

## Genetic Counseling and Prenatal Diagnosis for the Mitochondrial DNA Mutations at Nucleotide 8993

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### Summary

Mitochondrial genetics is complicated by heteroplasmy, or mutant load, which may be from 1%–99%, and thus may produce a gene dosage–type effect. Limited data are available for genotype/phenotype correlations in disorders caused by mtDNA mutations; therefore, prenatal diagnosis for mtDNA mutations has been hindered by an inability to predict accurately the clinical severity expected from a mutant load measured in fetal tissue. After reviewing 44 published and 12 unpublished pedigrees, we considered the possibility of prenatal diagnosis for two common mtDNA mutations at nucleotide 8993. We related the severity of symptoms to the mutant load and predicted the clinical outcome of a given mutant load. We also used the available data to generate empirical recurrence risks for genetic counseling, which may be used in conjunction with prenatal diagnosis.

### Introduction

During the past decade, many pathogenic mutations have been identified in mitochondrial (mt) DNA (Shoffner and Wallace 1995). Despite the progress in mtDNA mutation detection and diagnosis, the therapy of patients with mitochondrial cytopathies remains largely ineffective. It is therefore necessary to offer families with an mtDNA mutation accurate counseling about prognosis, recurrence risk, and prevention of further cases. However, genetic counseling of families with mtDNA mutations is difficult. Because mtDNA is inherited mater-

nally, preventing the transmission of mtDNA disorders is problematic, and little is known about recurrence risks. Nuclear transfer between oocytes and preimplantation diagnosis are possibilities, but, at present, in vitro fertilization (IVF), by means of oocytes donated by a woman with no mutant mtDNA, is the most feasible method of ensuring an unaffected child. IVF, however, is not suitable for all women, because of the financial and emotional hardships involved and the lack of oocyte donors.

Prenatal diagnosis for mtDNA mutations is technically possible; however, such tests have been performed with reluctance. Predicting the severity, type, and age at onset of symptoms caused by mtDNA mutations has been hampered by the difficulty in interpreting the level of heteroplasmy (the coexistence of at least two species of mtDNA molecules within the cell). The level of heteroplasmy is also referred to as the “mutant load,” which is the proportion of mutant mtDNA molecules. There are also concerns that the mutant load may vary among tissues and may change over time (Harding et al. 1992; Poulton and Morten 1993). To date, there have been few attempts to review the data on mtDNA mutations in order to generate predictive data for genetic counseling. Recently, Chinnery et al. (1997) reviewed the published data on two common mtDNA mutations (A3243G and A8344G) and identified a relation between mutant load in muscle (but not in blood) and the presence of specific clinical symptoms.

In the present study, we have reviewed the data from 56 pedigrees with the two mtDNA mutations at nt 8993—8993(T→G) and 8993(T→C)—in the ATPase6 gene. These mutations are associated with symptoms such as neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP; MIM 551500) (Holt et al. 1990) and Leigh syndrome (MIM 516060) (de Vries et al. 1993). The 8993C mutation is generally considered to be clinically milder than the 8993G mutation, and together they represent the most common mtDNA mutations identified in children (Rahman et al. 1996; Santorelli et al. 1996). The issues of tissue and age-related variation have been addressed for the mutations at nt

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8993 and appear to be minimal (White et al., in press). The data from the 48 8993G pedigrees and from the 8 8993C pedigrees have been used to gauge the relationship between the mutant load and the severity of symptoms and to generate empirical recurrence risks for the mutations at nt 8993. This information should be useful to clinicians and genetic counselors for helping families to make their reproductive choices and for determining the prognosis of a child born with a mutation at nt 8993.

