Electronic letter

Mitochondrial DNA inheritance in patients with deleted mtDNA

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EDITOR—Mitochondrial diseases encompass a large group of clinical disorders resulting from numerous genotypes, with variable age of onset and all possible modes of heredity, which have been extensively studied over the past decade.[1–3] So far, more than 50 point mutations and a significant number of complex rearrangements, including deletions and duplications, have been identified in mitochondrial DNA (mtDNA) of patients with oxidative phosphorylation diseases (MITOMAP[4]). In most cases, there is a cell to cell variable load of mutant to wild type (wt) mtDNA, a condition called heteroplasmy. The severity of the resulting mitochondrial defects depends not only on the nature of the mutation but also on the proportion of mutant to wild type mtDNA.

The origin and mechanism of the accumulation of mutant mtDNA (up to 99% in some tissues) in patients with mitochondrial diseases are still a matter of debate.[5] Different hypotheses have been proposed. First, the mutant mtDNA may already be present in the mother's oocytes. Then, by simple genetic drift, mutant mtDNA could finally represent a high proportion of the total mtDNA in a specific organ or tissue. It has already been shown that a small amount of founder mtDNA can populate the organism and that the number of segregating units (n) could be as low as a single mitochondrion.[6 7] This proposal of a genetic bottleneck has been supported by the observation of significant levels of rearranged mtDNA in the oocytes of a patient with Kearns-Sayre syndrome[8] and by studies of mtDNA heteroplasmy in mice.[7] Another explanation could be that the mutational event occurs later during embryogenesis. Accordingly, the accumulation of mutant molecules could reflect a proliferative advantage for the mutant mtDNA over the wild type during replication or segregation. This hypothesis comes from studies of mutant mtDNA segregation in vitro.[9–11]

A new alternative hypothesis is based on the particular pattern of inheritance of mtDNA. This is assumed to be strictly maternally inherited with no apparent paternal contribution to the mtDNA pool of the offspring. It is known that paternal mitochondria entering the egg are rapidly eliminated by active processes during the first stages of mammalian embryogenesis.[12 13] However, the presence of paternal mtDNA has been detected at the blastocyst stage in some abnormal human embryos.[14] Furthermore, in contrast to the orthodox view of clonal maternal inheritance, several recent articles have shown that recombination events can occur in human mtDNA, casting doubts on the strict maternal inheritance of mtDNA.[15–17] However, while attracting a lot of attention, these results remain contentious.[18–22]

Based on these observations, it could be hypothesised that the presence of mutant mtDNA in the zygote could result from a partial degradation of paternal mtDNA after fertilisation. To address this question, we have ascertained the parental origin of the deleted mtDNA (Δ-mtDNA) in a group of patients with Pearson syndrome, villous atrophy, and/or encephalomyopathy.[23–27] All patients had a proportion of Δ-mtDNA higher than 80% of the total mtDNA pool in the tissue studied, except for one case where the proportion of Δ-mtDNA was 30%. We first looked for the presence of Δ-mtDNA in the blood cells of the mothers by PCR amplification of the relevant fragments, using primers flanking the deletion. The Δ-mtDNA was absent in all tested samples. In the case of a paternal origin of the Δ-mtDNA, we expected to find a paternal haplotype in the patients. The mitochondrial control region (CR) haplotypes were therefore defined in six families where DNA samples from both parents of the affected child were available. Variation in the CR sequence was determined by direct sequencing of a total of 401 bp (from nucleotide position (np) 16 000 to np 16 400) encompassing the CR hypervariable segment I (HVS-I), as described previously.[28] Sequence variants were ascertained by alignment and comparison with the Cambridge Reference Sequence (CRS).

The mtDNA CR haplotypes in the six families analysed are reported in table 1 together with the proportion of Δ- *v* wt-mtDNA. Out of the six families analysed, two (families 2 and 4) turned out to be uninformative since all members shared the same mtDNA lineage. Among the four informative cases (families 1, 3, 5, and 6), all patients showed only the maternal mtDNA lineage. Thus, all mtDNA molecules present in the affected children were transmitted following strict maternal inheritance, ruling out the possibility of a paternal origin of the Δ-mtDNA molecules found in the offspring. Our results also show that mtDNA deletions occur on different mtDNA backgrounds, for example, the common deletion (4977 bp) was

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Table 1 HVS-I haplotypes in the six families analysed. Relevant information on % of deleted mtDNA, extent of the deleted region, and clinical presentation is also given

