MELAS and MERRF
The relationship between maternal mutation load and the frequency of clinically affected offspring

Patrick F. Chinnery, Neil Howell, Robert N. Lightowlers and Douglass M. Turnbull

Summary
The majority of pathogenic mitochondrial DNA (mtDNA) mutations are heteroplasmic, with both mutant and wild-type alleles present within the same individual. MtDNA is transmitted only from females to their offspring but a single female can bear offspring who harbour different levels of mutant mtDNA and have a variable phenotype. In single families, this complex genetic and phenotypic variability has confounded the identification of any relationship between the level of mutant mtDNA (mutation load) in the mother and the clinical features of her offspring. To obtain a more accurate description of the inheritance of pathogenic mtDNA mutations, we studied a large number of pedigrees that carried either the mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (A3243G MELAS) or the myoclonus epilepsy with ragged-red fibres (A8344G MERRF) mutations. We made two principal observations. First, for both mutations, higher levels of mutant mtDNA in the mothers’ blood were associated with an increased frequency of affected offspring. Secondly, at any one level of maternal mutation load there was a greater frequency of affected offspring for the A3243G MELAS mutation than for the A8344G MERRF mutation. Although these results should not be used to give absolute risks to a female contemplating pregnancy, they suggest that the outcome of pregnancy is related to the level of mutant mtDNA in the mother and that the risks of having affected offspring may differ between different mtDNA mutations.

Keywords: mitochondrial encephalomyopathies; MELAS; MERRF; mitochondrial inheritance; maternal inheritance

Abbreviations: MELAS = mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; MERRF = myoclonus with epilepsy and ragged-red fibres; mtDNA = mitochondrial DNA; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; tRNA = transfer RNA

Introduction
Since 1988, more than 200 different mitochondrial DNA (mtDNA) defects have been associated with a wide variety of human disorders (Schon et al., 1997; Servidei, 1998). These genetic defects are often inherited, producing a progressive disabling neurological syndrome which results in premature death. At present we have no effective treatment for these disorders. The development of genetic counselling is, therefore, of prime importance.

MtDNA mutations fall into two groups: rearrangements (deletions and duplications) and point mutations. The majority of mtDNA rearrangements are sporadic and they are not transmitted to subsequent generations. In contrast, mtDNA point mutations are inherited exclusively down the maternal line. Individuals with Leber hereditary optic neuropathy usually harbour only mutant mtDNA (homoplasmic mutant) and extensive studies have characterized the risks of developing an optic neuropathy in related individuals (Harding et al., 1995; Macmillan et al., 1997). In contrast, transfer RNA (tRNA) gene mutations are usually heteroplasmic, both mutant and wild-type mtDNA being present within an affected individual (Larsson and Clayton, 1995). In vitro studies have shown that a critical threshold level of mutant mtDNA (mutation load) must be exceeded before a cell expresses a mitochondrial respiratory chain defect (Attardi et al., 1995). There is also evidence that the clinical features in mitochondrial disease are related to the mutation load within affected individuals (Chinnery et al., 1997). However, a female who harbours a heteroplasmic mtDNA mutation may transmit a low amount of mutant mtDNA to one offspring (who may not be clinically affected).
and a high mutation load to another offspring (who may develop clinical features of mtDNA disease). As a result, there is extensive genotypic and phenotypic variation among siblings in a single pedigree (Ciafaloni et al., 1992; Larsson et al., 1992), and it has not been possible to discern a clear relationship between the mutation load in the mother and the frequency of affected offspring (Warner and Schapira, 1997).

To gain greater insight into the inheritance of pathogenic mtDNA mutations, we have studied the transmission of the two most common mtDNA point mutations in a large number of different pedigrees: the mutation A3243G MELAS (mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes) and the mutation A8344G MERRF (myoclonus with epilepsy and ragged-red fibres). These mutations are relatively rare at the population level and the frequency of affected offspring (Warner and Schapira, 1997).

Data collection and evaluation

We minimized the effects of ascertainment bias by excluding mother–child pairs in which the mother or the child was the index case. Each mother–child pair was then analysed critically using the following criteria. We included mother–child pairs only when the clinical data were sufficient to allow a confident clinical classification by a neurologist with extensive experience of mitochondrial disease. Furthermore, mother–child pairs were analysed only where the level of heteroplasmy was quantified by an established method that was described in sufficient detail. On this basis, we identified six MELAS and MERRF mother–child pairs from our own database and 109 through an extensive search of the literature (Shoffner et al., 1990; Seibel et al., 1991; Ciafaloni et al., 1992; Kobayashi et al., 1992; Larsson et al., 1992; Martinuzzi et al., 1992; Reardon et al., 1992; Graf et al., 1993; Hammans et al., 1993, 1995; Moraes et al., 1993; Mosewich et al., 1993; Campos et al., 1995; Piccolo et al., 1993; Remes et al., 1993; Shanske et al., 1993; Silvestri et al., 1993; Tanno et al., 1993; Chu et al., 1994; de Vries et al., 1994; Degoul et al., 1994; Dougherty et al., 1994; Huang et al., 1994, 1996; Liou et al., 1994; Matthews et al., 1994; Kishimoto et al., 1995; Mariotti et al., 1995; Ozawa et al., 1995; Rusanen et al., 1995; Traff et al., 1995).