## Material and Methods

### *Subject Groups*

To assess genotype/phenotype correlations and to estimate recurrence risks, we examined reports on members from all families in the literature with either the 8993G or the 8993C mutation (van Erven et al. 1987; Holt et al. 1990; Sakuta et al. 1992; Shoffner et al. 1992; Tatuch et al. 1992; Ciafaloni et al. 1993; de Vries et al. 1993; Ortiz et al. 1993; Puddu et al. 1993; Santorelli et al. 1993, 1994, 1996, 1997; Tatuch and Robinson 1993; Yoshinaga et al. 1993; Fryer et al. 1994; Klement et al. 1994; Lodi et al. 1994; Pastores et al. 1994; Degoul et al. 1995; Houstek et al. 1995; Makela-Bengs et al. 1995; Tulinius et al. 1995; Bartley et al. 1996; de Co0 et al. 1996; Mak et al. 1996; Vazquez-Memije et al. 1996; Blok et al. 1997; Degoul et al. 1997; Ferlin et al. 1997; Seller et al. 1997; Uziel et al. 1997). Data from an additional nine 8993G and an additional three 8993C pedigrees were also included (White et al., in press). Except where specified, separate analyses were done for each mutation group. Unexplained infant deaths were excluded from all analyses, unless developmental delay, lactic acidosis, seizures, or dystonia were noted in the clinical history. Miscarriages and stillbirths were excluded from all analyses.

The relationship between an individual's clinical symptoms (see the Clinical Scores and Outcomes subsection, below) and mutant load was investigated in all individuals from the above-mentioned studies in whom the mutant load had been measured. When more than one tissue from an individual had been analyzed, the mutant load used was that obtained in blood or that obtained in muscle when blood was not available. The relationship between the mutant load of each mother and that of her children was investigated by means of the mother/child pairs in which both the mother and her offspring had a quantified mutant load. The relationship between the mutant load of each mother and the clinical symptoms of her children was investigated by means of the mother/child pairs in which the mother had a quantified mutant load and her offspring had clinical symptoms or a symptomatic status (see the Clinical Scores and Outcomes subsection, below). Probands were ex-

cluded from all mother/child relationship analyses. Mother/child relationships in which the mother had a mutant load of zero were included if one or more of her offspring (other than a proband) had a detectable mutant load.

### *Clinical Scores and Outcomes*

The severity of symptoms was measured by assigning a score on the basis of each individual's clinical symptoms or symptomatic status. A score of 1 (the least affected) was assigned to asymptomatic adults; a score of 2 was assigned to asymptomatic children. These asymptomatic groups were separated because an asymptomatic child may develop symptoms at a later age. A score of 3 was assigned to individuals who were affected by mild symptoms associated with NARP (e.g., problems with night vision and proximal muscle weakness) or with symptoms that may or may not be due to 8993G or 8993C (e.g., depression, migraine, and bulimia). Individuals with marked symptoms of NARP (e.g., developmental delay, mental retardation, dystonia, and ataxia), who were alive and lacked a confirmed diagnosis of Leigh syndrome, were assigned a score of 4. Individuals with Leigh syndrome, those who had died in childhood as a result of Leigh-like symptoms, or those who died in infancy (and whose deaths were not attributed to sudden infant death syndrome) were assigned a score of 5.

These clinical scores were dichotomized into "mild" and "severe" outcomes for the logistic regression analyses. The mild-outcome group included those individuals with a clinical score of 1 or 2 and all individuals but one with a score of 3. The severe-outcome group included those individuals with a clinical score of 4 or 5 and one individual with a score of 3. That individual was a 6-year-old child who had hypotonia in infancy, delayed motor skills, poor vision (but no optic atrophy or retinitis pigmentosa), delayed speech, and dysarthria (Fryer et al. 1994). She had no ataxia or pyramidal tract dysfunction; however, because of her age, it was thought that any physical or mental deterioration would place her in the symptom range of a clinical score of 4.

### *Statistical Analyses*

Analyses were performed with SPSS for Windows (version 7.0), Stata (version 5.0), and MLn (Rasbash et al. 1995). The distributions of mutant load for all individuals within categories defined by symptom severity score (1–5) have been presented as box plots. To assess the significance of differences in the median level of mutant load across categories of symptom severity, Kruskal-Wallis one-way analyses of variance were performed. The probabilities of a severe clinical outcome for individuals with a given mutant load were estimated by

means of a logistic regression model (see Appendix A) fitted to the dichotomized clinical score.

To assess the relationship between the maternal mutant load and the mutant load of the children, the 8993G and 8993C subject groups were combined, since there is no evidence to suggest that the two mutations are transmitted from mother to child by a different mechanism. Mothers were divided into five categories on the basis of their mutant load (0%–20%, 21%–40%, 41%–60%, 61%–80%, or 81%–100%), and the categories are presented as pie charts. The proportion of children whose mothers have mutant loads that fall into each of these five categories is represented by the slices of the pies.