Family	Clinical presentation	Tissue	Deleted mtDNA	Deletion extent	HVS-I haplotype
P1	Pearson syndrome	Lymphocytes	90%	8484–13460	239G 256 311
M1					239G 256 311
F1					069 097G 104A 126 261
P2	Villous atrophy +	Muscle	90%	10665–14856	CRS
M2	encephalomyopathy				CRS
F2					CRS
P3	Villous atrophy +	Lymphocytes	30%	10744–14124	291
M3	encephalomyopathy				291
F3					224 270
P4	Progressive	Lymphocytes	90%	(Multiple deletions)	126 193 278
M4	encephalomyopathy			7845–15761, 7931–15761,	126 193 278
F4				7619–15032, 9844–16064, 9966–15801	298 311
P5	Pearson syndrome	Bone marrow	80%	5793–12767	069 126 145 222 261
M5					069 126 145 222 261
F5					069 126 145 222 261
P6	Pearson syndrome	Lymphocytes	85%	8484–13460	224 311 362
M6					224 311 362
F6					192 224 311

found in families 1 and 6 on two different mtDNA genetic backgrounds. Although sample size was rather small and deletion breakpoints heterogeneous, these observations suggest an absence of correlation between a particular mtDNA background and higher occurrence of mtDNA deletion.

Strict maternal transmission could be an advantage for preventing the spread of deleterious mitochondrial genomes from the spermatozoa.[29] Nevertheless, assisted reproductive techniques, such as intracytoplasmic sperm injection (ICSI), using immature spermatozoa during fertilisation, could increase the risk of a leakage of paternal mtDNA in the zygote.[30] Moreover, in two independent studies, no paternal mtDNA could be detected in the blood of children born after ICSI.[31 32] In contrast, paternal mtDNA was still detectable in polyploid embryos generated by standard in vitro fertilisation or ICSI.[14] Moreover, both recipient and donor female mtDNA was present in the first babies born after egg cytoplasmic donation.[33]

In conclusion, we report the absence of paternally transmitted mtDNA in children with mitochondrial deletions. While we only studied sporadic cases of mitochondrial disease, this should be extended to familial cases with more than one affected child harbouring multiple or single Δ-mtDNA. Characterising the origin of the mutant mtDNA molecules could help in understanding the genetic complexity of mitochondrial diseases and improve genetic counselling and prenatal diagnosis in families with mtDNA heteroplasmy.

