

BIOLOGICAL AND STATISTICAL STUDIES FOR DISEASES INVOLVING MTDNA MUTATIONS

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Abstract. Mitochondrial DNA (mtDNA) mutations have been shown to be involved in several rare and complex diseases. This paper first presents a brief review on mitochondrial genetics, heteroplasmic mtDNA transmission and biological studies for diseases related to mtDNA mutations. Then we present a detail review on statistical methods for testing mtDNA mutation involvement in diseases and for estimating their contribution to the disease if mtDNA mutations are involved. Available methods for studying the interaction between nuclear and mtDNA mutations are also discussed. The purpose of this paper is to stimulate research in the statistical studies of mitochondrial diseases.

Key words. mitochondrial genetics, heteroplasmic mtDNA transmission, mathematical modeling, statistical power.

AMS(MOS) subject classifications. 60K99, 62P10, 92D10, 92D30.

1. Introduction. Several diseases have been shown to be related to mitochondrial DNA (mtDNA) mutations. MtDNA plays a vital role in producing ATP via the complex oxidative phosphorylation (OXPHOS) pathway. ATP production is important in the function of brain, skeletal muscle, heart, and other organs. Therefore it is hypothesized that diseases related to mtDNA mutations might be a common cause of human degenerative diseases and aging (Taylor 1992, Wallace 1992). The study of mtDNA mutation involvement in diseases is a rapidly developing field. On the other hand, statistical studies of testing mtDNA mutation involvement in diseases lag far behind. In this paper, we present a review of biological and statistical studies on diseases related to mitochondrial DNA (mtDNA) mutations with the intention to stimulate further statistical studies to detect mtDNA mutation involvement and to estimate their contribution to the disease if mtDNA mutations are involved.

2. Mitochondrial Genetics. Human MtDNA is a 16,569 base pair (bp) closed circular molecule located within the matrix of the double membrane mitochondrion. The complete human mtDNA has been sequenced (Anderson et al. 1981). It encodes 37 genes, including a small (12S) and a large (16S) rRNA, 22 tRNAs, and 13 polypeptides. MtDNA encoded polypeptides are subunits of the respiratory chain which is embedded in the inner membrane of the mitochondrion and consists of about 90 different subunits. About 77 subunits of the respiratory chain are also encoded

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by nucleus-encoded polypeptides. Because of the dual roles of nuclear and mitochondrial encoded proteins in the respiratory chain, mutations in both nuclear and mitochondrial DNA can cause diseases related to the respiratory chain. Bu and Rotter (1991) and Bu et al. (1992) classified mitochondrial related diseases into three main classes. The first class involves mutations only in the mitochondrial genome. Because mtDNA is maternally inherited, this type of diseases exhibits maternal inheritance. The second class involves mutations in both the nuclear genome and the mitochondrial genome. The interaction of the mutations in the nuclear genome and the mitochondrial genome gives the disease phenotype. This class of diseases usually presents excess maternal inheritance. The third class involves mutations only in the nuclear genome, in which the nuclear genome produces defective subunits for mitochondrial function. This class of diseases does not necessarily show excess maternal inheritance and can usually be studied by methods for nuclear diseases. In this paper we mainly consider the first two classes of diseases.

Mitochondrial genetics is quite different from nuclear genetics. MtDNA is predominantly transmitted through oocyte cytoplasm and therefore it is maternally inherited (Case and Wallace 1981, Giles et al. 1980). Although low levels of paternal mtDNA transmission has been reported in interspecific mouse crosses (Kaneda et al. 1995), it is considered rare in human. Mutations accumulate extremely rapidly in mtDNA, about 10-20 times the mutation rate in nuclear genes (Neckelmann et al. 1987, Wallace et al. 1987). The high mutation rate is thought to be resulted from the mtDNA's lack of protective histones, inefficient DNA repair system, and continuous exposure to the mutagenic effects of the oxygen radicals generated by oxidative phosphorylation.

Unlike nuclear genes which are present in diploid in each cell, each human cell contains hundreds of mitochondria and thousands of mtDNAs. If a mutation occurs in some of the mtDNAs in a cell, it creates a mixture of normal and mutant mtDNAs: a state known as heteroplasmy. When heteroplasmic cells divide, the mtDNA genotype undergoes replicative segregation and the proportion of mutant mtDNAs drifts, such that cells tend towards having either all mutant or normal mtDNAs: a state known as homoplasmy. The mechanism of heteroplasmic mtDNA transmission is complicated and is a topic of current research. Several biological studies have been done to understand the transmission of heteroplasmic mtDNAs in model organisms.

