Letters to the Editor

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Genetic Counseling and Prenatal Diagnosis for mtDNA Disease

To the Editor:

Over the last decade, clinicians have seen an increasing number of patients who have been diagnosed with mtDNA disease (Chinnery and Turnbull 1997*b*). As a consequence, clinicians also have seen more and more women of childbearing age carrying a pathogenic mtDNA mutation who seek advice about the potential risks to future offspring (Chinnery and Turnbull 1997*a*). With this trend in mind, the recent editorial by Poulton et al. (1998) was timely, tackling the difficult but intriguing problem of the origin, segregation, and inheritance of heteroplasmic mtDNA mutations. The authors placed particular emphasis on the transmission of pathogenic mutations, and, on the basis of their interpretation of mechanisms, they suggested an approach to the counseling for and prenatal diagnosis of mtDNA disease that could be used in clinical practice. However, because our understanding of the mechanisms governing these processes is rudimentary at best, caution must be used when counseling families with mtDNA disease.

At the most general level, two processes contribute to the marked intrafamilial variation of genotype and phenotype that is the hallmark of mtDNA disease (Chinnery and Turnbull 1997*b*). The first process occurs during the early embryonic development of a female. Between the formation of the zygote and the maturation of the oocyte lineage, the cellular copy number of mtDNA is reduced and then amplified. This results in a high level of variability in the level of mutated mtDNA that is transmitted to the subsequent generation. Poulton et al. (1998) refer to this process as the bottleneck, but whether the variability results from selection events (e.g., see Hauswirth and Laipis 1985) or from random drift (Jenuth et al. 1996) is the subject of debate. In the second process, further diversity is generated as mutated genomes differentially replicate and segregate during histogenesis and organ maturation (for a review, see Lightowlers et al. 1997).

These two processes are of intense interest to those scientists who seek to understand the mechanisms that determine the inheritance of mtDNA. Not surprisingly, their importance also has been recognized by clinicians who counsel women at risk of transmitting heteroplasmic pathogenic mtDNA mutations. At present, unfortunately, very little guidance can be offered to these women. There are three possible approaches to resolving the clinical problem: (1) application of our understanding of the mitochondrial genetic bottleneck; (2) empirical investigation of the relationship between the maternal mutation load and the mutation load and clinical phenotypes among offspring; and (3) the use of prenatal diagnostic tests.

Although our rudimentary understanding of the bottleneck might be tempting to use when counseling patients, it is, in fact, of limited practical use at present. There are a number of theoretical and experimental problems that beset the bottleneck phenomenon. First, differences in the number of mtDNA molecules and in the number of cell divisions during germ-line development may have a profound effect on the size of the bottleneck, thus weakening any simple extrapolation of data from animal studies to humans (Austin 1995; Strachan and Lindsay 1997). Second, undefined differences among embryos may result in bottlenecks of different sizes (Herbert et al. 1995); there is no evidence of a simple, one-size-fits-all bottleneck (Howell et al. 1992). Finally, and perhaps most importantly, although mathematical models of the bottleneck can be used to predict the range of possible levels of mutant mtDNA in an offspring, the resulting range is so wide as to be of limited value in counseling. For the specific example described by Poulton et al. (1998)—namely, a female with 21% mutant mtDNA in her blood—the 95% confidence interval of the mutation load in her offspring is 0%–50%. Furthermore, even if prediction of the precise level of mutant mtDNA in the blood of the offspring was possible, the clinical outcome from a particular mtDNA mutation load cannot be predicted with a high level of confidence, at the present time (Chinnery et al. 1997). For example, even for the A8344G mutation, there is considerable overlap between the levels of mutant mtDNA detected in the blood of clinically affected individuals and the levels in their unaffected relatives (fig. 1). Thus, the complex and multisystem clinical phe-

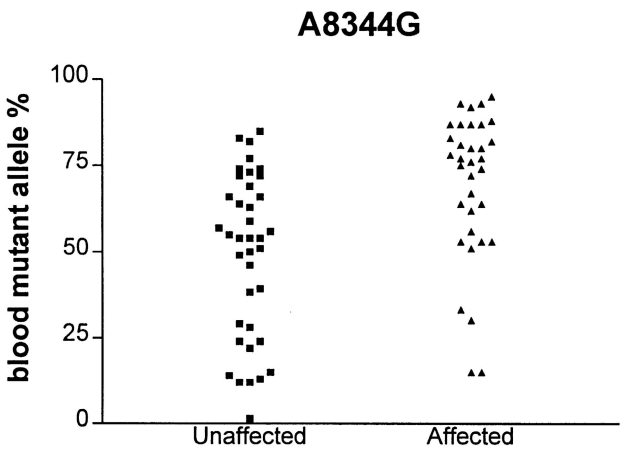

Figure 1 Percentage of mutant mtDNA in blood, for 72 individuals harboring the A3243G MERRF (myoclonic epilepsy with ragged red fibers) mutation. For the details of data acquisition, see the article by Chinnery et al. (1997).

notypes are difficult to predict on the simple basis of blood levels of mutant mtDNA in an individual harboring an mtDNA mutation.

An alternative, more empirical approach would be to study the outcome of pregnancy in a large number of women with heteroplasmic mtDNA mutations, *without* making any assumptions about the mechanism of the bottleneck. Thus, even with the limitations of a retrospective multicenter study, the frequency of clinically affected offspring born to women who harbor the A3243G or the A8344G point mutation recently has been shown to be related to the level of mutant mtDNA in the mothers' blood (Chinnery et al., in press). However, the relationship clearly differed for the two pathogenic mutations, which indicates significant differences in the expression of the two mutations, for a given inherited mutation load. Although the use of this retrospective data to give precise estimates of the risks involved for a particular female is premature, these observations underscore the potential value of more-extensive longitudinal, tissue-distribution, and, especially, prospective analyses.

Finally, preimplantation testing of a chorionic villus biopsy may prove useful for counseling; however, at present, there is very little data to support its use. If the level of mutant mtDNA is distributed evenly to all the tissues of a developing embryo and if the mutation load stays constant with time, then this technique may be reliable (as may be the case for the T8993G/C point mutations). However, it is already known that the level of mutant mtDNA is not distributed evenly in most patients with mtDNA disease and that this differential segregation probably occurs at the later stages of development. Even subtle variations in tissue mutation load may lead to a profound variation in the phenotype, and sampling of a single cell or chorionic villus may not reflect the load in clinically relevant organs such as the brain. Further studies are needed to establish the value of these potentially hazardous techniques in the counseling of patients with mitochondrial disease.

The prevalence of pathogenic mtDNA defects is at least 1/10,000 in the general population of northern Europe (Majamaa et al. 1998; P.F.C. and D.M.T., unpublished data). Many of these individuals are women of childbearing age who urgently need genetic counseling and advice, with regard to both the prognosis for their children and the risk of disease in subsequent offspring. Poulton et al. (1998) have highlighted some of the difficulties encountered in counseling these patients, and their discussion has given us much food for thought. However, their recommended acceptance of a proposed simple bottleneck model and its application to prenatal mitochondrial diagnosis is premature.

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