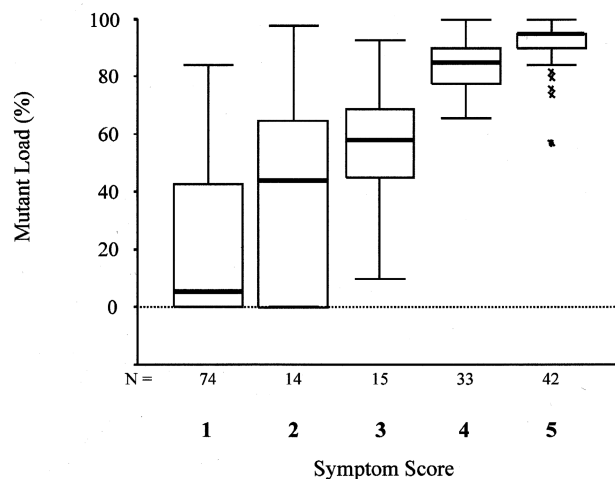
Recurrence risks, defined as the probability of a severe outcome in a child given the mutant load in the child's mother, were estimated for each mutation. To allow for variation between mothers in terms of their propensity to pass on the mutant load (e.g., the size of their mtDNA bottleneck), we fitted logistic regression models that included a random-effect term for mothers (see Appendix B). There were no children (excluding probands) with severe outcomes whose mothers had a mutant load of zero (a total of 18 mothers with 37 children with the 8993G mutation and 1 mother with 3 children with the 8993C mutation). The logistic regression implied a linear relationship between the mother's and the child's mutant load when the mothers with a mutant load of zero were excluded from the analyses. When the "zero mothers" were included, the data no longer fit the statistical model implied by the data from mothers with 1%–100% mutant load. These zero mothers were therefore excluded from the logistic regression analyses used to generate the log odds of adverse outcome given the mother's mutant load.

## Results

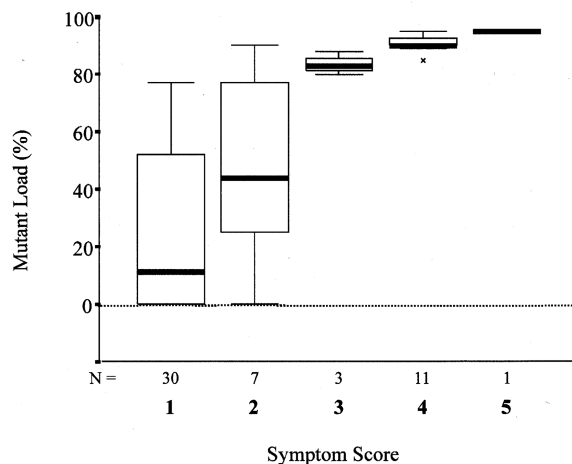
### Genotype/Phenotype Correlation

The relationship between the mutant load and the clinical score for individuals was investigated for both the 8993G ( $n = 178$ ) and the 8993C ( $n = 52$ ) mutations. Figure 1 shows a significant increase in median values of percent mutant load with increasing severity of symptoms. This is true for individuals with either the 8993G mutation or the 8993C mutation ( $P < .0001$ , for both). The ranges of mutant load are wide, and interquartile ranges overlap in symptom score categories 1 and 2, and 2 and 3 for the 8993G mutation, and in symptom score categories 1 and 2 for the 8993C mutation. In the higher-symptom score categories, individuals with the 8993C mutation showed less variation in mutant load than those with an 8993G mutation, which may be partly because of the small number of subjects. All individuals with the 8993C mutation and a symptom

a) 8993G



b) 8993C



**Figure 1** Box plots showing distributions of mutant load (%) within categories defined by clinical score for a, the 8993G mutation, and b, the 8993C mutation. The horizontal line across the box is the median, and the box represents the interquartile range (25th to 75th percentile). The bars extend to either (1) the maximum and the minimum values or (2) the 1.5-fold range of interquartile distance. Outliers are represented by (x) (1.5–3 box widths away from the edge of the box) or (\*) (>3 box widths away from the edge of the box).

score of  $\geq 3$  had a mutant load of  $\geq 80\%$ , whereas some individuals with the 8993G mutation had quite severe symptoms with lower mutant loads. Separating patients by sex in categories 4 and 5 showed no significant difference between the median mutant loads of males and females (data not shown), implying that there is no major difference in genotype/phenotype correlation between males and females.