Solignac et al. (1984) studied the heteroplasmic mtDNA transmission in a strain of *D. mauritiana* of *Drosophila*. Volz-Lingenhohl et al.(1992) studied heteroplasmic mtDNA transmission for a large-scale deletion in the coding region of *Drosophila subobscura* mtDNA. In both studies, the heteroplasmic mtDNA transmission is very stable in *Drosophila* and no homoplasmy was found after several generations. Rand and Harrison (1986) studied heteroplasmic mtDNA transmission in crickets trying to find the

distribution of the fraction of mutant in the offspring from heteroplasmic females. As in *Drosophila*, heteroplasmic mtDNA transmission is also stable in *crickets*. The Wright-Fisher model from population genetics fits the mtDNA transmission data well. Extensive studies have been done on heteroplasmic mtDNA transmissions in bovines and Holstein cows (Hauswirth and Laipis 1982, 1985, Ashley et al. 1989, Laipis et al. 1988, Koehler et al. 1991). In contrast to the studies in *Drosophila* and *crickets*, heteroplasmic mtDNAs can rapidly achieve homoplasmic state in a few generations. They proposed that at some stage in oogenesis the number of mitochondrial genomes within any one developing oocyte is reduced to as few as five or less. This is called the bottleneck model. To understand when the bottleneck occurs, Jenuth et al. (1996) created a heteroplasmic mouse model to study mtDNA distributions in mature and progenitor oocytes. They concluded that random segregation and a bottleneck of about 200 mtDNAs can explain their data. They also concluded that the major changes in the distribution of heteroplasmic mtDNAs occurs during the development from primary oocyte to mature oocyte. The study of heteroplasmic mtDNA transmission in human is more difficult due to the small number of heteroplasmic individuals available. Rapid changes in mtDNA genotype have been observed in families affected with Leber hereditary optic neuropathy (LHON) before and were taken as evidence that the bottleneck might be very narrow in humans. On the other hand, Howell et al. (1992) studied the segregation of a heteroplasmic silent mtDNA polymorphism in a multi-generation family with a homoplasmic LHON mtDNA mutation and found that heteroplasmy is maintained within this family. They concluded that the bottleneck might not be too narrow. In a more recent study skewed segregation of the mtDNA mutation in human oocytes was reported (Blok et al. 1997). More studies need to be done to understand heteroplasmic mtDNA transmission in humans.

In an individual harboring heteroplasmic mtDNAs, different tissues may have different fractions of mutant mtDNAs. This is called tissue specificity. Even different cells in the same tissue may have different fractions of mutant mtDNAs. Different tissues and organs rely on mitochondrial energy to various extents, with the central nervous system, followed by heart, muscle, kidney, and endovine. As the proportion of mutant mtDNAs increases, mitochondrial energy output declines. When the ATP-generating capacity of the tissue falls below the energy threshold necessary for normal tissue function, disease occurs. As a result, these tissues are more likely to be affected by mitochondrial mutations. In most of the mitochondrial diseases discovered so far, one or more of these tissues are affected. Due to the variability of heteroplasmic mtDNA transmission and tissue specificity, diseases related to mtDNA mutations can show a wide range of variability even within the same family.

3. Mitochondrial Diseases. MtDNA mutations were first shown to be involved in LHON and mitochondrial myopathies using molecular techniques in 1988 (Holt et al. 1988, Wallace et al. 1988). Three mtDNA mutations: a G-A transition at nucleotide 3460, a G-A transition at nucleotide 11778 and a T-G transition at nucleotide 14484, account for about 70-90% of the worldwide LHON patients. The three mutations have never been found in non-LHON controls and are thus referred as primary mutations. About 15 other mtDNA mutations have been found to be associated with LHON but the pathological role of these mutations are not established yet as they either were found only in a single pedigree or are present in low frequency in controls. These mutations are referred as secondary mutations. Secondary mutations may either be simple polymorphism and their association with LHON reflects historical genetic structure, or they may have pathological role in the development of LHON. There is no consensus about the classification of primary and secondary mutations.

