Am. J. Hum. Genet. 72:1515–1526, 2003

A Novel Class of Tests for the Detection of Mitochondrial DNA–Mutation Involvement in Diseases

Fengzhu Sun,[1] Jing Cui,[2] Haralambos Gavras,[2] and Faina Schwartz[2]

[1]Molecular and Computational Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles; and [2]Department of Medicine, Hypertension Section, Boston University School of Medicine, Boston

We develop a novel class of tests to detect mitochondrial DNA (mtDNA)–mutation involvement in complex diseases by the study of affected pedigree members. For a pedigree, affected individuals are first considered and are then connected through their relatives. We construct a reduced pedigree from an original pedigree. Each configuration of a reduced pedigree is given a score, with high scores given to configurations that are consistent with mtDNA-mutation involvement and low scores given to configurations that are not consistent with mtDNA-mutation involvement. For many pedigrees, the weighted sum of scores of the pedigrees is calculated. The tests are formed by comparing the observed score with the expected score under the null hypothesis that only nuclear autosomal mutations are involved. We study the optimality of score functions and weights under the heterogeneity model without phenocopies. We also develop a method to estimate the contribution that mtDNA mutations make if they are involved under a heterogeneity model. Finally, we apply our methods to three data sets: Leber hereditary optic neuropathy, a disease that has been proved to be caused by mtDNA mutations; non–insulin-dependent diabetes mellitus (NIDDM); and hypertension (HTN). We find evidence of mtDNA-mutation involvement in all three diseases. The estimated fraction of patients with NIDDM due to mtDNA-mutation involvement is 22% (95% confidence interval [CI] 6%–38%). The fraction of patients with HTN potentially due to mtDNA-mutation involvement is estimated at 55% (95% CI 45%–65%).

Introduction

mtDNA mutations have been found to cause various rare diseases, such as Leber hereditary optic neuropathy (LHON [MIM 535000]), Kearns-Sayre syndrome, and NARP (neurogenic muscle weakness, ataxia, and retinitis pigmentosa) (Wallace 1994, 1995). mtDNA mutations have also been found to be associated with common diseases, such as non–insulin-dependent diabetes (NIDDM [MIM 125853]) (Ballinger et al. 1992; Reardon et al. 1992; van den Ouweland et al. 1992; Kadowaki et al. 1994) and Alzheimer disease (Davis et al. 1997). Recently, the involvement of mtDNA mutations has been suggested in hypertension (HTN [MIM 145500]) (DeStefano et al. 2001). As we continue to understand more about the genetic basis of common diseases and mitochondrial genetics, it is suspected that mtDNA mutations will be found to play a role in many common diseases (Wallace 1992).

The available statistical methods are not adequate for the testing of mtDNA-mutation involvement and for the study of contributions that mtDNA mutations make to complex diseases. Ottman et al. (1988) have proposed an epidemiological method to identify excess maternal inheritance of epilepsy in nuclear families. Mili et al. (1996) have extended this method to screen for excess maternal inheritance in extended pedigrees. McMahon et al. (1995) have found that there were more affected mothers than affected fathers for the probands in the bipolar pedigrees that they studied; they also hypothesized that mtDNA mutations may be involved in bipolar affective disorder. These methods split large pedigrees into nuclear families, and the information on mtDNA-mutation involvement from large pedigrees is not adequately considered. Schork and Guo (1993) have presented a general framework for segregation analysis to test mtDNA-mutation involvement. Recently, Haghighi and Hodge (2002) have provided a likelihood-based method to test for parent-of-origin effects in complex diseases. The likelihood-based approaches require assumptions about the mode of inheritance of the disease of interest and about the interactions between nuclear and mtDNA mutations; these assumptions are generally not known. Sun et al. (1998) have studied the statistical properties of a test by comparing the risk carried by one type of relative in the mitochondrial lineage of the probands with the risk carried by the same type of rel-

Received January 6, 2003; accepted for publication March 27, 2003; electronically published April 30, 2003.

Address for correspondence and reprints: Dr. Fengzhu Sun, Molecular and Computational Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089. E-mail: fsun@hto.usc.edu

0002-9297/2003/7206-0015$15.00

ative in the nonmitochondrial lineage. The mitochondrial lineage of a proband is defined as the relatives who share the same mtDNA as the proband when homoplasmic mtDNA transmission is assumed; the other relatives form the nonmitochondrial lineage. Sun et al. (1998) found that, under the heterogeneity model, in which a disease is caused by either nuclear autosomal mutations or mtDNA mutations, the power of the test increases as the relationship between the relatives and the probands becomes distant; however, they did not provide methods to combine information from different types of relatives.

In the present study, we develop a novel class of tests to detect mtDNA-mutation involvement in complex diseases by the study of affected pedigree members. These tests are applicable to pedigrees ascertained through various sampling schemes, such as families with affected sibling pairs or families with multiple affected individuals, as long as the inclusion and/or exclusion of pedigrees does not depend on the sex of affected individuals. The tests do not assume the mode of inheritance of the disease, although the power of the tests certainly depends on the mode of inheritance. In this sense, the tests developed here are nonparametric. We also develop methods to combine information from different pedigrees. Our approach is similar to that proposed by Weeks and Lange (1988, 1992), Whittemore and Halpern (1994), and Teng and Siegmund (1997) for linkage studies of nuclear mutations when affected pedigree members are included.

First, we present the class of statistical tests, which depend on score functions defined for the relationship among affected individuals. Then, we study the optimality of score functions under the heterogeneity model, which assumes that the disease can be caused by either autosomal mutations or mtDNA mutations. We also present methods to estimate the contribution of mtDNA mutations, under the heterogeneity model, once evidence has been found for their involvement. Finally, we apply our tests to simulated data and real data on LHON (which has been proved to be related to mtDNA mutations), NIDDM, and HTN.

Tests

To avoid pedigrees with disease due to different mutations, we consider only those pedigrees in which individuals who marry into the pedigrees and their first-degree relatives are not affected. If that happens, then we remove affected married-in individuals and their descendants from the study.

Score Function and Test Statistic with One Pedigree

For a given pedigree, we construct another pedigree as follows:

1. Remove the married-in individuals.
2. Connect any two affected individuals through their closest relatives and mark the individuals between them as affected.
3. If a sibship has at least one affected individual and none of them have affected offspring, then denote the sibship with a diamond.
4. Remove unaffected individuals in the resulting pedigree.

