker; at this screening density, LD with a dominant mutation of the same age is detectable only if multiallelic markers are used. It is worth noting that sample sizes required for detection of LD with a dominant mutation are quite large. However, for a given disease-allele frequency, a dominant disease is much more prevalent in the popu-

Table 5

No. of Cases Required in Order to Achieve 80% Power to Detect LD, When $\alpha = .001$, for Dominant Disease, for $m = 2$ and $m = 8$

	No. of Cases Required (for $m = 2/m = 6$), for Marker Spacing of[a]			
t	1 cM	2 cM	5 cM	10 cM
10	−	−	−	184/74
20	−	−	183/73	525/176
40	−	−	511/172	+
100	181/73	503/170	+	+

[a] A minus sign (−) denotes that, for both values of *m*, <150 cases were required; a plus sign (+) denotes that, for both values of *m*, >300 cases were required.

lation than is a recessive disease, so collecting the larger number of cases may not be a major problem.

The foregoing summary shows that, in the assessment of the feasibility of genome screens for LD, it is very important to consider the expected age of a mutation, conditional on its existence in the current population. Factors such as selection, rate of population growth, and current disease-allele frequency will affect the expected age of a mutation and, consequently, the expected size of the region with detectable LD. Theoretical considerations predict that mutations will be relatively young both in populations that have undergone rapid and steady population growth (Thompson and Neel 1997) and in very small stable populations (Ewens 1979, p. 166). The latter, however, are unlikely to be useful for the sorts of case-control studies considered here, precisely because of the small population size. In populations that have experienced a slower growth rate, mutations are expected to be somewhat older. However, because of the overall population growth in the human species, the age of few mutations would be expected to be $\geqslant$150 generations, and the age of many mutations will be younger than 40–80 generations (Thompson and Neel 1997). Although our calculations do not allow for an increase in mutation frequency, we do not expect that such a scenario would change our results substantially, as long as the mutation is still rare and monophyletic. Since the sampled chromosomes are still removed from the ancestral chromosome by the same number of meioses, the observable patterns of single-locus disequilibria should be similar.

A growing body of data supports these general theoretical predictions about the likely ages of mutations existing in human populations. In cases in which there is evidence of monophyletic mutation, the sizes of genomic regions with evidence of LD are generally 5–12 cM in populations that have experienced recent, rapid population growth and that, therefore, may be expected to have young disease mutations. Such populations often are composed of relatively recent ($t \leqslant 40$) immigrants into environments that then allow rapid population growth. They are also generally populations without significant gene flow from other populations, thereby retaining the monophyletic origin of rare disease mutations. Examples include diseases showing strong founder effects in French Canadians (Casaubon et al. 1996), Ashkenazi Jews (Risch et al. 1995), Roma (Kalaydjieva et al. 1996), Volga Germans (Bird et al. 1988), and the Amish (Sulisalo et al. 1994). The Finns, with a somewhat older initial immigration time ($80 < t < 100$), also show evidence of founder effects through large regions of LD around disease loci (Varilo et al. 1996), possibly because of both a history of extremely rapid population growth and additional recent migration, within the past 20 generations, into the northern parts of the country (Norio 1991). Ages of mutations in some of these populations have been estimated to be between $t = 14$ (Risch et al. 1995) and $t = 20$–30 (Varilo et al. 1996), although extreme caution must be used in the interpretation of such estimates, since their variance is enormous. These observations of extended regions of LD are consistent with young mutation age and suggest that a 10-cM genome screen for LD, with use of multiallelic markers, may be a reasonable way to approach the localization of Mendelian disease genes in populations such as these.