1 Larsson NG, Clayton DA. Molecular genetic aspects of human mitochondrial disorders. *Annu Rev Genet* 1995;**29**:151-78.
2 Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999;**283**:1482-8.
3 Munnich A, Rötig A, Chretien D, Saudubray JM, Cormier V, Rustin P. Clinical presentations and laboratory investigations in respiratory chain deficiency. *Eur J Pediatr* 1996;**155**:262-74.
4 MITOMAP. A human mitochondrial genome database. Center for Molecular Medicine, Emory University, Atlanta, USA. http://www.gen.emory.edu/mitomap.html (2000).
5 Chinnery PF, Thorburn DR, Samuels DC, White SL, Dahl HM, Turnbull DM, Lightowlers RN, Howell N. The inheritance of mitochondrial DNA heteroplasmy: random drift, selection or both? *Trends Genet* 2000;**16**:500-5.
6 Jenuth JP, Peterson AC, Fu K, Shoubridge EA. Random genetic drift in the female germline explains the rapid segregation of mammalian mitochondrial DNA. *Nat Genet* 1996;**14**:146-51.
7 Shoubridge EA. Mitochondrial DNA segregation in the developing embryo. *Hum Reprod* 2000;**15**:229-34.
8 Marchington DR, Macaulay V, Hartshorne GM, Barlow D, Poulton J. Evidence from human oocytes for a genetic bottleneck in an mtDNA disease. *Am J Hum Genet* 1998;**63**:769-75.
9 Yoneda M, Chomyn A, Martinuzzi A, Hurko O, Attardi G. Marked replicative advantage of human mtDNA carrying a point mutation that causes the MELAS encephalomyopathy. *Proc Natl Acad Sci USA* 1992;**89**:11164-8.
10 Bourgeron T, Chretien D, Rotig A, Munnich A, Rustin P. Fate and expression of the deleted mitochondrial DNA differ between human heteroplasmic skin fibroblast and Epstein-Barr virus-transformed lymphocyte cultures. *J Biol Chem* 1993;**268**:19369-76.
11 Moraes CT. What regulates mitochondrial DNA copy number in animal cells? *Trends Genet* 2001;**17**:199-205.
12 Kaneda H, Hayashi JI, Takahama S, Taya C, Lindahl KF, Yonekawa H. Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. *Proc Natl Acad Sci USA* 1995;**92**:4542-6.
13 Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Ubiquitin tag for sperm mitochondria. *Nature* 1999;**402**:371-2.
14 St John J, Sakkas D, Dimitriadi K, Barnes A, Maclin V, Ramey J, Barratt C, De Jonge C. Failure of elimination of paternal mitochondrial DNA in abnormal embryos. *Lancet* 2000;**355**:200.
15 Ankel-Simons F, Cummins JM. Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. *Proc Natl Acad Sci USA* 1996;**93**:13859-63.
16 Awadalla P, Eyre-Walker A, Smith JM. Linkage disequilibrium and recombination in hominid mitochondrial DNA. *Science* 1999;**286**:2524-5.
17 Strauss E. Human genetics. mtDNA shows signs of paternal influence. *Science* 1999;**286**:2436.
18 Macaulay V, Richards M, Sykes B. Mitochondrial DNA recombination - no need to panic. *Proc R Soc Lond B Biol Sci* 1999;**266**:2037-9.
19 Parsons TJ, Irwin JA. Questioning evidence for recombination in human mitochondrial DNA. *Science* 2000;**288**:1931.
20 Kumar S, Hedrick P, Dowling T, Stoneking M. Questioning evidence for recombination in human mitochondrial DNA. *Science* 2000;**288**:1931.
21 Kivisild T, Villems R. Questioning evidence for recombination in human mitochondrial DNA. *Science* 2000;**288**:1931.
22 Jorde LB, Bamshad M. Questioning evidence for recombination in human mitochondrial DNA. *Science* 2000;**288**:1931.
23 Rotig A, Cormier V, Blanche S, Bonnefont JP, Ledeist F, Romero N, Schmitz J, Rustin P, Fischer A, Saudubray JM, Munnich A. Pearson's marrow-pancreas syndrome. A multisystem mitochondrial disorder in infancy. *J Clin Invest* 1990;**86**:1601-8.
24 Cormier-Daire V, Bonnefont JP, Rustin P, Maurage C, Ogler H, Schmitz J, Ricour C, Saudubray JM, Munnich A, Rotig A. Mitochondrial DNA rearrangements with onset as chronic diarrhea with villous atrophy. *J Pediatr* 1994;**124**:63-70.
25 Cormier V, Rotig A, Geny C, Cesaro P, Dufier JL, Munnich A. mtDNA heteroplasmy in Leber hereditary optic neuroretinopathy. *Am J Hum Genet* 1991;**48**:813-14.

26 Cormier V, Rotig A, Quartino AR, Forni GL, Cerone R, Maier M, Saudubray JM, Munnich A. Widespread multi-tissue deletions of the mitochondrial genome in the Pearson marrow-pancreas syndrome. *J Pediatr* 1990;**117**: 599-602.
27 Rotig A, Bourgeron T, Chretien D, Rustin P, Munnich A. Spectrum of mitochondrial DNA rearrangements in the Pearson marrow-pancreas syndrome. *Hum Mol Genet* 1995;**4**:1327-30.
28 Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC. African populations and the evolution of human mitochondrial DNA. *Science* 1991;**253**:1503-7.
29 Allen JF. Separate sexes and the mitochondrial theory of aging. *J Theor Biol* 1996;**180**:135-40.
30 Bourgeron T. Mitochondrial function and male infertility. *Results Probl Cell Differ* 2000;**28**:187-210.
31 Houshmand M, Holme E, Hanson C, Wennerholm UB, Hamberger L. Is paternal mitochondrial DNA transferred to the offspring following intracytoplasmic sperm injection? *J Assist Reprod Genet* 1997;**14**:223-7.
32 Danan C, Sternberg D, Van Steirteghem A, Cazeneuve C, Duquesnoy P, Besmond C, Goossens M, Lissens W, Amselem S. Evaluation of parental mitochondrial inheritance in neonates born after intracytoplasmic sperm injection. *Am J Hum Genet* 1999;**65**:463-73.
33 Barritt JA, Brenner CA, Willadsen S, Cohen J. Spontaneous and artificial changes in human ooplasmic mitochondria. *Hum Reprod* 2000;**15**:207-17.