The resulting pedigree will be referred to as a "reduced pedigree." Individuals having at least one descendant in the reduced pedigree will be referred to as "informants," since only these individuals provide information on mtDNA-mutation involvement. Only pedigrees with at least two affected sibships in the reduced pedigrees are considered in the analysis. Pedigrees with only one affected sibship in the reduced pedigrees are not used. We emphasize here that an affected sibship can contain one or more affected individuals. Figure 1*a* shows a true pedigree consisting of an affected grandparent and an affected grandchild with an unaffected mother; the corresponding reduced pedigree is shown in figure 1*b*. Figure 2*a* provides an example of a pedigree with affected cousin pairs; the corresponding reduced pedigree is given in figure 2*b*. It is reasonable to assume that all members in the reduced pedigree carry the genetic mutation(s) responsible for the disease if it is a genetic disease. In this sense, we include only affected pedigree members in the present study. In complex diseases, it is often difficult to determine unambiguously if an individual is

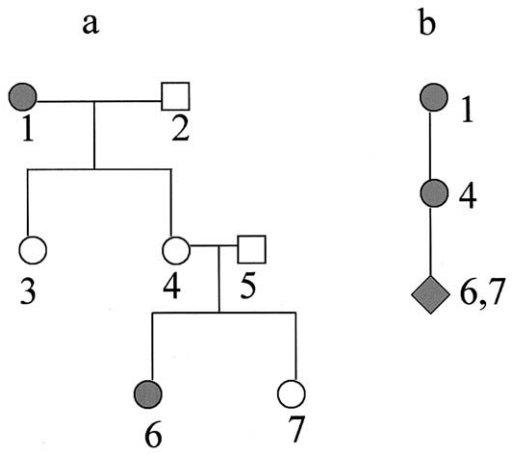

Figure 1 A reduced pedigree constructed from a true pedigree that includes an affected grandparent and an affected grandchild. *a*, True pedigree. *b*, Reduced pedigree. Construction of the reduced pedigrees is as follows: (i) married-in individuals (2 and 5) are removed; (ii) individual 4 is labeled as affected, because individuals 1 and 6 are connected through individual 4; (iii) the sibship consisting of individuals 6 and 7 is labeled with a diamond; and (iv) individual 3 is removed, to obtain the reduced pedigree. Individuals 1 and 4 are the informants.

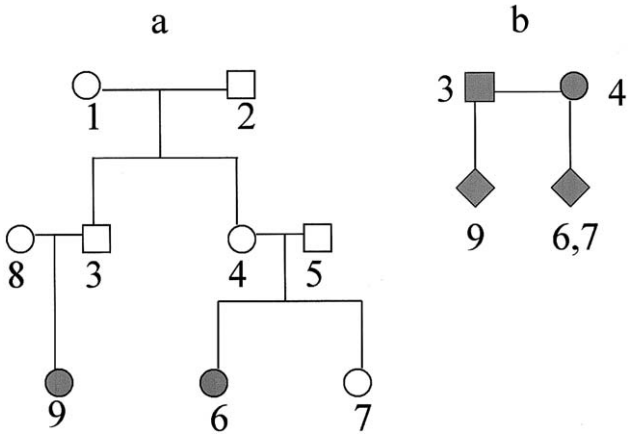

Figure 2 A reduced pedigree constructed from a pedigree with affected cousin pairs. *a*, True pedigree. *b*, Reduced pedigree. Construction of the reduced pedigrees is as follows: (i) married-in individuals (5 and 8) are removed; (ii) individuals 3 and 4 are labeled as affected, because individuals 6 and 9 are connected through individual 4; (iii) the sibship consisting of individuals 6 and 7 and the sibship consisting of individual 9 are labeled with a diamond; and (iv) individuals 1, 2, 5, and 8 are removed, to obtain the reduced pedigree. Individuals 3 and 4 are the informants.

affected. For late-onset diseases, although an individual may carry the genetic mutation, he or she may not be affected, because he or she has not yet reached the age of onset. The inclusion of only affected members may reduce these problems. The reasons for consideration of affected pedigree members are the same as those in the linkage analysis of nuclear mutations.

We next neutralize the sex of informants. Let k be the number of informants in the reduced pedigree. There are 2^k possible configurations in which each informant in the reduced pedigree is either male or female. For the reduced pedigree in figure 1*b*, we have $k = 2$. The $2^k = 4$ possible configurations are given in figure 3. Similarly, for the reduced pedigree in figure 2*b*, $k = 2$, and the $2^k = 4$ possible configurations are given in figure 4. If only nuclear autosomal mutations are involved and there are no sex differences with respect to prevalence and fertility, then these 2^k configurations should be equally probable, with probability 2^{-k}.

A score function is defined by giving a score $S(\phi_j)$ to each of the 2^k configurations $\phi_j, j = 1,2,\ldots,2^k$. High scores are given to configurations that are consistent with mtDNA-mutation involvement, and low scores are given to configurations that are not consistent with mtDNA-mutation involvement. For example, a higher score is given to configuration *a* than to the other three configurations in figure 3. Similarly, a higher score is given to configuration *c* than to the other three configurations. Let T be the score for a reduced pedigree with k informants. Under the null hypothesis of only nuclear-autosomal-mutation involvement, all the 2^k configurations are equally likely, with probability $1/2^k$, because all the informants can be either male or female with the same probability, 1/2. The mean score $E(T)$ for a reduced pedigree with k informants is $E(T) = 2^{-k}\sum_{\phi_j} S(\phi_j)$. The summation is over all the 2^k configurations. The variance is $\mathrm{Var}\,(T) = 2^{-k}\sum_{\phi_j} S^2(\phi_j) - [E(T)]^2$.

Score Function and Test Statistic with Several Pedigrees

Now, suppose that we have n pedigrees. We first generate the corresponding reduced pedigrees. Suppose that the n reduced pedigrees can be classified into I classes, $C_1, C_2, \ldots, C_I$, with n_i reduced pedigrees in the ith class, $n_1 + n_2 + \cdots + n_I = n$. The pedigrees within each class have the same reduced pedigree except that the sex of the informants may be different. Let ϕ_{ij} be the configuration in the jth reduced pedigree of the ith class, when $j = 1,2,\ldots,n_i$ and $i = 1,2,\ldots,I$. Then, $S(\phi_{ij}), j = 1,2,\ldots,n_i$ are independent identically distributed random variables and have the same distribution under the null hypothesis of only nuclear-autosomal-mutation involvement. Let T be the weighted sum of scores for all the pedigrees with weight w_i for pedigrees in the ith class; that is,

$$T = \sum_{i=1}^{I} w_i \sum_{j=1}^{n_i} S(\phi_{ij}) \ .$$

The idea is to reject the null hypothesis that only nuclear autosomal mutations are involved if T is large compared with its mean.

We consider the following statistic:

$$\mathcal{T} = \frac{T - E(T)}{\sqrt{\mathrm{Var}\,(T)}} \ ,$$

where $E(T)$ and $\mathrm{Var}\,(T)$ are the expectation and variance of T under the null hypothesis of only nuclear-autosomal-mutation involvement. From equations (1)

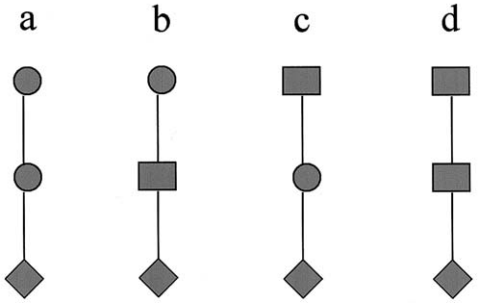

Figure 3 The four reduced pedigrees after neutralizing the sex of the informants corresponding to the reduced pedigree in figure 1*b*.