To minimize the effect of inaccurate quantifications and the nonuniformity of clinical investigation on the analyses, and for ease of modeling, we dichotomized

clinical scores into mild (which included asymptomatic individuals) and severe, to generate predictive figures for the clinical outcome. Figure 2 shows the fitted probability, with 95% confidence intervals, of a severe outcome for the range of individual's mutant loads, for the 8993G mutation (fig. 2a) and the 8993C mutation (fig. 2b). The probability of having severe symptoms is low until the mutant load reaches 60%–70% for the 8993G mutation and 80%–90% for the 8993C mutation, at which point there is a steep increase in the probability with increasing mutant load. The regression line for the 8993G data in figure 2a predicts a probability of .25 for a severe outcome at a mutant load of 63%, with the probability increasing to .50, .75, and .95 at mutant loads of 71%, 78%, and 91%, respectively. Because of the small sample size, the relationship could not be precisely estimated for the 8993C mutation.

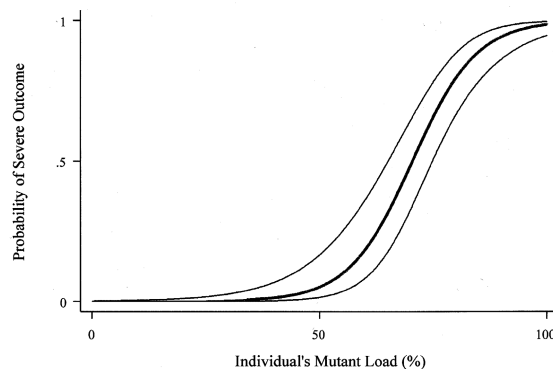
#### *Relationship between Mother's and Offspring's Mutant Loads*

In the mother-child analyses (from which all probands were excluded), there were 53 mothers with the 8993G mutation and 122 children. The number of children per mother was in the range of 1–7 (25 mothers had only one child other than the proband). Of these 122 children, 80 had both a measured mutant load and a clinical score; 42 had only a clinical score. Eighteen mothers with 46 children had the 8993C mutation, and each mother had between one and seven children (five mothers had only one child other than the proband). Of these 46 children, 38 had both a measured mutant load and a clinical score; 8 had only a clinical score. We combined the 8993G and 8993C data sets in which both mother and child had a measured mutant load ( $n = 118$ ) to assess the proportion of children in five categories of mutant load (0%–20%, 21%–40%, 41%–60%, 61%–80%, or 81%–100%) whose mothers were in the same categories (fig. 3). Each pie chart represents a single category of mothers. It is apparent that there is a greater proportion of children with a high mutant load as the mothers' mutant load increases, although it is possible for a mother with a high mutant load to have a child with a low mutant load and vice versa.

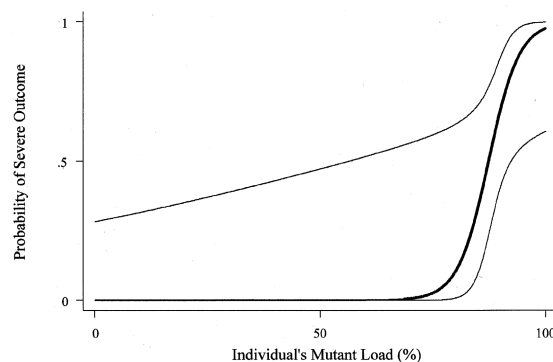
#### *Recurrence Risks for nt 8993 Mutations*

