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Transmission of mitochondrial DNA heteroplasmy in normal pedigrees

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Abstract The presence of multiple mitochondrial genotypes (heteroplasmy) has been studied in normal individuals. Six multigenerational normal families were screened for heteroplasmy by PCR of the mitochondrial control region and the cytochrome c oxidase intergenic regions. Two individuals from different families exhibited multiple length polymorphisms in a homopolymeric tract at positions 16 184–16 193 and a grandmother in a third family was heteroplasmic for both cytosine and thymidine at position 15 945. Although the 15 945 T variant comprised 28% of the grandmother's mitochondrial DNA, this sequence was not present in any of her descendants. Heteroplasmy was detected in 2.5% of the 96 mother-offspring pairs, consistent with the possibility that it may not be rare.

Introduction

Studies of human mitochondrial DNA (mtDNA) transmission in families with mitochondrial encephalomyopathies (Wallace 1992) have revealed that multiple mtDNA genotypes (heteroplasmy) may be present in the same individual (Blok et al. 1997; Ghosh et al. 1996; Goto et al. 1992; Howell et al. 1996; Larsson et al. 1990; Poulton et al. 1991). The level of heteroplasmy can vary among family members and among different tissues within the same individual. Unbiased mtDNA transmission cannot be studied in these kindreds, since the mutations themselves have the potential to alter the degree of heteroplasmy by affecting oxidative phosphorylation and, as a consequence, cell viability (Shoubridge et al. 1990). The proportions of the heteroplasmic mtDNA forms may also be altered by differences in the efficiency of DNA replication of the mutant and normal genomes (Yoneda et al. 1992).

The present study attempts to remove bias in germ-line mtDNA segregation by analyzing neutral mtDNA substitutions in kindreds unaffected by mitochondrial disease. Rare examples of multiple mtDNA types (heteroplasmy) within normal individuals have been identified (Comas et al. 1995; Gill et al. 1994; Jazin et al. 1996; Monnat et al. 1985; Monnat and Reay 1986; Mumm et al. 1997; Wilson et al. 1997) although length heterogeneity of two imperfect homopolymeric stretches has been noted repeatedly (Bendall and Sykes 1995; Hauswirth and Clayton 1985; Marchington et al. 1997). Since normal humans have been assumed to be homoplasmic (Laipis 1996), we and others (Gocke et al. 1994; Bendall et al. 1996; Parsons et al. 1997) have studied the incidence and segregation of mtDNA heteroplasmy in maternally related individuals.

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Materials and methods

Pedigrees

We have screened highly polymorphic segments of the mitochondrial genome in six multigenerational families to identify mtDNA heteroplasmy. DNA was extracted from lymphoblast cell lines of CEPH pedigrees 1340, 1329 and 1331 (Dausset et al. 1990; BIOS Laboratories, New Haven, Conn.) and leukocytes from a single two-generation family (T) and two four-generation families (L and H). Differences in maternal and offspring genotypes were examined in 6 maternal-offspring pairs in CEPH 1340, 35 pairs in CEPH 1329, 9 pairs in CEPH 1331, 22 pairs in family L, 19 pairs in family H, and 2 pairs in family T. The entire study group comprised 119 individuals from 25 maternal lineages, with 1 (unrelated male spouse) to 21 individuals in each lineage.

Amplification and detection of heteroplasmy

mtDNA was amplified with seven different sets of (^{32}P) end-labeled oligonucleotide primers that span the highly polymorphic mtDNA control region (D-loop) and intergenic regions adjacent to

Table 1 List of mtDNA oligonuclotide primers used (coordinates according to Anderson et al. 1981)

Primers	Coordinates
D 1	15882–15905
D 2	16280–16260
D 3	16271–16291
D 4	151– 129
D 6	668– 646
D 7	401– 421
D 9	16133–16111
D10	16034–16054
D11	16521–16502
D12	16459–16478
CO3	8108– 8127
CO4	8598– 8577
CO5	8379– 8399
CO6	8380– 8359