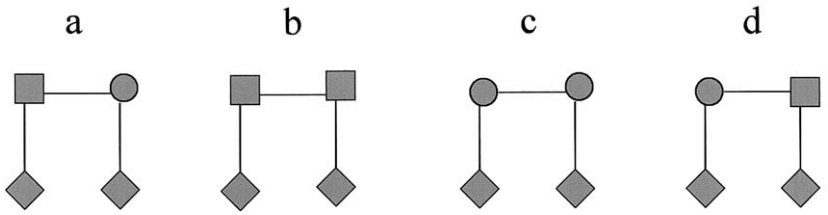

Figure 4 The four reduced pedigrees after neutralizing the sex of the informants corresponding to the reduced pedigree in figure 2*b*

and (2), we have $E(T) = \sum_{i=1}^{I} w_i n_i E(T_i)$ and $\mathrm{Var}(T) = \sum_{i=1}^{I} w_i^2 n_i \mathrm{Var}(T_i)$.

$\mathcal{T}$ tends to the standard normal distribution, with mean 0 and variance 1, when $n_i, i = 1,2,\ldots,I$ tend to ∞. Thus, it is relatively easy to obtain the significance level for an observed T. When n_i is not large and normal approximation is in doubt, we can use the following formula to calculate the significance level for an observed T:

$$\alpha = \sum \prod_{i=1}^{I} \prod_{j=1}^{n_i} P(\phi_{ij}) = N(T) 2^{-\sum_{i=1}^{I} n_i k_i}, \qquad (3)$$

where k_i is the number of informants in the reduced pedigree for the ith class and $N(T)$ is the number of configurations $\{\phi_{ij}, j = 1,2,\ldots,n_i, i = 1,2,\ldots,I\}$ such that $\sum_{i=1}^{I} \sum_{j=1}^{n_i} w_i S(\phi_{ij})$ is at least T. Equation (3) holds because $P(\phi_{ij}) = 2^{-k_i}$ and the summation in the middle is over all the configurations such that $\sum_{i=1}^{I} \sum_{j=1}^{n_i} w_i S(\phi_{ij})$ is at least T.

Different score functions and weights give different tests. The problem becomes how to choose score functions and weights. We will consider the choice of score functions and weights under the heterogeneity model in the next section, "Score Functions and Power Analysis." The resulting statistics have several features:

First, as shown above, the tests permit calculation of exact P values when the number of pedigrees are small and normal approximations are in doubt.

Second, because we use only affected members and their closest relatives, the tests are applicable to pedigrees ascertained through multiple affected members. This feature is particularly important, since many pedigrees collected for linkage analysis are ascertained through multiple affected members. The tests are affected by neither late onset nor variable onset of a disease, although the power does depend on these factors. The tests are valid as long as the ascertainment does not depend on the sex of individuals to be included. However, we note that different rates of participation in a study, as well as recall bias of the affection status for males and females, can invalidate the results.

Third, the separation of probability distribution and score functions for the configurations of reduced pedigrees is another feature of the tests. This feature makes it possible to choose appropriate probability distribution and score functions under different scenarios. For example, we can modify the score function according to different assumptions about the etiology of the disease. Thus, if we were to assume no phenocopies, we might give a high score to the configuration in which all affected informants are females and a common low score to other configurations. If we were to allow phenocopies, we might let the score of a configuration be a decreasing function of the number of different possible types of mtDNAs among the affected individuals.

Fourth, the tests combine information from different pedigrees. A suitable weighting scheme for different classes of pedigrees allows us to give different weights according to each class's importance to the goal of detecting mtDNA-mutation involvement. In the next section ("Score Functions and Power Analysis"), we show that, under the heterogeneity model, the weights should increase with the number of informants in a reduced pedigree. This is consistent with our previous study, in which distant relatives had higher power than close relatives under the heterogeneity model (Sun et al. 1998).

As with other tests for excess maternal inheritance, the significance of the tests does not lead us directly to the conclusion that mtDNA mutations are involved in the etiology of the disease. There are other confounding factors that can lead to the significance of the test: X linkage, maternal imprinting, differential prevalence of the disease among males and females, differential fertility for affected males and females, intrauterine effect, and other genetic or environmental factors can all lead to the rejection of the null hypothesis. Some of these factors can be circumvented through the tests, and some cannot.

Differential prevalences and fertilities among males and females can be circumvented by changing the probability distribution for the configurations of reduced pedigrees. If the prevalence of the disease among males, p_m, and the prevalence of the disease in females, p_f, are different, then we can incorporate this by defining the probability for a configuration with k affected mothers and $n - k$ affected fathers as

$$(nk)\left(\frac{p_f}{p_f + p_m}\right)^k \left(\frac{p_m}{p_f + p_m}\right)^{n-k}.$$

Similarly, we can incorporate differential fertilities and recall bias for males and females into the probability distribution. However, we emphasize that p_m and p_f have to be known to be incorporated into our model.

Using reduced pedigrees comprising three or more generations, we can distinguish between maternal imprinting and mitochondrial inheritance. For example, for the four configurations in figure 3, let $S(a) = 1$, $S(b) = -1$, $S(c) = -3$, $S(d) = -1$. Then, under the hypothesis of only nuclear autosomal mutations and no imprinting, the four configurations are equally likely, with probability 1/4. The mean score of such reduced pedigrees is $(1 - 1 - 3 - 1)/4 = -1$. For an autosomal maternally imprinted gene, we can see only configurations *a* and *c,* since males do not transmit the disease to their offspring (Sleutels et al. 2000). The two configurations have the same probability, 1/2. Suppose that the disease can be caused by nuclear mutations, with probability $1 - f$, or maternally imprinted nuclear autosomal mutations, with probability f. Then, the mean score of one such pedigree is

$$(1 - f)(-1) + \frac{f(1 - 3)}{2} = -1 \ .$$

The two means are the same. It is highly unlikely that the test yields significant results. In contrast, under the heterogeneity model with autosomal and mtDNA mutations, the mean score is

$$(1 - f)(-1) + 1f = -1 + 2f > -1 \ . \qquad (4)$$

Therefore, with a sufficient number of such reduced pedigrees, the test can yield significant results. Therefore, using the above score function (eq. [4]), we eliminated the confounding factor due to imprinting. For other types of reduced pedigrees, we can similarly define score functions so that imprinting is not a confounding factor. Note that the above score function (eq. [4]) reduces the power of the test when mtDNA mutations are actually involved. Also note that we have to use reduced pedigrees with at least three generations to distinguish between maternal imprinting and mtDNA-mutation involvement.

Score Functions and Power Analysis

In the previous section ("Tests"), we have presented the general idea of how to construct the class of tests. For a given score function, the power of the corresponding test depends on the underlying disease-transmission model. For a given disease-transmission model, we say that test T_1 (corresponding to a score function S_1) is better than test T_2 (corresponding to a score function S_2) if T_1 is more powerful than T_2 under that disease-transmission model.