To understand the effect of the three primary mtDNA mutations on LHON under different genetic backgrounds, we recently performed a phylogenetic approach for LHON cases and non-LHON controls (Brown et al. 1997). Extensive molecular studies have shown that each population can be divided into several different lineages according to their evolutionary history. For example, it has been shown that about 65% of North American Caucasians belong to four major lineages: H, I, J, and K. Each lineage is defined by one or two neutral mtDNA mutations which are specific to that lineage. We collapse all the other individuals who do not belong to the above four lineages into one lineage denoted by R. Using 175 controls and 17 11778-, 10 3460-, and 8 14484-positive patients, we found that 3460-positive individuals distribute along the different lineages proportional to that of controls. In contrast, 6 out of the 8 14484-positive individuals belong to lineage J while only 16 out of the 175 controls belong to lineage J. The difference is statistically significant even after adjusting for multiple comparisons (p -value = 0.0001). 11778-positive individuals are also more likely to belong to lineage J than non-LHON controls although the difference is not statistically significant after adjusting for multiple comparisons (p -value = 0.0442). To confirm our observations, we collected all the data on LHON patients from the literature and found that the same conclusions hold for this data set too. The reason(s) for the difference is not clear and needs to be further studied. This approach may prove to be useful to study the pathogenicity of certain mtDNA mutations. In order for this method to work, accurate methods for defining the different lineages are needed.

Although many studies have been done on LHON, its pathogenesis is still not well understood. LHON is strictly maternally inherited and therefore mtDNA mutations must account for most of the LHON cases if not all. On the other hand, males are more likely to be affected than females. It has been found that about 80-85% of LHON patients are males. Thus mtDNA mutations can not be the only factor causing LHON. Nuclear mutations

might also be involved in LHON. It is hypothesized that an X-linked factor interacting with mtDNA mutations to give the LHON phenotype. An initial linkage to DXS7 on the X-chromosome was reported (Vilkki et al. 1991) and was later reevaluated and excluded (Juvonen et al. 1993). This locus was also excluded in other families (Sweeney et al. 1992). No mechanisms have been found to explain the predominant prevalence of LHON among males over females.

Besides LHON, several other rare diseases including Kearns-Sayre syndrome (KSS), myoclonic epilepsy and ragged-red fibre disease (MERRF), and mitochondrial encephalomyopathy, lactic acidosis, and stroke like episodes (MELAS) have been shown to be associated with mtDNA mutations.

MtDNA mutations were also found in certain types of diabetes mellitus patients (Ballinger et al. 1992, Reardon et al. 1992, van den Ouweland et al. 1992, Gerbitz et al. 1995). Indications of mtDNA mutation involvement in diabetes mellitus came from two sources. First, epidemiological studies always find excess maternal inheritance for non-insulin dependent diabetes mellitus (NIDDM). Second, diabetes mellitus is frequently associated with mitochondrial diseases. A mutation at nuclear position 3243 was first found in a large diabetes pedigrees in 1992 (van de Ouweland et al. 1992). About 1.5% of diabetes families have been shown to harbor this mutation (Gerbitz et al. 1995). Although mtDNA mutation involvement in diabetes can explain excess maternal inheritance in NIDDM, this small fraction of mtDNA mutation in NIDDM can not explain the strong excess maternal inheritance observed for NIDDM. In a recent study, we estimated that about 22% of NIDDM maybe due to mtDNA mutations with 95% confidence interval 6 to 38% (Sun, unpublished data). Therefore more studies need to be done to estimate the contribution of mtDNA mutations to diabetes mellitus.

4. Statistical Methods for Studying Mitochondrial Diseases.

Compared to molecular studies of mitochondrial diseases, statistical study on mtDNA mutation involvement in diseases is an under developed subject although several investigators previously addressed this problem. In this section, we review statistical studies to test mtDNA mutation involvement in diseases.

4.1. Heteroplasmic mtDNA transmission. As discussed above, the mechanism of heteroplasmic mtDNA transmission is not well understood. It is important both in itself theoretically and in studying diseases related to mtDNA mutations. Given a female having a fixed fraction of mutant mtDNAs in her oocyte, what is the distribution for the fraction of mutant mtDNAs in her offspring? This question can be compared with the Mendelian segregation rule for nuclear genes although the later is much simpler and well understood. To understand the mechanism of heteroplasmic mtDNA transmission mathematically, Hopfenmuller (1978, 1979) constructed a mathematical model for heteroplasmic mtDNA transmission

in germ cells and somatic cells based on the knowledge of oocyte development. The oocyte development was divided into four stages: the cleavage period, the cell propagation period, the cell growth period, and the cell turn over period.

