not sporadic and occur in tissue-specific stem cells during fetal development or in the postnatal maintenance or postinjury repair stage). Such mutations would be expected to be propagated only within the progeny of that stem cell and affect a particular tissue within a given individual, without evidence of heritability.

PROSPECTS FOR CLINICAL MANAGEMENT OF mtDNA DISEASE

TREATMENT OF mtDNA DISORDERS

No specific curative treatment for mtDNA disorders is currently available; therefore, the management of mitochondrial disease is largely supportive. Management issues may include early diagnosis and treatment of diabetes mellitus, cardiac pacing, ptosis correction, and intraocular lens replacement for cataracts. Less specific interventions in the case of other disorders involve combined treatment strategies including dietary intervention and removal of toxic metabolites. Cofactors and vitamin supplements are widely used in the treatment of diseases of mitochondrial oxidative phosphorylation, although there is little evidence, apart from anecdotal reports, to support their use. This includes administration of artificial electron acceptors, including vitamin K_3, vitamin C, and ubiquinone (coenzyme Q10); administration of cofactors (coenzymes) including riboflavin, carnitine, and creatine; and use of oxygen radical scavengers, such as vitamin E, copper, selenium, ubiquinone, and idebenone. Drugs that could interfere with mitochondrial function, such as the anesthetic agent propofol, barbiturates, and high doses of valproate, should be avoided. Supplementation with the nitric oxide synthase substrate, L-arginine, has been advocated as a vasodilator treatment during stroke-like episodes. The physician should also be familiar with environmental interactions, such as the strong and consistent association between visual loss in LHON and smoking. A clinical penetrance of 93% was found in men who smoked. Asymptomatic carriers of an LHON mtDNA mutation should, therefore, be strongly advised not to smoke and to moderate their alcohol intake. Although not a cure, these interventions might stave off the devastating clinical manifestations of the LHON mutation. Another example is strict avodiance of aminoglycosides in the familial syndrome of ototoxic susceptibility to aminoglycosides in the presence of the mtDNA m.1555A>G mutation of the 12SrRNA encoding gene.

GENETIC COUNSELING, PRENATAL DIAGNOSIS, AND PREIMPLANTATION GENETIC DIAGNOSIS IN mtDNA DISORDERS

The provision of accurate genetic counseling and reproductive options to families with mtDNA mutations is challenging due to the unique genetic features of mtDNA inheritance that distinguish it from Mendelian genetics. mtDNA defects are transmitted by maternal inheritance. mtDNA de novo mutations are often large deletions, affect one family member, and usually represent no significant risk to other members of the family. In contrast, mtDNA point mutations or duplications can be transmitted down the maternal line. Accordingly, the father of an affected individual has no risk of harboring the disease-causing mutation, and a male cannot transmit the mtDNA mutation to his offspring. In contrast, the mother of an affected individual usually harbors the same mutation but might be completely asymptomatic. This wide phenotypic variability is primarily related to the phenomena of heteroplasmy and the mutation load carried by different members of the same family. Consequently, a symptomatic or asymptomatic female harboring a disease-causing mutation in a heteroplasmic state will transmit to her offspring variable amounts of the mutant mtDNA molecules. The offspring will be symptomatic or asymptomatic primarily according to the mutant load transmitted via the oocyte and, to some extent, subsequent mitotic segregation during development. Interactions with the mtDNA haplotype background or nuclear human genome (as in the case of LHON) serve as an additional important determinant of disease penetrance. Because the severity of the disease phenotype associated with the heteroplasmic mutation load is a function of the stochastic differential segregation and copy number of mutant mtDNA during the oogenesis bottleneck and, subsequently, following tissue and organ development in the offspring, it is rarely predictable with any degree of accuracy. For this reason, prenatal diagnosis (PND) and PGD techniques that have evolved into integral and well-accepted standards of practice are severely hampered in the case of mtDNA-related diseases.

The value of PND and PGD is limited, partly due to the absence of data on the rules that govern the segregation of wild-type and mutant mtDNA species (heteroplasmy) among tissue in the developing embryo. Three factors are required to ensure the reliability of PND and PGD: (1) a close correlation between the mutant load and the disease severity, (2) a uniform distribution of mutant load among tissues, and (3) no major change in mutant load with time. These criteria are suggested to be fulfilled for the NARP m.8993T>G mutation but do not seem to apply to other mtDNA disorders. In fact, the level of mutant mtDNA in a chorionic villous or amniotic fluid sample may be very different from the level in the fetus, and it would be difficult to deduce whether the mutational load in the prenatal samples provides clinically useful information regarding the postnatal and adult state.

PREVENTION OF MITOCHONDRIAL DISEASE INHERITANCE BY ASSISTED REPRODUCTIVE TECHNOLOGIES

Because the treatment options for patients with mitochondrial disease are rather limited, preventive interventions that eliminate the likelihood of transmission of affected mtDNA into offspring are desirable. The lack of utility of PND and PGD techniques to reliably diagnose and predict mitochondrial disorders at preimplantation-stage products of conception has resulted in the search for alternative preventive approaches for the same problem. One possible approach to "diluting" or even entirely eliminating the mutant mtDNA is applicable only in the earliest embryonic state and in effect represents a form of germline preventive therapy (Fig. 85e-9). This possibility has been explored by using alternative assisted reproduction techniques such as ooplasmic transfer (OT), metaphase chromosome transfer (CT), pronuclear transfer (PNT), and germinal vesicle transfer (GVT) in animal models and, to an extent, in humans. OT is a technique wherein a certain volume (5–15%) of healthy donor oocyte cytoplasm with normal mitochondria is injected into the patient oocyte containing mutated mitochondria. The reasoning behind OT is to supplement the patient's oocyte with uncompromised cytoplasmic factors such as mtDNA, mRNA, proteins, and other molecules by injecting cytoplasm from healthy oocytes. In PNT, following fertilization, pronuclei of a patient's zygote are removed with a cytoplasm ("karyoplast"). The karyoplast is transferred to the perivitelline space of a donated zygote, which has been already enucleated. The karyoplast is then fused with enucleated zygote by electric pulses or inactivated Sendai viruses (HVJ). The reconstructed zygote contains a nucleus from the patient (patient nuclear DNA) and cytoplasm from the donor. Thus, the majority of the patient mtDNA is replaced with mtDNA from the donor oocyte. In CT, meiosis II stage of oocyte maturation provides an opportunity for the reconstruction of oocytes with different nuclear and cytoplasmic components before fertilization takes place. Reconstructed oocytes by metaphase chromosome transfer are then fertilized to produce embryos with desired mtDNA haplotypes. In GVT, replacement of compromised cytoplasm with healthy cytoplasm through germinal vesicle transfer before the start of chromosome segregation is carried out.

These approaches have not yet met with widely reported clinical success, yet there is room for optimism. As noted above, analysis of heteroplasmy and inheritance patterns indicates that even a small increase in copies of nonmutant mtDNA can exceed the threshold required to ameliorate serious clinical disease. All of the approaches described above show promise in achieving this goal and thus reducing the burden of clinical mtDNA disease in the future.