In the present section, we consider the heterogeneity model. Let f be the fraction of pedigrees with disease due to mtDNA mutations and let $1 - f$ be the fraction of pedigrees with disease due to nuclear autosomal mutations with no phenocopies. To consider the optimality of score functions, we first assume that all reduced pedigrees are of the same class, with i informants in each reduced pedigree. There are 2^i possible configurations for the reduced pedigrees. If a pedigree has disease that is due to nuclear autosomal mutations, then all the configurations have the same probability, 2^{-i}. If a pedigree has disease that is due to mtDNA mutations, then only one configuration is possible; that is, all the informants are female. Under the above assumptions, theoretical studies have shown that the optimal score function is to assign a high score to the configuration in which all the informants are females and to assign a common low score to the other $2^k - 1$ configurations. The power of the resulting test does not depend on the specific values of the high and low scores. Therefore, we choose the high score to be 1 and the low score to be -1. Let T_i be the resulting statistic for reduced pedigrees having i informants. Under the heterogeneity model, we have

$$P\{T_i = 1\} = f + (1 - f)p_i, P\{T_i = -1\} = (1 - f)q_i \ ,$$

where $p_i = 2^{-i}$ and $q_i = 1 - p_i$.

$$E(T_i) = f + (1 - f)(p_i - q_i) \ ,$$

and

$$\mathrm{Var}\,(T_i) = 4(1 - f)q_i[f + (1 - f)p_i] \ .$$

We classify all reduced pedigrees with the same number, i, of informants into one class, called the ith class. Suppose that there are I classes of pedigrees with n_i pedigrees in the ith class. Next, let us consider how to choose the weight, w_i, for the ith class. Recall that $T = \sum_{i=1}^{I} w_i \sum_{j=1}^{n_i} S_{ij}$, where S_{ij} is the score for the jth pedigree in the ith class. $S_{ij}, j = 1,2, \ldots, n_i$ are independent identically distributed random variables and have the same distribution as T_i. Therefore,

$$E(T) = \sum_{i=1}^{I} w_i n_i[f + (1 - f)(p_i - q_i)] \ ,$$

and

$$\mathrm{Var}\,(T) = 4(1 - f)\sum_{i=1}^{I} w_i^2 n_i q_i[f + (1 - f)p_i] \ .$$

Let E_0 and V_0 be the mean and variance of T under the null hypothesis that $f = 0$. Then,

$$T = \frac{T - E_0}{\sqrt{V_0}} \qquad (5)$$

Table 1

Efficiency of Test Statistics

	Efficiency[a] When f Is										
f'	.00	.10	.20	.30	.40	.50	.60	.70	.80	.90	1.00
.00	1.00	.97	.93	.90	.87	.82	.81	.79	.78	.77	.77
.25	.91	.98	1.00	1.00	.99	.99	.98	.97	.96	.95	.95
.50	.84	.94	.98	.99	1.00	1.00	1.00	1.00	.99	.99	.99
.75	.79	.90	.95	.98	.99	1.00	1.00	1.00	1.00	1.00	1.00
1.00	.76	.87	.93	.96	.98	.99	.99	.99	1.00	1.00	1.00

Note.—Results are based on test statistics in equation (5), using different weights $w_i = 1/[f' + 2^{-i}(1 - f')]$, $i = 1,2,3$ when the ratio for the numbers of families with 1, 2, and 3 informants is 3:2:1.

[a] Calculated using equation (6), where f is the underlying fraction of individuals affected owing to mtDNA-mutation involvement.

is approximately normal when $n_i, i = 1,2,\ldots,I$ are large. We reject the null hypothesis when

$$T = \frac{T - E_0}{\sqrt{V_0}} \geqslant z_\alpha ,$$

for a significance level α, where z_α is the $1 - \alpha$ percentile of the standard normal distribution.

The power of this test is given by

$$1 - \beta = 1 - \Phi\left(\frac{-f\sum_{i=1}^{I} w_i n_i q_i}{\sqrt{(1-f)\sum_{i=1}^{I} w_i^2 n_i q_i [f + (1-f)p_i]}} + z_\alpha \sqrt{\frac{\sum_{i=1}^{I} w_i^2 n_i q_i p_i}{\sum_{i=1}^{I} w_i^2 n_i q_i [f + (1-f)p_i]}}\right),$$

where $\Phi(\cdot)$ is the cumulative distribution function for the standard normal distribution. The smaller the term inside Φ is, the larger the power is. When $n_i, i = 1,2,\ldots,I$ are large, the dominant term inside Φ is the first one. Therefore, the best weights, w_i, should maximize

$$\frac{(\sum_{i=1}^{I} w_i n_i q_i)^2}{\sum_{i=1}^{I} w_i^2 n_i q_i [f + (1-f)p_i]} .$$

Mathematical analysis has shown that w_i should be proportional to $1/[f + (1 - f)p_i]$.

In reality, we do not know the actual value of f. Therefore, we cannot use the above weights in the test. There are several possible choices for the weights. On the one hand, if we are interested in detecting a very small fraction of mtDNA-mutation involvement, then we may take $f = 0$, which gives weights $w_i = 1/p_i$; for example, if there are i informants in the reduced pedigree, then we let $w_i = 2^i$. On the other hand, if we are interested in testing for f to be in a certain range, then we may take f to be the middle point of that range; for example, we may take $f = 0.25$ if we are interested in testing $0 < f < 0.5$. When f is relatively large, such as $f > 0.5$, it should be relatively easy to detect the involvement of mtDNA mutations, and different weights may not be very important for the power of the test.

Using the idea of Feingold and Siegmund (1997) and Teng and Siegmund (1997), we define the "efficiency" of a test, T, using weights w_i as the limit of the ratio between the number of pedigrees n, using test T, and the number of pedigrees n_0, using the optimal weights $w_i = 1/[f + (1 - f)p_i]$, to obtain the same power as T when n tends to ∞. Let λ_i be the fraction of ith-class pedigrees. From the power function, the efficiency of a test T with weights, w_i, is given by

$$\frac{(\sum_{i=1}^{I} w_i \lambda_i q_i)^2}{\{\sum_{i=1}^{I} \lambda_i q_i / [f + (1-f)p_i]\}\{\sum_{i=1}^{I} w_i^2 \lambda_i q_i [f + (1-f)p_i]\}} . \quad (6)$$

Table 1 shows the efficiency of five tests using weights $w_i = 1/[f' + (1 - f')p_i]$ with $f' = 0,0.25,0.50,0.75,1.00$ at different values of f when $\lambda_1:\lambda_2:\lambda_3 = 3:2:1$. From table 1, we see that the efficiency is close to 1 when f' is close to f. When $f' = 0$, the efficiency decreases rapidly, and the efficiency is ~80% when $f > 0.50$. When $f' = 0.25$, the efficiency is always >91%, and the efficiency is >95% when $f > 0.05$. When $f' = 1$, the efficiency is <87% when $f < 0.10$.