In the published nt 8993 pedigrees, many members did not have a quantified mutant load, but essentially all had a known clinical status. To minimize the ascertainment bias inherent in our sample population, we investigated recurrence risks using the clinical status rather than the mutant load of the offspring. The clinical scores were dichotomized into mild and severe outcomes, and the data were then used to estimate recurrence risks via a logistic regression model (see fig. 4 and Appendix B for equations). The model predicts an in-

a) 8993G



b) 8993C



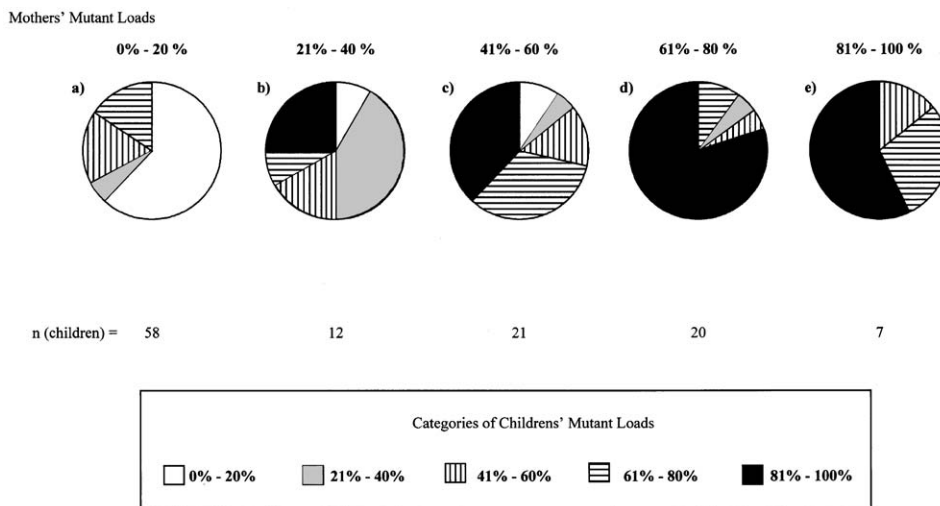
**Figure 2** Estimated probability of severe outcome (with 95% confidence intervals) on the basis of the mutant load of the sample derived from the logistic regression model for *a*, the 8993G mutation ( $n = 178$ ), and *b*, the 8993C mutation ( $n = 52$ ).

creasing risk of a severe outcome with increasing mutant load in the mother. The fitted probability of a severe outcome in the child is plotted for a 95% range of mothers' propensity to pass on the mutation in figure 4. The central line on this plot represents the fitted probability for a child whose mother is "average" with respect to passing on the mutation. The 95% range shows that at a given mutant load, there is considerable variation in the propensity of a woman to have a child with a severe outcome; hence, there is a high degree of uncertainty in the prediction. The two plots suggest a higher probability of a severe outcome at a given maternal mutant load for the 8993G mutation than for the 8993C mutation.

## Discussion

### *Reliability of Mutant Load Estimates*

Predicting the clinical severity or risk of recurrence from the mutant load measured in a particular tis-



**Figure 3** Pie charts for mothers with mutant loads in categories of *a*, 0%–20%; *b*, 21%–40%; *c*, 41%–60%; *d*, 61%–80%; and *e*, 81%–100%. Each pie chart shows the proportion of children with these mutant loads whose mothers are in the same categories.

sue—that is, CVS or blood—depends on that mutant load being representative of the other fetal or adult tissues. A review of the published 8993G and 8993C pedigrees provides strong evidence to suggest that (1) there is usually no substantial variation of the 8993G or 8993C mutant load among different fetal and adult tissues and (2) the nt 8993 mutations show no substantial age-related variation (White et al., in press). Therefore, the mutant load of a mutation at nt 8993 measured in a CVS or woman's blood can be considered as representative of the mutant load in the other fetal or adult tissues. These observations cannot be extrapolated to all mtDNA mutations. For example, the difference between blood and muscle mutant loads of the MELAS 3243G mutation increases with age (Poulton and Morten 1993), and the 3243G blood mutant load does not correlate with symptoms (Chinnery et al. 1997).