In the cell cleavage period, it was assumed that a human oocyte containing about 30,000 mitochondria is divided into 2^6 to 2^8 cells after 6 to 8 cell divisions. After the cleavage period, each cell contains about 300 mitochondria. The daughter cells were referred as primordial germ cells (PGC) in the biology literature. During this period, each mitochondria belongs to a PGC with equal probability 2^{-i} , where i is the number of cell divisions in the cleavage period. Let A_F be the number of mutant mitochondria in the original cell. Then the number of mutant mitochondria in a PGC will be binomially distributed, $B(A_F, 2^{-i})$.

In the cell propagation period, it is assumed that each PGC derived from the cell cleavage period undergoes mitotic cell division without changing the total number of mitochondria in the cell. It is assumed that a PGC goes through 17-21 cell mitochondrial cell divisions in this period. The resulting daughter cells are called primary oocytes. Let n be the number of mitochondria in a cell and β be the number of mutant mitochondria after the k -th cell division. In the $k+1$ -st mitotic cell division the cell first double the number of mitochondria to $2n$ mitochondria with 2β mutant. The model assumes that these $2n$ mitochondria separates into two cells with n mitochondria in each cell. The number of mutant mitochondria in each of the two cells is modeled as a geometric random variable. The probability of having m mutant mitochondria is given by

$$p_{k+1}(m) = \sum_{\beta=m/2} \binom{2\beta}{m} \binom{2n-2\beta}{n-m} p_k(\beta) / \binom{2n}{n}.$$

In the growth period, the primary oocytes with about 300 mitochondria develop into mature oocytes with about 30,000 mitochondria. It was assumed that, during this period, each time a mitochondria is randomly sampled and then divides into two mitochondria until the cell is mature. If the initial cell has n mitochondria, the cell will contain $n+k$ mitochondria after the k -th cell division. Let $p_k(m)$ be the probability of having m mutant mitochondria after the k -th cell division. Then $p_k(m)$ satisfies the following recursive equation

$$p_{k+1}(m) = \frac{m-1}{n+k} p_k(m-1) + \frac{n+k-m}{n+k} p_k(m).$$

The first term is the probability that there are $m-1$ mutant after the k -th cell division and one mutant mitochondria was selected to duplicate. The second term is the probability that there are m mutant after the k -th cell division and one wide type mitochondria was selected to duplicate.

In the turn over process, it was assumed that first a mitochondria is randomly sampled and duplicated, and then one of the resulting mito-

chondria is selected to be lost. Let $p_k(m)$ be the probability of having m mutants after the k -th turn over process assuming that there are a total of n mitochondria in the cell. Then

$$p_{k+1}(m) = \left(1 - \frac{m}{n+1}\right) \left\{ p_k(m-1) \frac{m-1}{n} + p_k(m) \left(1 - \frac{m}{n}\right) \right\} \\ + \frac{m+1}{n+1} \left\{ p_k(m) \frac{m}{n} + p_k(m+1) \left(1 - \frac{m+1}{n}\right) \right\}.$$

Like any mathematical model, this model can not be completely consistent with the real mechanism underlying heteroplasmic mtDNA transmission. The question is whether this model captures the main features of heteroplasmic mtDNA transmission. It is also too complicated to obtain any practical results. The validity of this model was not compared to real data sets. The model received little attention since its publication. Now data on heteroplasmic mtDNA transmissions are available on model systems such as mouse (Jenuth et al. 1996). It is interesting to see if this model fits the data or not. From their data set, Jenuth et al. (1996) concluded that the main changes in the fraction of mutant mitochondria occurs from the transition from PGC to primary oocytes, while the above mathematical model predicts that the main changes occur from primary oocytes to mature oocytes. Thus the model for the propagation period may not represent the real mechanism. Jenuth et al. (1996) also concluded that the Wright—Fisher model on random drift can explain their data. If this is true for all model organisms and human, the model for heteroplasmic mtDNA mutations might be much simpler than had been thought. Both biological and mathematical understanding of heteroplasmic mtDNA transmission are needed.