the cytochrome c oxidase genes, which together comprise 11% of the mitochondrial genome (Table 1; Horai and Hayasaka 1990; Vigilant et al. 1989). Total cellular DNA (10 ng) was amplified for 30 cycles in 10-μL PCR reactions at the calculated annealing temperature for these primers. Single-strand conformational polymorphism analysis, which has been used to detect mtDNA sequence variation among different individuals (Jaksch et al. 1995; Kim et al. 1995), was performed on 5% polyacrylamide gels containing 10% glycerol, run at 300–400 V for 6–22 h at either room temperature or 6° C. At least 5% of a heteroplasmic population of mtDNA was detectable in mixtures containing two different genotypes (Gocke et al. 1994; results not shown). Selected variant conformers were isolated and cloned into pGEM-T (Promega, Madison, Wis.). The DNA sequences of 4–8 clones were determined for each PCR product and at least four clones with the same variant sequence were identified. The proportion of the variant mtDNA species was determined using a previously published method (Tanno et al. 1991).

Results

The majority of individuals studied exhibited homoplasmy (97.5%). Members of CEPH 1331, L, H, or T families did not demonstrate heteroplasmy or discordant maternal-offspring genotypes. Several mother-offspring pairs demonstrated more than one mtDNA genotype, however. The analysis of a total of 96 maternal-offspring pairs (which included a comparison among three siblings whose mother was unavailable) identified three distinct examples of heteroplasmy, two of which were length polymorphisms. In one family, heteroplasmy resulted in discordant mtDNA genotypes between a mother and three of her offspring.

DNA sequence analysis revealed that one of the grandmothers in CEPH 1329 (UP-072) was heteroplasmic at position 15945 for C (the reference sequence) and T (Gocke et al. 1994; results not shown). Since the 15945 T variant destroyed a *Sty*I restriction site, the relative quantities of the reference and variant populations were compared (Tanno et al. 1991). The 15945 T variant was a prominent grandmaternal mtDNA type (28%), but was undetectable in three of her children and six grandchildren (Fig. 1). These discordant maternal-offspring genotypes in this family suggest that heteroplasmy may segregate to

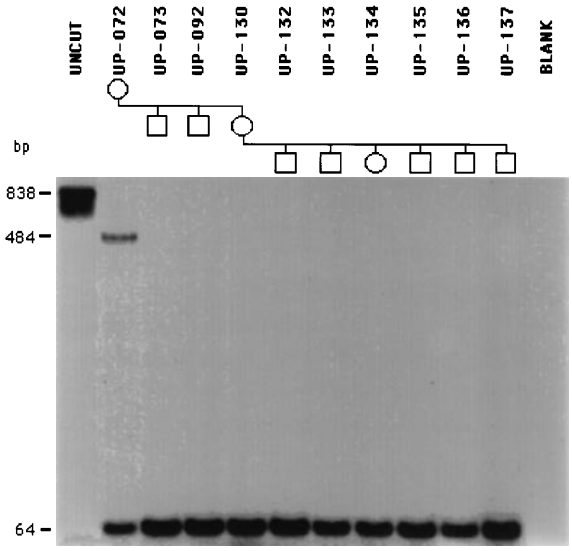

Fig. 1 PCR-RFLP analysis of a mtDNA single nucleotide variant found in CEPH 1329. Total lymphoblast DNA was amplified by PCR with primers D1–D4 for 30 cycles with (^{32}P)-labeled D1 added at the final cycle (Tanno et al. 1991). The products were digested with the restriction enzyme *Sty*I and subjected to electrophoresis on a 2% agarose gel that was dried and autoradiographed. The 15945 T variant in UP-072 destroys a restriction site at position 15945, releasing a labeled 484-bp fragment. The 484-bp band represents 28% of the background-corrected total intensity. This site is completely cleaved in UP-073, UP074 and UP-130, indicating that this variant is not detected in her offspring (< 5%)

homoplasmy in a single generation in the absence of any obvious replicative advantage for either mitochondrial variant. Segregation of a presumably neutral heteroplasmic allele in fewer than five generations has been reported (Ivanov et al. 1996).