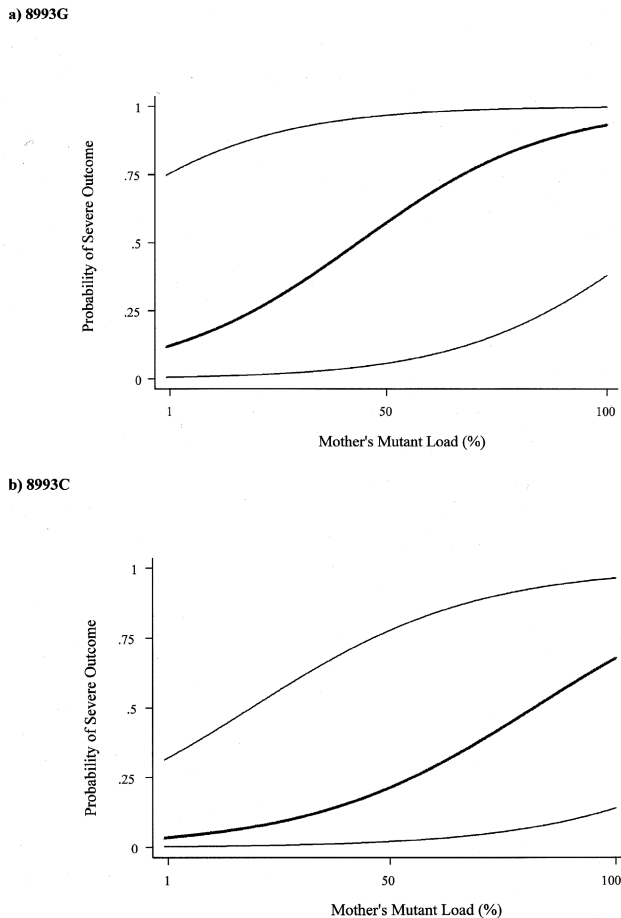
Some inconsistency is likely when comparing results of 8993G or 8993C mutant loads estimated by different techniques in different laboratories. Such differences are unlikely to substantially affect the predicted correlation curves relating mutant loads with outcome; however, they will increase the uncertainty around predictions for both the predictive clinical outcomes and recurrence risks. The greatest variabilities in mutant load quantifications are likely to be at the extreme ends of the mutant load range—that is, the underestimation of a low mutant load (<10%) and an overestimation of a high mutant load (>90%). However, the phenotypic variability at each of these mutant load ranges is quite small (as evidenced by the narrow confidence intervals), and modest variations are unlikely to alter the predicted curves substantially.

When using the predictive curves with patient data, it is essential to obtain an accurate quantification. We recommend avoiding the use of cultured cells or paraffin-embedded tissues in predicting risk, as these samples show more artifactual variation than blood or fresh tissues (White et al., in press).

#### *Relationship between Mutant Load and Clinical Symptoms*

Clinical scores assigned to individuals were sometimes subjective, because of the nonuniformity of clinical investigation. Only some of the probands were investigated for brain lesions, which would result in a score of 5 (for a diagnosis of Leigh syndrome), rather than 4 (for a diagnosis of NARP). It appears that some asymptomatic adults were not investigated fully, for example, for ophthalmologic changes, which, if present, would have resulted in a clinical score of 3 rather than 1. There was also uncertainty as to the age of some of the asymptomatic members of the families and, therefore, whether the clinical score should have been 1 or 2.

Overlap between the asymptomatic clinical score groups (fig. 1) was partly because of the difficulty in accurately classifying symptom severity in these groups but also reflects the variability in symptoms resulting from low mutant loads. In figure 1*a* (8993G), most subjects with symptom scores of 4 or 5 had mutant loads of  $\geq 75\%$ . One individual, marked “\*”, is an extreme outlier, with a mutant load of 57% and a diagnosis of Leigh syndrome (score of 5). Inspection of the figures from the original papers (Sakuta et al. 1992; Yoshinaga et al. 1993) suggests that the mutant load is considerably



**Figure 4** Predicted recurrence risks, with 95% range of propensity to pass on the mutation, determined by estimated variability between mothers with identical mutant loads of between 1% and 100% of the *a*, 8993G mutation and the *b*, 8993C mutation.

>57% in both blood and muscle. There are four other outliers (marked “x” on fig. 1a) with clinical scores of 5: two probands with mutant loads of 80% and 76% (families 6 and 10 in the study by White et al., in press), and a proband and her sister with mutant loads of 77% and 82%, respectively (Shoffner et al. 1992). Only one outlier—a proband with a confirmed diagnosis of NARP and a mutant load of 85% (family 12 in the study by White et al., in press), who was marked “x” on figure 1b—was seen with the 8993C mutation. These outliers may be due to factors affecting the quantifications or may represent variations in the clinical progress of the disorder. As with other metabolic diseases, environmental factors (particularly viral infections) and genetic background would be expected to contribute some variability to the genotype/phenotype correlation.