4.2. Testing for mtDNA mutation involvement in diseases.

Because mtDNA is maternally inherited, diseases related to mtDNA mutations must show excess maternal inheritance. Excess maternal inheritance indicates mtDNA mutation involvement although several other factors also result in excess maternal inheritance. Several investigators studied this problem for various diseases. It was observed long ago that LHON is exclusively maternally inherited leading to the hypothesis that mtDNA mutations might be involved in LHON. It was later confirmed using molecular approaches. Ottman et al. (1988) used standard epidemiologic methods to demonstrate that offspring of mothers with epilepsy are more likely to be affected than those with affected fathers. Based on this observation and other facts, they proposed that mtDNA mutations or intrauterine, neonatal, or early childhood environmental factors might be involved in the etiology of epilepsy. Mili et al. (1996) extended their approach to screen for excess maternal inheritance in extended pedigrees. They compared the risk of the disease among individuals who have affected mother or maternal grandmother or maternal aunts or uncles with the risk of the disease among individuals with affected father or paternal grandparents or paternal aunts

or uncles. They applied the approach to a data set on LHON disease and found excess maternal inheritance while they did not find any excess maternal inheritance in a data set on bipolar affective disorder, a psychiatric disorder having a population prevalence of about 1%. MtDNA mutations have been implicated for bipolar affective disorder. Through unilineal family studies of bipolar affective disorder, two groups (McMahon et al. 1995, Gershon et al. 1996) proposed a novel hypothesis that mtDNA mutations might play a role in bipolar affective disorder. The scheme used by McMahon et al. (1995) was to sample probands with at least two affected sibs or one affected sib and one (only one) affected parent. They found more affected mothers than affected fathers of the probands. They also found that maternal relatives are more likely to be affected than non-maternal relatives of the probands. In Gershon et al. (1996), they sampled pedigrees with at least six affected individuals. Similar results were obtained in this data set as that in McMahon et al. (1995).

The above studies did not consider the power of the proposed test under various disease transmission models involving mtDNA mutations. One question is what type of sampling schemes are most powerful in detecting the involvement of mtDNA mutations if it exists. To address this problem, we recently studied the power of the test by comparing the recurrence risk of certain type relatives of probands along the mitochondrial lineage with that of the same type relatives of probands along the non-mitochondrial lineage (Sun et al. 1998). The mitochondrial lineage of a proband is defined as the proband's relatives who share the same mtDNA assuming homoplasmic mtDNA transmissions. The other relatives belong to the non-mitochondrial lineage. In order to study the power of the tests under the hypothesis of mtDNA mutation involvement using current statistical theory and to remove the dependency among family members, we assumed that only one randomly chosen relative of the proband was selected from each family. In that study, we considered a heterogeneity model in the sense that the disease can either be caused by mutations at nuclear loci or in the mitochondrial genome. Under this model, the power of the test increases as the relationship between the probands and their relatives becomes more distant. On the other hand, under a multiplicative epistatic model in which mutations at the nuclear loci and the mitochondrial genome interact with each other to give the disease phenotype, the power of the test decreases as the relationship between the probands and the relatives becomes distant. From the above study we see that the usefulness of different types of proband-relative pairs using the proposed test to detect mtDNA mutation involvement depends on the underlying disease transmission model. To find the best sampling scheme, it is important to distinguish heterogeneity model from multiplicative model. Risch's method (1990) might be adapted to achieve this goal.

The problem with the above approach is that factors other than mtDNA mutation involvement might also result in excess maternal inheritance such

as X-linkage, maternal imprinting with activation of disease alleles transmitted by the mother, recall biases, different prevalences of the disease in males and females, etc.. In Sun et al. (1998) we proposed methods to distinguish mtDNA mutation involvement from these factors using proband-relative pairs.

Schorck and Guo (1993) considered testing mtDNA mutation involvement in diseases in the traditional segregation analysis framework. There are three main components in traditional segregation analysis: (i) the penetrance function, ϕ , which gives the probability of having the disease given an individual's genotype; (ii) the transmission probability, τ , which gives the probability distribution of an individual's genotype given both parents' genotype, and (iii) the allele frequency parameters, γ . Given the above three components, it is possible to calculate the likelihood of the individuals' phenotypes in a given pedigree. For multiple pedigrees, the log likelihood function is the sum of the log likelihood over all the pedigrees. Elston and Steward (1971) presented efficient algorithms to calculate the log likelihood function for complex pedigrees. Schork and Guo (1993) provided a variety of pedigree models for diseases involving mtDNA mutations. In the simple maternal inheritance model, the transmission probability function is defined by

$$\tau(g_i = 1 | g_m, g_f) = \begin{cases} 1 & \text{if } g_m = 1 \\ \psi & \text{if } g_m = 0 \end{cases}$$

where g_i, g_m and g_f denote the mitochondrial genotypes of the individual, mother and father, respectively and "1" and "0" denote that the individual has the mutant and non-mutant mitochondrial, respectively. ψ is usually set to be very small and it represents mutation rate at the mitochondrial locus. The penetrance function is defined by