We use simulations to confirm the theoretical results. In the simulations, n_i reduced pedigrees each having i informants, $i = 1,2,3$ with $n_1 \geqslant n_2 \geqslant n_3$, were simulated according to the heterogeneity model. Twenty sets of simulations, corresponding to $n_1,n_2,n_3 = 5$–20 by step 5, were done. Five test statistics, $T_k, k = 1,2,3,4,5$ corresponding to weights $w_i = 1/[f' + (1 - f')p_i]$, $i = 1,2,3$ for $f' = 0,0.25,0.50,0.75,1.0$, respectively, were studied. First, the critical values $c_k, k = 1,2,3,4,5$ of the five test statistics $T_k, k = 1,2,\ldots,5$ for significance level $\alpha = 0.05$ were found, respectively, by 10,000 repetitions under the null hypothesis of only nuclear-autosomal-mu-

tation involvement. Once the critical values were found, the same number of pedigrees were simulated under the heterogeneity model for $f = 0.05$–0.45 by step 0.05, for 10,000 times each. In each simulation, the five statistics were calculated. The power of test T_k was the fraction of times $T_k \geq c_k, k = 1,2,3,4,5$. Table 2 gives the powers of the five tests for $n_1 = 15$, $n_2 = 10$, and $n_3 = 5$, $f = 0.05$–0.45 by step 0.05. Table 2 is typical in that, in most of the 20 sets of simulations, T_2 was the best test statistic, followed by T_3, T_4, and T_1. T_5 almost always had the least power in the range of $0 < f < 0.5$. This is consistent with our theoretical predictions.

Table 2

Simulated Power of Five Tests $T_k, k = 1,2,\ldots,5$

Test Statistic	Approximate Power[a] When f[b] Is								
	.05	.10	.15	.20	.25	.30	.35	.40	.45
T_1	.10	.20	.32	.45	.59	.73	.83	.90	.95
T_2	.12	.22	.35	.50	.64	.77	.88	.93	.97
T_3	.12	.21	.33	.48	.62	.76	.86	.92	.97
T_4	.12	.21	.33	.48	.62	.76	.86	.92	.97
T_5	.07	.13	.22	.35	.49	.64	.77	.86	.93

Note.—Results are based on test statistics in equation (5), using different weights $w_i(k) = 1/\{f'(k) + 2^{-i}[1 - f'(k)]\}$, $k = 1,2,\ldots,5$, $i = 1,2,3$. Type I error rate is set at .05.

[a] Based on 10,000 simulations consisting of 15, 10, and 5 reduced pedigrees with 1, 2, and 3 informants, respectively.

[b] True fraction of individuals affected owing to mtDNA-mutation involvement.

Estimating the Contribution of mtDNA Mutations

In the previous sections, we have considered the problem of the testing of mtDNA-mutation involvement in a disease. Once evidence of mtDNA-mutation involvement has been found, it is important to estimate the contribution that mtDNA mutations make to the disease. We use an attributable fraction to measure this contribution. The attributable fraction due to mtDNA mutations is defined as the difference between the prevalence of the disease among the general population and the prevalence among individuals without the mtDNA mutations divided by the prevalence of the disease among the general population. Under the heterogeneity model, the attributable fraction due to mtDNA mutations is equal to the fraction of individuals affected owing to mtDNA mutations (Khoury et al. 1993). In the present section, we propose a method to estimate the attributable fraction under the heterogeneity model without phenocopies, using affected members only.

First, we assume that all reduced pedigrees are of the same class, with i informants in the reduced pedigrees. Let $p_i = 2^{-i}$ and f be the fraction of pedigrees with disease due to mtDNA mutations. Then, the probability of having k_i pedigrees with score 1 and $n_i - k_i$ pedigrees with score -1 among n_i randomly sampled pedigrees is

$$L_i(f) = (n_i k_i)[f + (1 - f)p_i]^{k_i}[(1 - f)q_i]^{n_i - k_i},$$

where $q_i = 1 - p_i$. The maximum-likelihood estimate of f is

$$f' = \frac{k_i}{n_i q_i} - \frac{p_i}{q_i}. \quad (7)$$

When the right-hand side of equation (7) is <0, we take $f' = 0$. Because $k_i \leq n_i$, f' is always ≤ 1. When $k_i = n_i$, $f' = 1$. f' is an unbiased estimator of f, with mean and variance

$$E(f') = f, \text{Var}(f') = \frac{(p_i + fq_i)(1 - f)}{n_i q_i}.$$

The $1 - \alpha$ CI of f can be approximated by

$$f' \pm z_\alpha \sqrt{\frac{(p_i + f'q_i)(1 - f')}{n_i q_i}}.$$

Now, suppose that there are I classes of reduced pedigrees, with i informants in the ith class, $i = 1,2,\ldots,I$. If the estimations of f by use of different classes of reduced pedigrees are not statistically different, then we combine the information from different classes as follows. Suppose that there are n_i pedigrees in the ith class and k_i of them have score 1. Then, the likelihood function is the product of the likelihood function across all the classes; that is,

$$L(f) = \prod_{i=1}^{I} L_i(f) = \prod_{i=1}^{I} (n_i k_i)[f + (1 - f)p_i]^{k_i}[(1 - f)q_i]^{n_i - k_i}.$$

The maximum point of this likelihood functions satisfies the following equation:

$$\sum_{i=1}^{I} \frac{q_i k_i}{p_i + q_i f} = \frac{\sum_{i=1}^{I}(n_i - k_i)}{1 - f}. \quad (8)$$

When $\sum_{i=1}^{I}(q_i k_i/p_i) < \sum_{i=1}^{I}(n_i - k_i)$, equation (8) does not have a solution, and we let $f' = 0$. Otherwise, equation (8) has a unique solution that determines the maximum-likelihood estimation of f. By use of Fisher's information

Table 3

Numbers of Pedigrees with Scores 1 and −1 and Estimation for Contributions of mtDNA Mutations—among the NIDDM Pedigrees

No. of Informants	No. of Pedigrees			f'	$\sigma_{f'}$	95% CI
	Total	Score 1[a]	Score −1[a]			
1	83	51 (41.5)	32 (41.5)	.23	.11	.02–.44
2	21	8 (4.25)	13 (16.75)	.17	.14	0–.45
3	5	3 (.6)	2 (4.4)	.54	.25	.05–1
4	1	0 (.06)	1 (.9)			
5	1	0 (.03)	1 (1.0)			
Combined	111	62	49	.22	.08	.06–.38

[a] Expected numbers of families under the null hypothesis of no parent-of-origin effect are given in parentheses.

theory (Bickel and Doksum 2001), the approximate variance of f' is given by $\sigma^2_{f'} = 1/\sum_{i=1}^{I}[n_i q_i/(p_i + fq_i)(1-f)]$ when $n_i, i = 1,2,\ldots,I$ are large. The $1-\alpha$ CI is approximately $f' \pm z_\alpha \sigma_{f'}$.

Applications

In the present section, we apply our proposed tests and the estimation method to three data sets: LHON, a disease that has been proved to be related to mtDNA mutations; NIDDM; and HTN. Our purpose is to see if the tests can detect the mtDNA-mutation involvement in LHON. Then, we want to see if mtDNA mutations are involved in NIDDM or HTN.