Some authors (Risch and Merikangas 1996; Brown

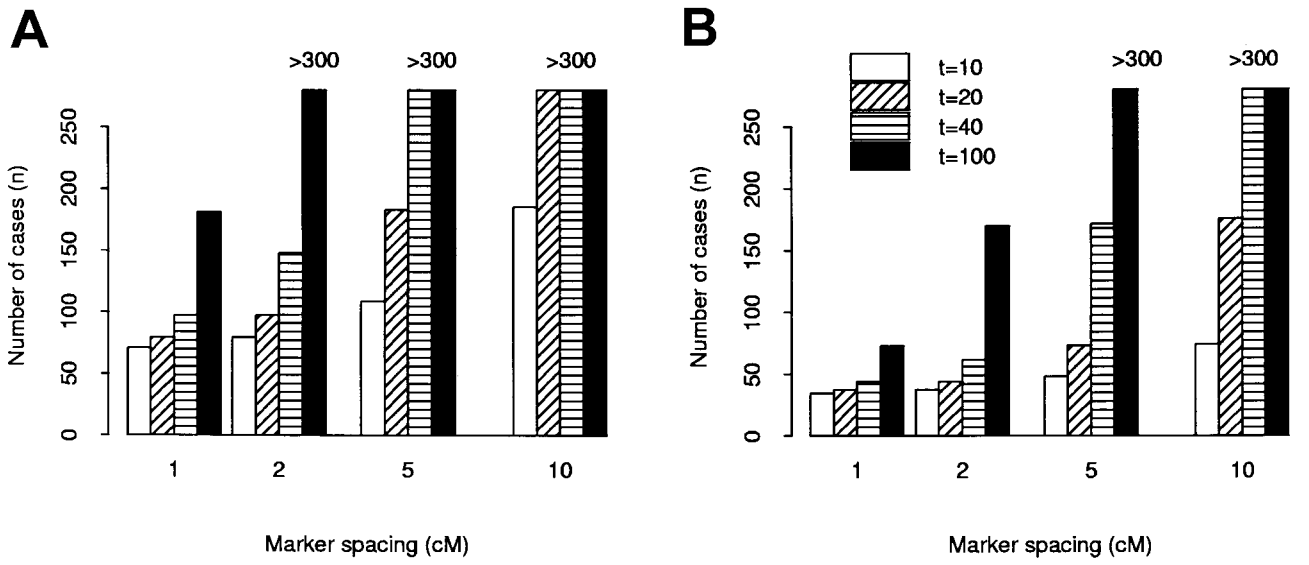

Figure 7 Sample size required in order to achieve 80% power to detect LD with a dominant mutation, when $\alpha = .001$, as a function of screen density and age of mutation, for diallelic (*A*) and eight-allele (*B*) markers.

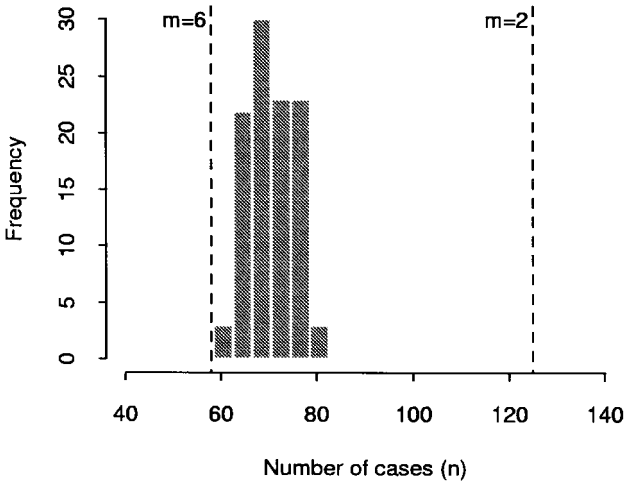

Figure 8 Sample sizes required in order to achieve 80% power to detect LD with a recessive mutation, when $\alpha = .001$, for a 5-cM screen when $t = 40$, for the six-allele GAW10 markers.

and Hartwell 1998) have suggested the use of LD screening methods in large randomly mating populations that are not genetic isolates. There is evidence that LD is detectable near Mendelian disease loci in such populations. When analysis is restricted to members of reasonably well-defined ethnic or political groups, evidence of LD can frequently be found with markers spanning the disease loci at 0.5–1.4-cM intervals (Wijsman 1997). Examples are found in the Japanese (Goddard et al. 1996), Italians (Pandolfo et al. 1990), British (Votruba et al. 1998), and Poles (Brzustowicz et al. 1993). These small intervals of detectable LD suggest older mutations, the existence of multiple mutations combined with admixture, or both, thereby inhibiting detection of LD, relative to the situation in isolated populations. Genome screens at the currently available density of 10 cM are clearly not adequate for detection of such LD, with reasonable sample sizes, but the existence of LD at small recombination fractions does suggest that, with a much denser map, especially of multiallelic markers, detection of LD with simple genetic diseases might be possible even in these nonisolated populations.

Even though our evaluation is based on highly optimistic conditions, some general extrapolation to more-complicated scenarios is possible. The results presented here are for fully penetrant recessive diseases and dominant diseases, but the methods used to calculate power can be easily applied to either diseases with reduced penetrance or more-complex models of inheritance. Comparison of conditions necessary to detect LD with a dominant versus recessive mutation provides insight into the effects of "contamination" of the sample of chromosomes available in the case population. For the parameters considered here, the dominant disease case required a 3.5–4.5-fold increase in sample size, compared with the recessive disease case. Clearly, this implies that, for mild contamination of the disease chromosomes, such as may occur because of a rare additional unlinked disease mutation, the sample size requirements will generally remain tolerable. However, this also suggests that a smaller sample of cases selected from a restricted population thought to be more homogeneous with respect to disease mutation may provide more power than is given by analysis of a larger but more heterogeneous sample. These results also suggest that, as the mixture fraction increases, as would be expected for a more complex trait, the sample sizes required may become extremely large.

LD testing as a fine-scale gene localization tool is well established, but LD testing as a genome screening tool has not yet been used to successfully map a disease gene. The results that we have presented here should further aid in the design of efficient studies aimed at fine-scale localization, but they raise questions about the feasibility of the use of single-marker LD testing in genome screens with anonymous markers. In a case-control design, LD is quickly obscured by even small complications, such as increasing mutation age and dominance. When added complications, such as multiple susceptibility loci and sporadic cases, are included, the required sample sizes and marker densities increase rapidly. In addition, as the difficulty in the detection of LD increases, so does the penalty inherent in the use of diallelic markers, as compared with the use of multiallelic markers. Together, these considerations suggest that the promise of diallelic markers for LD screens may be difficult to achieve. However, we have considered only single-marker tests here, and it is possible that future work on multipoint methods and methods that use haplotypes could change this picture. Until such studies have been performed, care should be taken to avoid overstatement of the potential of LD screening as a gene mapping tool, particularly in the case of complex traits.

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Electronic-Database Information

URLs for data in this article are as follows:

Research Genetics, Inc. (Cooperative Human Linkage Consortium), http://www.resgen.com

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