The level of mutant mtDNA had been measured in the blood of all 115 mothers but very few had undergone a muscle biopsy. Because of the paucity of muscle data, it was not possible to study the relationship between mutation load in the mothers’ muscle and the frequency of affected offspring. The following laboratory methods were used to quantify the level of mutant mtDNA in blood: (i) dot-blotting (n = 4; 3%, where n is the number of pairs) or Southern blotting of restriction digestion fragments (n = 24; 21%); (ii) restriction fragment length polymorphism (RFLP) analysis and densitometry of ultraviolet-transilluminated ethidium bromide-stained polymerase chain reaction (PCR) products (n = 10; 9%); (iii) RFLP analysis and densitometry or phosphorimage analysis of radiolabelled PCR products (n = 21; 18%); (iv) DNA sequencing of multiple (>20) clones (n = 1; 1%); (v) RFLP analysis and phosphorimage analysis of PCR products labelled in the last cycle of PCR (‘hot last cycle’) (n = 55; 48%).

Method

Potential confounding effects

For both mutations, the mean ages of affected and unaffected individuals were not statistically significantly different. The A3243G MELAS mutation yielded a mean age of unaffected individuals of 23.1 years (SD = 8.9), whereas the mean age of affected individuals was 26.4 years (SD = 18.8 years). The results for the A8344G MERRF mutation were 33.5 years (SD = 19.3) and 22.8 years (SD = 11.2) for unaffected and affected individuals, respectively. It does not appear, therefore, that this analysis has been biased by over-representation of asymptomatic offspring who became clinically affected as they grew older. Furthermore, it is likely that any underestimation of risk due to the subsequent progression of unaffected individuals will, at least in part, be counterbalanced by the small amount of bias that arises from the use of different methods for quantifying heteroplasmy. ‘Non-last hot cycle’ PCR methods tend to underestimate mutation load by a mean of between 10 and 14% (Tanno et al., 1995). To overcome these small effects and to minimize the effect of any other minor
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Fig. 1 Frequency distribution histograms showing the percentage of affected offspring born to mothers harbouring different levels of mutant mitochondrial DNA. (A) A3243G MELAS mutation measured in the mothers’ blood. (B) A8344G MERRF mutation measured in the mothers’ blood.

confounding variables on the overall trend, we categorized the mothers into large (20%) subgroups.

Observed frequency of affected offspring for the A3243G MELAS and A8344G MERRF mutations

We observed a statistically significant relationship between the mutation load in the mothers’ blood and the frequency of affected offspring (Fig. 1). The following results were calculated from the data set after the deletion of mother–child pairs that included the proband/index case, although similar trends were obtained for the larger data set, which included index cases.

A3243G MELAS

Mothers with <20% mutant mtDNA in their blood had a 25% incidence of affected offspring (Fig. 1A). The incidences of affected offspring were greater for mothers with a higher percentage of mutant mtDNA in their blood up to a level of 60%, reaching a frequency of 57% (χ²TR = 4.96, P = 0.02).

A8344G MERRF

Not one of the 22 offspring born to mothers with <40% mutant mtDNA in their blood was affected (Fig. 1B). Above this level (≥40% mutant mtDNA), mothers with greater levels of mutant mtDNA in their blood had a higher proportion of affected offspring, reaching a maximum of 78% affected offspring for mothers with ≥80% mutant mtDNA in their blood (χ²TR = 16.42, P = 5.09 × 10⁻⁵). Mothers with ≥80% mutant mtDNA in blood were 22.56 times more likely to have an affected child than were mothers with <60% mutant mtDNA in their blood (95% confidence interval = 12.17–41.80).

Comparison between A3243G MELAS and A8344G MERRF

Mothers with ≥60% A3243G MELAS mutation in their blood were 11.4 times more likely to have affected offspring than mothers with the same mutation load of the A8344G MERRF mutation in their blood (95% confidence interval = 3.72–34.96, χ²TR = 18.16, P = 2.03 × 10⁻⁵).

Discussion

We have shown here that, for both the A3243G MELAS and the A8344G MERRF mutations, the frequency of affected offspring is related to the level of mutant mtDNA in the mothers’ blood. We have also shown that, for a particular level of maternal mutation load in the blood, the frequency of affected offspring is different for the two pathogenic mutations. Both conclusions are supported by the high level of statistical significance attained in our analyses of a large number of mother-to-offspring transmissions.