Example 1. LHON

As did Mili et al. (1996), we used pedigrees from studies published before the first mtDNA mutation that causes LHON was identified (Wallace et al. 1988; Singh et al. 1989). These pedigrees may be regarded as a random sample of the LHON-affected pedigrees, because investigators only speculated on the mode of inheritance of LHON. We defined the disease as present if an individual was affected and as absent otherwise. Only pedigrees with at least two affected sibships were useful for our analysis. We obtained a total of 18 pedigrees from the literature (Kwittken and Barest 1958; Wilson 1963, 1965; Went 1964; Adams et al. 1966; Wallace 1970; Nikoskelainen et al. 1977, 1987; McLeod et al. 1978; Muller-Jensen et al. 1978; Johnston et al. 1979; Cotticelli et al. 1984; Novotny et al. 1986). Then, we constructed the corresponding reduced pedigrees according to the methods given above. We found seven reduced pedigrees with 4 informants, four reduced pedigrees with 1 informant, two reduced pedigrees with 3 informants, and one reduced pedigree each with 2, 6, 8, 15, and 18 informants. In all the reduced pedigrees, the informants are females. If we do not consider that males are more likely to be affected than females, then, under the null hypothesis that only nuclear autosomal mutations are involved, the probability of seeing the configurations is $2^{-[(4\times7)+(1\times4)+(3\times2)+2+6+8+15+18]} = 2^{-87}$, which is extremely small. If we consider that males are more likely to be affected than females, then this probability is even smaller. Therefore, we reject the null hypothesis that only nuclear autosomal mutations are involved in LHON. Actually, all reduced pedigrees are consistent with mtDNA-mutation involvement.

We used equation (8) to estimate the contribution of mtDNA mutations. Because all the reduced pedigrees have score 1, the maximum-likelihood estimation of f is equal to 1. Almost 100% of the LHON cases are due to mtDNA mutations. Molecular studies have found that mutations at nucleotide positions 11778, 3460, and 14484 in the mitochondrial genome account for >97% of LHON cases worldwide. Our results are consistent with molecular studies.

Example 2. NIDDM

Many studies have found excess maternal inheritance for NIDDM (for a recent review, see Alcolado et al. 2002). In the present article, we applied our tests to an NIDDM data set provided by the National Diabetes Association GENNID (Genetics of NIDDM) Study (Raffel et al. 1996). The phenotypes of individuals in the GENNID data set were carefully defined by historical data, physical-examination findings, and basic metabolic and biochemical testing. (For details on data collection, see Raffel et al. 1996.) From population data, no significant differences were observed for the prevalence of NIDDM between males and females, although the prevalence of NIDDM can be very different among major ethnic groups. We used 161 pedigrees in this data set. We constructed the reduced pedigrees according to the method described above. Table 3 gives the number of pedigrees with scores 1 and −1 for each class of reduced pedigrees with one to five informants; in parentheses, we also give the expected number of pedigrees with scores 1 and −1

for each class of pedigrees under the null hypothesis of only nuclear autosomal mutations. For n_i reduced pedigrees with i informants, the expected number of pedigrees with score 1 is $2^{-i} \times n_i$. From table 3, we see that there are always more reduced pedigrees with score 1 than expected for reduced pedigrees with one, two, and three informants. In this data set, there is only one reduced pedigree each with four and five informants, and we cannot see the pattern here. This observation is consistent with mtDNA-mutation involvement or other maternal effects for NIDDM.

We then applied our statistics to reduced pedigrees with one, two, and three informants with weights $w_i = 1/[0.25 + (0.75 \times 2^{-i})], i = 1,2,3$. We choose these weights on the basis of our results in a previous section ("Score Functions and Power Analysis"). The score for all the pedigrees is 19.11. Simulations showed the P value to be ~.0009. We also used other weights $w_i = 1/[f' + (1 - f')2^{-i}], f' = 0,0.5,0.75,1.0$ for reduced pedigrees with one, two, and three informants; the corresponding P values are .0005, .0005, .0015, and .0026, respectively. Thus, the null hypothesis can be rejected using any weights. It should be noted that the P values are higher for $f' = 0.75,1.0$ than for $f' = 0,0.25,0.5$.

Next, we studied the contribution that mtDNA mutations make to NIDDM. Using the above methods, we estimated the fraction of pedigrees with disease due to mtDNA mutations, its SD, and the corresponding 95% CI by using both the classes of pedigrees with one, two, and three informants separately and the combined data set. The results are given in the last three columns of table 3. For example, using the 83 reduced pedigrees with one affected informant, we estimate the fraction of pedigrees with disease due to mtDNA mutations to be 23% (95% CI 2%–44%). The numbers of reduced pedigrees with two and three informants are small, 21 and 5 pedigrees, respectively. The estimation may not be accurate. Although the three estimations seem different, the three 95% CIs overlap in a large region (5%–45%). The difference may be caused by the small sample size in the last two classes of pedigrees. We then combined the three classes by using the above proposed method. Using the combined data set, we estimated the fraction of pedigrees with disease due to mtDNA mutations to be 22% (95% CI 6%–38%). Although the point estimation of this fraction was roughly the same as that using only reduced pedigrees with one informant, the 95% CI was much narrower than the one using only reduced pedigrees with one informant. Molecular studies have found that ⩾5% of patients with NIDDM harbor pathological mtDNA mutations, and our results are consistent with molecular studies. Furthermore, our results indicate that more NIDDM cases may be found to be due to mtDNA mutations.

Example 3. HTN

HTN, presently defined in the United States as a blood pressure value ⩾140/90 mmHg, represents the higher end of the blood pressure distribution in the general population. Several investigators noted significant mother-offspring correlations in blood pressure levels and little or no father-offspring correlations, indicating maternal effect. Recently, DeStefano et al. (2001) studied the contributions from mothers and fathers to HTN in whites and African Americans. In the study, hypertensive probands were ascertained without respect to family history of HTN from clinics at the Boston Medical Center and collaborating sites in Greece. Individuals were classified as affected with primary HTN on the basis of standard clinical criteria (i.e., unequivocal elevation of blood pressure ⩾140/90 on at least three different occasions or chronic hypertensive treatment with previously documented high blood pressure in the medical records) and on the basis of the absence of findings suggestive of HTN secondary to organic causes. Probands were identified by a clinic physician. By consideration of these individuals as index subjects, the pedigrees of multigeneration families with HTN were collected. Data collected on relatives included date of birth, date and cause of death (if applicable), hypertensive status, age at diagnosis (for hypertensive individuals), and risk-factor information. DeStefano et al. (2001) found that, among parents with known hypertensive status, the fraction of affected mothers is significantly higher than the fraction of affected fathers among hypertensive probands of African Americans, U.S. whites, and Greek whites. They also studied the affection status of siblings of the probands and found that the siblings of probands with mother only affected are more likely to be affected than are the siblings of probands with father only affected in Greek whites. However, no significant difference was found in African Americans and U.S. whites. Molecular studies have found significant associations between some pathological mtDNA mutations and HTN-associated end-stage renal disease (Watson et al. 2001).

We applied our method to the HTN data sets for whites and African Americans, respectively (DeStefano et al. 2001). Individuals with unknown status were treated as unaffected. (For details on data collection, see DeStefano et al. 2001.) A total of 350 white pedigrees and 98 African American pedigrees are available. The numbers of noninformative pedigrees are 172 and 43 for whites and African Americans, respectively. The fraction of noninformative pedigrees in whites (172/350 [49%]) is very close to the fraction of noninformative pedigrees in African Americans (43/98 [44%]). Among the informative pedigrees, the numbers of ped-

igrees with different numbers of informants and with scores 1 and −1 are given in table 4. Among the pedigrees with one informant, the fraction of pedigrees with mother only affected in whites (133/178 [75%]) is very close to that in African Americans (42/55 [76%]). We then applied our test statistics to the reduced pedigrees, and the P value was $<10^{-7}$ when any weights were used. Thus, maternal effect for HTN is much stronger than that for NIDDM.