The presence of a “threshold” effect with mtDNA mutations was postulated in 1983 (Howell 1983) and has been verified by subsequent cybrid studies (Boulet

et al. 1992; Yoneda et al. 1995). This theory suggests that there is a minimum proportion of wild-type mtDNA molecules sufficient for ATP production, and that when the proportion of wild-type mtDNA molecules falls below this threshold, ATP production is compromised. A threshold effect is apparent in figure 2a. The data for the 8993C mutation are consistent with a threshold at a higher mutant load than the 8993G mutation. There is a higher probability of severe outcome at a given mutant load for the 8993G than the 8993C mutation (fig. 2). This finding is consistent with suggestions of others (Rahman et al. 1996; Santorelli et al. 1996) that the 8993G mutation is more severe than the 8993C mutation.

#### *Relationship between Mother's and Offspring's Mutant Load*

Rapid switching of mtDNA type, often in one generation, has been seen in Holstein cows and in humans and has led to the formulation of the “bottleneck theory.” This theory suggests that at some stage in oocyte development, a small number of mtDNA molecules are preferentially amplified to “repopulate” the oocyte (Hauswirth and Laipis 1982). The bottleneck theory is supported by the analysis of an individual's seven oocytes that showed a highly skewed distribution of the 8993G mutation (Blok et al. 1997). The theory is also supported by the finding that ~20% of families with the 8993G mutation appear to result from the expansion of a de novo mutation (de Coo et al. 1996; Degoul et al. 1997; Santorelli et al. 1997; Seller et al. 1997; Uziel et al. 1997; White et al., in press).

If only a small number of mtDNA molecules are being replicated to repopulate the oocyte, one would expect that a clonal expansion of the mother's mtDNA would result in a woman with a high mutant load having more oocytes with high mutant loads. Similarly, one would expect a woman with a low mutant load to have more oocytes with low mutant loads, since the “source” of the clonal expansion would consist mainly of wild-type mtDNA molecules. Can we therefore gain an indication of the risk of having an affected child from the maternal mutant load?

The data from the mother/child relationships are likely to be affected by the ascertainment biases inherent in the sample population. There is almost certainly an underascertainment of clinically unaffected siblings with the mutation (because of ethical concerns about presymptomatic testing) and an overascertainment of families with multiple severely affected individuals in the published literature. Although one should be mindful of the biases, there appears to be a strong positive relationship between a mother's blood load and the mutant load of a mutation at nt 8993 in her offspring (fig. 3).

The data in figure 3e are all the offspring ( $n = 7$ ) of one woman with a mutant load in blood of 84% and a clinical score of 1—that is, she was asymptomatic (DeGoul et al. 1995). The figure from the original report shows that the mtDNA fragments in the Southern blot were very faint, and the low signal intensity may have made the quantification unreliable. This woman gave birth to eight children, two of whom were also asymptomatic but had high mutant loads. This family shows considerable inconsistencies with the other families in this study, both in the correlation between the mutant load and the severity of symptoms and in the relationship between the maternal mutant load and the clinical symptoms of the offspring.

#### Recurrence Risks for Mutations at nt 8993

Predicted recurrence risks for both mutations at nt 8993 were generated (fig. 4) and indicate an increasing probability of severe outcome with increasing mother's mutant load. However, there is a wide 95% range of predicted probabilities, reflecting the variation in mutant loads of children whose mothers have a mutation at nt 8993. This finding is consistent with a small bottleneck size. It also indicates that caution is needed when making predictions about severity of symptoms in children on the basis of a woman's mutant load. The model gives us an idea of the outcome for an "average" woman, but clients must be counseled about the wide range of possible outcomes. The size of the bottleneck could be calculated for each family by use of the data obtained in this study (Poulton et al. 1998). However, the calculations would be affected by the inherent ascertainment biases and small sample size in the families and would be unlikely to provide more-specific recurrence risks to a couple requiring genetic counseling than those generated in figure 4.

A number of women had no detectable mutant load and no children, other than the proband, with symptoms or a detectable mutant load. These cases are likely to arise from de novo mutations that appear to be common (see the Relationship between Mother's and Offspring's Mutant Load subsection, above). However, it is possible that some mothers of children with apparently de novo cases had a mutant load below the limit of detection. Since many of their children have not had mutation testing (because of ethical concerns about testing presymptomatic or asymptomatic children), these women cannot be regarded as having a zero recurrence risk, and we think it reasonable to quote a recurrence risk figure of <10%.