Integrity of the data set

We believe that the results from this large group of maternal transmissions reliably represent the inheritance and expression of the A3243G MELAS and A8344G MERRF mutations on a population level. First, the clinical features...
of mitochondrial disease were similar among the different pedigrees and they were in concordance with recognized diagnostic criteria (Chinnery and Turnbull, 1997a). Secondly, established methods were used to quantify the level of heteroplasmy in all cases. Thirdly, the 115 mother–child pairs were derived from 63 unrelated pedigrees. Fourthly, we took steps to minimize the effects of ascertainment bias. Fifthly, we categorized mother–child pairs into large subgroups (20% maternal mutation load) to minimize the effects of any quantification inaccuracies or variation on the overall trend. Finally, we found no evidence that age was a significant confounding factor in the classification of offspring into affected and unaffected groups. Although we recognize that some of the individuals classified as unaffected might develop features of mtDNA disease in the future, we believe that this potential bias will be counterbalanced by any residual effects of incomplete ascertainment.

**The frequency of affected offspring and maternal mutation load**

Our observations indicate that, at least for these two mutations, the level of mutant mtDNA in a mother’s blood correlates with the risk of having clinically affected offspring. Intuitively, one might expect mothers who harbour higher levels of mutant mtDNA to transmit higher levels of mutant mtDNA and thus have an increased frequency of clinically affected offspring. However, extreme variability in the segregation of mitochondrial genomes between offspring within individual pedigrees has confounded the interpretation of previous, smaller studies. This variability is, at least in part, due to the reduction and subsequent proliferation of mitochondrial genomes in the developing oocyte (called the ‘genetic bottleneck’ by some authors; for a review see Lightowlers et al., 1997). In contrast to the smaller studies carried out by others, we have analysed the relationship between the maternal mutation load and the level of mutant mtDNA in the offspring in a large population sample of mother–child transmissions. Our results demonstrate that, despite a degree of scatter, there is also a highly statistically significant relationship between the level of mutant mtDNA in mothers and in their offspring (P. F. Chinnery, N. Howell, D. M. Turnbull, unpublished observations).

It is of interest that we identified only two individuals who were born to mothers with >60% of the A3243G MELAS mutation in their blood. This paucity may either be because women with mutation loads of 60% or higher are so severely clinically affected that they have a markedly reduced capacity for reproduction or because there is an early developmental loss of foetuses which harbour high levels of mutant mtDNA. However, mothers harbouring high levels of the A8344G MERRF mutation were still able to have children. Finally, whilst there was a trend for affected mothers to have more affected children than unaffected mothers, this difference did not reach statistical significance. This may be due to the small number of affected mothers who were available for analysis after the deletion of probands.

**Comparison between A3243G MELAS and A8344G MERRF**

The pathogenic A3243G MELAS and A8344G MERRF mutations both involve mitochondrial tRNA genes, and they have similar effects on respiratory chain function in vitro (Chomyn et al., 1991; Attardi et al., 1995). It is not immediately clear, therefore, why they should have such different inheritance patterns. We suspect that the differences that we observed in this study reflect differences in the expression of these two mutations within an affected individual, and not different transmission mechanisms of the mtDNA molecules. Evidence to support this suggestion comes from a comparison of the similar clinical features for patients harbouring the A3243G MELAS and A8344G MERRF mutations (Chinnery et al., 1997). Individuals with <70% of the A3243G MELAS mutation in skeletal muscle often have ataxia, epilepsy and dementia. By contrast, ataxia, epilepsy and dementia are uncommon in individuals who harbour <70% of the A8344G MERRF mutation. Because the level of mutant mtDNA in skeletal muscle closely correlates with the level in other postmitotic tissues (such as central neurons), this comparison provides evidence, albeit indirect, that the level of mutant mtDNA is not the only factor involved in the clinical expression of these two mutations. Different molecular pathogenic mechanisms (Enriquez et al., 1995; Kaufmann et al., 1996) or different tissue segregation patterns (Hammans et al., 1993; Silvestri et al., 1993; Holme et al., 1995; Chinnery et al., 1997) may contribute to the different frequencies of clinically affected offspring between the A3243G MELAS and A8344G MERRF mutations that we have observed.

**Conclusion**

It should be stressed that it is not appropriate to use the data presented here to give absolute risks to females contemplating pregnancy. Despite the fact that we have demonstrated the validity of our data set, any retrospective study will be subject to a degree of bias. However, our results strongly support the view that maternal mutation load is important in determining the likelihood of having affected offspring. We have also shown that mothers who harbour the A3243G MELAS mutation have a high (>25%) frequency of affected offspring whatever the level of mutant mtDNA in their blood. In contrast, mothers with <40% A8344G MERRF in their blood have a relatively low frequency (zero in this analysis) of having an affected child. It remains to be seen whether other point mutations behave in a similar way. Extensive prospective studies should be carried out to provide a solid foundation for genetic counselling of the mitochondrial encephalomyopathies.
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References


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