Assuming that a fraction f of HTN cases are caused by mtDNA mutations and that others are caused by nuclear autosomal mutations, we can use our method to estimate f. The estimated values of f obtained using data on families with one or two informants for whites and African Americans are given in table 4. The estimated values of f obtained using families with two informants vary widely and seem very different from the estimated values of f obtained using reduced pedigrees with one informant. This may be due to the small number of families with two informants. The 95% CIs of f overlap with each other. Thus, we combine the data to give an overall estimate of f. Using all the data with one or two informants in both whites and African Americans, we estimate f to be 55% (95% CI 45%–65%).

Discussion

In the present article, we have developed a novel class of statistics to test mtDNA-mutation involvement in a disease. The class of tests has several features. First, the tests do not depend on the mode of inheritance of the disease, although their power certainly differs according to different modes of inheritance. Second, they are applicable to pedigrees ascertained through multiple affected members. This feature is particularly important, since many pedigrees collected for linkage studies were ascertained through multiple affected members. Third, they combine information from different pedigrees by choosing weights for different reduced pedigree structures on the basis of their importance to the goal of finding mtDNA-mutation involvement. Finally, the tests can be adapted to circumvent some of the confounding factors, such as differential prevalence and fertility (if they are known) among affected males and females.

The class of tests is intended to be nonparametric in that it does not depend on the mode of inheritance of the disease and other parameters, such as prevalence and penetrance of the mutant alleles. The power of the tests certainly depends on these parameters. In the present study, we considered the heterogeneity model without phenocopies. Under this model, we studied the optimality of score functions and weighting schemes. With reduced pedigrees of the same type, we found that the optimal score function is to give a high score to the configuration in which all the informants in the reduced pedigree are females and to give a common low score to other configurations. The power of the resulting test does not depend on the particular values of the high and low scores. Therefore, we give score 1 to the former and score −1 to the latter. We also studied the efficiency of different weighting schemes. Once evidence has been found for mtDNA-mutation involvement, we propose a method to estimate the contribution of mtDNA mutations. Although our assumption based on the model without phenocopies is strong, the results obtained in the present study should be useful for well-designed studies, such as the GENNID Study (Raffel et al. 1996). The present study is also useful in that it provides insights into how to choose score functions according to different assumptions about the disease-transmission mechanism.

There are several limitations of the proposed tests. First, although the tests are valid if the ascertainment scheme does not explicitly include or exclude individu-

Table 4

Numbers of Pedigrees with Scores 1 and −1 and Estimation for Contributions of mtDNA Mutations—among the Pedigrees with HTN

No. of Informants	No. of Pedigrees			f'	$\sigma_{f'}$	95% CI
	Total	Score 1[a]	Score −1[a]			
Whites:						
1	178	133 (89)	45 (89)	.49	.065	.36–.62
2	10	8 (2.5)	2 (7.5)	.73	.17	.40–1.00
African Americans:						
1	55	42 (27.5)	13 (27.5)	.53	.12	.30–.76
2	9	9 (2.25)	0 (7.75)	1.0	...	.72–1.00
Whites and African Americans:						
1	233	175 (116.5)	58 (116.5)	.50	.057	.39–.61
2	19	17 (4.75)	2 (14.25)	.86	.094	.68–1.00
Combined	252	192	60	.55	.05	.45–.65

[a] Expected numbers of families under the null hypothesis of no parent-of-origin effect are given in parentheses.

als on the basis of sex, differential rates of participation in a study and recall bias between males and females can all lead to misleading results. Differential prevalences of the disease and different fertility among affected males and females can be incorporated into our model if they are known; however, they are generally not known, and these parameters have to be estimated from the data. Second, the statistically significant results do not lead us directly to the conclusion of mtDNA-mutation involvement in a disease. Some confounding factors can be tested using other statistics, and some cannot. Third, we assumed a heterogeneity model in the present study. For complex diseases, such as diabetes and HTN, the disease model can be much more complicated, and the heterogeneity model may not be valid. If nuclear and mtDNA mutations interact with each other in a disease, then we will see excess maternal inheritance, as well as father-to-offspring transmissions of the disease. The father-to-offspring transmissions are not counted toward mtDNA-mutation involvement under the heterogeneity model, and, thus, the true fraction of affected individuals with mtDNA-mutation involvement is underestimated on the basis of the heterogeneity model. Therefore, the fraction of affected individuals with mtDNA-mutation involvement as estimated on the basis of the heterogeneity model represents a lower bound for its true value. Fourth, the attributable fraction estimated in the present study may not apply to the whole population, but only to pedigrees that meet the inclusion criteria. As pointed out by Whittemore and Halpern (2001), the definition and interpretation of the proportion of families segregating a nuclear variation of interest makes sense only under very strict—and, most likely, unrealistic—assumptions. The same concerns apply here to heterogeneous diseases involving both nuclear and mitochondrial mutations. Finally, the methods developed in the present study can test only if mtDNA mutations are involved in a disease of interest. They cannot be used to distinguish between the contributions of inherited mtDNA mutations and a more general contribution of certain common mtDNA haplotypes.

We have applied our test statistics and estimation methods to three data sets: LHON, NIDDM, and HTN. For LHON, we have found strong evidence of mtDNA-mutation involvement, and almost all the pedigrees have disease that may be due to mtDNA mutations. For NIDDM and HTN, we have also found strong evidence of mtDNA-mutation involvement. For NIDDM, we have estimated the fraction of pedigrees with disease due to mtDNA mutations to be ~22% (95% CI 6%–38%). For HTN, we have estimated the fraction of pedigrees with disease potentially due to mtDNA mutations to be ~55% (95% CI 45%–65%). Thus, further studies of the mtDNA-mutation involvement in NIDDM and HTN are warranted.

Although the proposed class of tests can circumvent some confounding factors, factors other than mtDNA-mutation involvement could still give significant results for our tests. Intrauterine effects, differential influence from mothers and fathers, and other gene-environment factors may all contribute to the statistics. These factors must be excluded before we confirm mtDNA-mutation involvement.

In the present article, we have considered only the heterogeneity model. The score function that we have proposed may not be good for the testing of mtDNA-mutation involvement if the disease is not heterogeneous. Other score functions are needed in the study of diseases that involve interactions between nuclear and mtDNA mutations. This is a topic of further research.

Acknowledgments

We wish to thank Dr. Douglas C. Wallace, for providing the pedigrees with LHON, and Dr. Matt Petersen, of the American Diabetes Association, for providing the pedigrees with NIDDM. We also thank the reviewers, for suggestions that greatly improved the presentation of the manuscript. This research was supported, in part, by National Institutes of Health grants R29 DK53392 and R01 AG18412.

Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for LHON, NIDDM, and HTN)

References

Adams JH, Blackwood W, Wilson J (1966) Further clinical and pathological observations on Leber's optic atrophy. Brain 89: 15–26

Alcolado JC, Laji K, Gill-Randall R (2002) Maternal transmission of diabetes. Diabet Med 19:89–98

Ballinger SW, Shoffner JM, Hedaya EV, Trounce I, Polak MA, Koontz DA, Wallace DC (1992) Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. Nat Genet 1:11–15

Bickel PJ, Doksum KA (2001) Mathematical statistics: basic ideas and selected topics, 2nd ed. Prentice Hall, Upper Saddle River, NJ

Cotticelli L, Rinaldi E, D'Alessandro L, Russo S, Libondi T, Costagliola C (1984) Red cell glutathione in Leber's optic atrophy. Metab Ophthalmol 8:31–34

Davis RE, Miller S, Herrnstadt C, Ghosh SS, Fahy E, Shinobu LA, Galasko D, Thal LJ, Beal MF, Howell N, Parker WD (1997) Mutations in mitochondrial cytochrome *c* oxidase genes segregate with late-onset Alzheimer disease. Proc Natl Acad Sci USA 94:4526–4531

DeStefano AL, Gavras H, Heard-Costa N, Bursztyn M, Manolis A, Farrer LA, Baldwin CT, Gavras I, Schwartz F (2001) Ma-

ternal component in the familial aggregation of hypertension. Clin Genet 60:13–21

Feingold E, Siegmund DO (1997) Strategies for mapping heterogeneous traits by allele-sharing methods. Am J Hum Genet 60:965–978

Haghighi F, Hodge SE (2002) Likelihood formulation of parent-of-origin effects on segregation analysis, including ascertainment. Am J Hum Genet 70:142–156

Johnston PB, Gaster RN, Smith VC, Tripathi RC (1979) A clinicopathologic study of autosomal dominant optic atrophy. Am J Ophthalmol 88:868–875

Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto YI, Hayakawa T, Matsuka K, Hagura R, Akanuma Y, Yazaki Y (1994) A subtype of diabetes mellitus associated with a pathogenic mutation of mitochondrial DNA. N Engl J Med 330:962–968

Khoury MJ, Beaty TH, Cohen BE (1993) Fundamentals of genetic epidemiology. Oxford University Press, New York, pp 77–79

Kwittken J, Barest HD (1958) The neuropathology of hereditary optic atrophy (Leber's disease): the first anatomic study. Am J Pathol 34:185–191

McLeod JG, Low PA, Morgan JA (1978) Charcot-Marie-Tooth disease with Leber optic atrophy. Neurology 28:179–184

McMahon FJ, Stine OC, Meyers DA, Simpson SG, DePaulo JR (1995) Patterns of maternal transmission in bipolar affective disorder. Am J Hum Genet 56:1277–1286

Mili F, Flanders WD, Sherman SL, Go RCP, Wallace DC (1996) Genetic epidemiology methods to screen for matrilineal inheritance in mitochondrial disorders. Genet Epidemiol 13:605–614

Muller-Jensen A, Bushart W, Steidinger J, Funk M, Hellner KA (1978) Leber's optic atrophy—an interdisciplinary study. Klin Monatsbl Augenheilkd 172:831–842

Nikoskelainen EK, Savontaus ML, Wanne OP, Katila MJ, Nummelin KU (1987) Leber's hereditary optic neuroretinopathy: a maternally inherited disease. Arch Ophthalmol 105:665–671

Nikoskelainen E, Sogg RL, Rosenthal R, Friberg TR, Dorfman LJ (1977) The early phase in Leber hereditary optic atrophy. Arch Ophthalmol 95:969–978

Novotny EJ, Singh G, Wallace DC, Dorfman LJ, Louis A, Sogg RL, Steinman L (1986) Leber's disease and dystonia: a mitochondrial disease. Neurology 36:1053–1060

Ottman R, Annegers JF, Hauser WA, Kurland LT (1988) Higher risk of seizures in offspring of mothers than of fathers with epilepsy. Am J Hum Genet 43:257–264

Raffel LJ, Robbins DC, Norris JM, Boerwinkle E, DeFronzo RA, Elbein SC, Fujimoto W, Hanis CL, Kahn SE, Permutt MA, Chiu KC, Cruz J, Ehrmann DA, Robertson RP, Rotter JI, Buse J (1996) The GENNID Study: a resource for mapping the genes that cause NIDDM. Diabetes Care 19:864–872

Reardon W, Ross RJM, Sweeney MG, Luxon LM, Pembrey ME, Harding AE, Trmbath RC (1992) Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet 340:1376–1379

Schork NJ, Guo SW (1993) Pedigree models for complex human traits involving the mitochondrial genome. Am J Hum Genet 53:1320–1337

Singh G, Lott MT, Wallace DC (1989) A mitochondrial DNA mutation as a cause of Leber's hereditary optic neuropathy. New Engl J Med 320:1300–1305

Sleutels F, Barlow DP, Lyle R (2000) The uniqueness of the imprinting mechanism. Curr Opin Genet Dev 10:229-233

Sun FZ, Ashley-Koch AE, Durham LK, Feingold E, Halloran ME, Manatunga AK, Sherman SL (1998) Testing for contributions of mitochondrial DNA mutations to complex diseases. Genet Epidemiol 15:451–469

Teng J, Siegmund D (1997) Combining information within and between pedigrees for mapping complex traits. Am J Hum Genet 60:979–992

van den Ouweland JM, Lemkes HH, Ruitenbeck W, Sandkuijl LA, de Vijlder MF, Struyvenberg PAA, van de Kamp JJP, Maassen JA (1992) Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat Genet 1:368–371

Wallace DC (1970) A new manifestation of Leber's disease and a new explanation for the agency responsible for its unusual pattern of inheritance. Brain 93:121–132

——— (1992) Mitochondrial genetics: a paradigm for aging and degenerative disease? Science 256:628–632

——— (1994) Mitochondrial DNA sequence variation in human evolution and disease. Proc Natl Acad Sci USA 91:8739–8746

——— (1995) Mitochondrial DNA variation in human evolution, degenerative disease, and aging. Am J Hum Genet 57:201–223

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AM, Elsas LJ 2nd, Nikoskelainen EK (1988) Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 242:1427–1430

Watson B, Khan MA, Desmond RA, Bergman S (2001) Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease. Am J Kidney Dis 38:529–536

Weeks DE, Lange K (1988) The affected-pedigree-member method for linkage analysis. Am J Hum Genet 42:315–326

——— (1992) A multilocus extension of the affected-pedigree-member method for linkage analysis. Am J Hum Genet 50: 859–868

Went LN (1964) A sex-linked heredo-degenerative neurological disorder associated with Leber's optic atrophy: genetic aspects. Acta Genet Basel 14:220–239

Whittemore AS, Halpern J (1994) A class of tests for linkage using affected pedigree members. Biometrics 50:118–127

——— (2001) Problems in the definition, interpretation, and evaluation of genetic heterogeneity. Am J Hum Genet 68: 457–465

Wilson J (1963) Leber's hereditary optic atrophy: some clinical and aetiological considerations. Brain 86:347–362

——— (1965) Skeletal manifestations in Leber's hereditary optic atrophy: a possible disorder of cyanide metabolism. Ann Phys Med 8:91–95