$$\phi(d_i = \text{affected} | g_i) = \begin{cases} \rho & \text{if } g_i = 1 \\ \hat{\phi} & \text{if } g_i = 0 \end{cases}$$

where $\hat{\phi}$ represents the phenocopy probability. For heteroplasmic mtDNA transmission, they proposed to divide the unit interval into several subintervals. The transmission probability is modeled as the probability that the fraction of mutant in an offspring is in interval j given the fraction of mutant in the mother is in interval i . This will introduce too many parameters into the likelihood function. Schork and Guo also proposed models for threshold effect and for interaction between nuclear and mtDNA mutations. The problem with these approaches is that a model needs to be specified for the analysis. If the model is correct, the proposed method is supposed to be most powerful in detecting the mtDNA mutation involvement. But in general the correct model is rarely known as a prior.

Once mtDNA mutations have been shown to be involved in the etiology of a disease, it is important to know if mtDNA mutations are the

primary cause of the disease or nuclear mutations might also be involved. Bu and Rotter (1991) and Bu et al. (1992) addressed this problem in their studies of LHON. As shown above that mtDNA mutations alone can not explain the LHON transmission data as males are more likely to be affected than females. They hypothesized that an X-linked mutation together with X-chromosome inactivation interacts with mtDNA mutations to give the LHON phenotype. To test this hypothesis, they considered individuals in the pedigrees who are genetically related through females which they called maternal line pedigrees. Because all the members in the maternal line pedigrees have the same mtDNA mutation, the study of the segregation pattern along the nuclear locus is reduced to the one locus model.

Some other diseases have long been shown to be related to nuclear mutations, but nuclear mutations alone can not explain the transmission pattern either. For example, Huntington disease is a classic example of autosomal dominant disease. It has been observed that offspring of affected females have a later age of onset than those of affected males which can not be explained by nuclear mutations alone. Several models have been proposed to explain this observation. Among these models, Boehnke et al. (1983) found that a model assuming a protective factor in the mitochondrial genome combined with the autosomal mutation delays the age of onset for Huntington disease is consistent with the transmission data. This model has not been confirmed by molecular studies.

5. Discussion. Identifying genes affecting complex diseases, such as cancer, diabetes, hypertension, and affective disorders, is an important topic in current genetic research. Biological studies have identified over 60 mtDNA point mutations and hundreds of mtDNA rearrangements associated with human diseases (Wallace 1994, 1995). Heteroplasmic mtDNA transmission is a complicated process and is not well understood. Due to heteroplasmic mtDNA transmission, the fraction of mutant mtDNAs can change over generations along matrilineal lineages. In a heteroplasmic individual, different tissues can have different fraction of mutant mtDNAs. Also different tissues have different needs for mitochondrial energy. Thus diseases related to mtDNA mutations can show complex transmission patterns. In studying complex diseases, not only do we need to consider nuclear complexities, such as reduced penetrance, phenocopies, polygenic effect, and heterogeneities, we also need to consider the possible involvement of mtDNA mutations. Failure to take mtDNA mutations into account may give misleading results.

In this brief review we present an outline of biological and statistical studies for diseases related to mtDNA mutations with an intention to stimulate research in developing statistical methods to test mtDNA mutation involvement and to estimate the contribution of mtDNA mutations in the etiology of a disease. Several problems have not been addressed in the literature and need to be studied. First, for common diseases, it is more

likely that both nuclear and mtDNA mutations are involved in the diseases. Then it is important to know how nuclear and mtDNA mutations interact with each other to give the disease phenotype: whether either mutation is enough to cause the disease or both mutations are needed to show the phenotype. This study is important for clinical purposes too. For example, a subset of diabetes related to mtDNA mutations have some distinct phenotypic features not showing in other types of diabetes and may represent a heterogeneity model. LHON represents another example that both nuclear and mtDNA mutations are needed to show the phenotype although the responsible nuclear mutation(s) has not been identified yet. Second, once mtDNA mutations have been identified to be involved in the disease, how to estimate the attributable fraction due to mtDNA mutations? Third, what effects can mtDNA mutation involvement have in our effort in identifying nuclear genes affecting the disease using current linkage analysis method such as pedigree method, sib-pair analysis and transmission disequilibrium test (TDT)? If these methods are not powerful enough, are there any better method to find linkage to nuclear genes in the presence of mtDNA mutation involvement? Due to the difficulties in dealing with gene-gene interaction in general, these problems are challenging.

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