The predictive data in figures 2 and 4 provide the only figures currently available to help with genetic counseling for families with a mutation at nt 8993. Although it must be stressed that there is uncertainty around pre-

dictions that can be made, they provide guidelines for use by clinicians dealing with families in this situation. The empirical recurrence risks can be used to help with decision making during prepregnancy counseling and to determine if prenatal diagnosis is appropriate for the family. Furthermore, the calculated risk of a severe clinical outcome made on the basis of mutant load provides figures for use in interpreting results from a prenatal diagnostic test, if the family chooses to take this option. Clearly, the uncertainty about the predictions needs to be communicated to the families, particularly for the 8993C mutation. The subject group consisting of the 8993C families was small; therefore, recurrence risks given to families with this mutation should be accompanied by careful counseling about the limitations of the data. However, as in many areas of genetic counseling, uncertainty and making decisions on the basis of imprecise risk estimates is a part of the process.

## Acknowledgments

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## Appendix A

The logistic regression model for an individual's probability ( $P$ ) of a severe clinical outcome, given the mutant load, is  $\ln \frac{P}{1-P} = \beta_0 + \beta_1 x$ , where  $x$  is the individual's mutant load. The exponential of the parameter  $b_1$  is the odds ratio of a severe clinical outcome for a unit increase in an individual's mutant load. The model was fitted separately for data from the 8993G and 8993C mutations, and the estimates are given in table A1. The estimates can be used to obtain an average fitted probability of severe outcome for individuals with a given mutant load (as done for fig. 2).

**Table A1**

**Estimate of Logistic Regression Model Parameters for the 8993G and the 8993C Mutations**

PARAMETER	ESTIMATE (STANDARD ERROR) FOR MUTATION	
	8993G	8993C
Coefficient for individual's mutant load ( $\beta_1$ )	.143 (.024)	.294 (.142)
Constant ( $\beta_0$ )	-10.092 (1.810)	-25.645 (12.606)

## Appendix B

The logistic regression model for the  $j$ th child's probability of a severe outcome,  $\Pr(y_{ij}=1)$ , given the mother's mutant load, denoted  $x_i$ , and including a mother-specific random effect,  $u_i$  (assumed to have a normal distribution with mean of zero and variance of  $\sigma^2$ ), is

$$\ln \frac{\Pr(y_{ij} = 1)}{1 - \Pr(y_{ij} = 1)} = \beta_0 + \beta_x x_i + u_i .$$

The exponential of the parameter  $\beta_x$  is the odds ratio of a severe outcome for a unit increase in mother's mutant load (given the mother's random effect  $u_i$ ). This model is fitted separately to data from 8993G and 8993C mutations. The estimates from table B1 can be used in the model to obtain fitted probabilities for an individual child, either for a range of typical mothers (as done for fig. 4) or for an average mother (i.e., when  $u_i = 0$ ).

**Table B1**

**Estimates of Random-Effect Model Parameters for the 8993G and 8993C Mutation Models**

PARAMETER	ESTIMATE (STANDARD ERROR) FOR MUTATION	
	8993G <sup>a</sup>	8993C <sup>b</sup>
Coefficient for mother's mutant load ( $\beta_x$ )	.046 (.018)	.041 (.019)
Constant ( $\beta_0$ )	-2.023 (0.866)	-1.709 (.578)
Variation between mothers ( $\sigma^2$ )	2.505 (1.331)	1.696 (1.628)

<sup>a</sup> We produced these estimates using the restrictive iterative generalized least squares (RIGLS) procedure and performed updates using 2d-order predictive quasilikelihood (PQL).

<sup>b</sup> Mother's mutant load was centered at 40 to aid the estimation of the model for the 8993C mutation (i.e.,  $x_i$  was replaced by  $[x_i - 40]$  in the model above). The estimation of the model for the 8993C mutation was RIGLS with 1st-order PQL. Estimation problems were encountered when we attempted 2d-order PQL, presumably because of the small sample size.

## Electronic-Database Information

Accession numbers and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for NARP [MIM 551500] and for Leigh syndrome [MIM 516060])

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