The 15945 T variant noted in UP-072 lies within the coding region for tRNA^{Thr} at the base of the TψC loop stem. This base change disrupts a G-C base pair, but still permits non-Watson-Crick G-U pairing. The G-U pair is common in tRNA of lower eukaryotic mitochondrial tRNAs, and it is particularly frequent at the base of the stem of the TψC loop (Clark 1978). The 5-base stem adjacent to TψC of tRNA^{Thr} is present in other organisms with a G-U pair at the corresponding position and in bovine tRNA^{Thr}, this stem is only 4 bp in length (Sprinzl et al. 1989). This sequence variant does not significantly alter predictions of the cloverleaf form of the molecule based on an energy maximization algorithm (Zuker 1989). By contrast, a pathologic mutation in the TψC loop at the analogous position of tRNA^{Lys} (Silvestri et al. 1992) is predicted to markedly alter the secondary structure. It is unlikely that the 15945 T variant would be deleterious in this normal CEPH kindred.

Two males who married into family L and CEPH 1340 exhibited heteroplasmy in the D loop region (Fig. 2). UP-031 (Fig. 2C; CEPH 1340) displayed length variants in the imperfect homopolymeric C tract at 16184–16193 ranging from 11 to 13 bp (Fig. 2D; the reference sequence

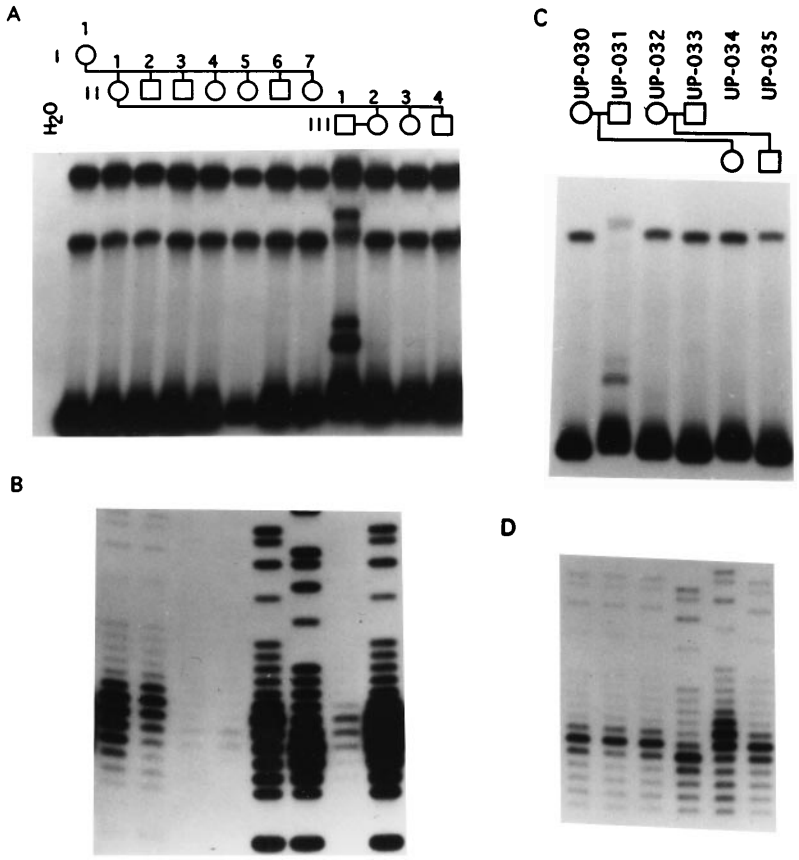

Fig. 2 A–D Length variants in the mtDNA control region in CEPH 1340 and pedigree L. SSCP analysis of individuals III-1 from pedigree L (**A**) and UP-31 from CEPH 1340 (**C**) reveals multiple conformers using the D2–D10 primer pair. DNA sequencing of independent cloned PCR products from these individuals demonstrates expansions of the imperfect homopolymer at coordinates 16 184–16 193 (based on the reference sequence of Anderson et al. 1981). Sequencing was performed with primer D10; only G lanes are shown. In individual III-1 (**B**), the length of the homopolymer ranges from 10 bp *(third lane from right)* to 13 bp *(left two lanes)*. In individual UP-031 (**D**), this sequence ranges from 11 bp *(third lane from right)* to 13 bp *(second lane from right)*. A strong polymerase pause at position 16 189 has prevented determining whether a T→C transition previously noted at this position (Bendall and Sykes 1995) is present

of Anderson et al. 1981 has an imperfect homopolymer 10 bp in length). The male spouse in family L (III-1; Fig. 2 A) exhibited multiple length variants in the same tract ranging from 10 to 13 bp (Fig. 2 B). Both individuals also exhibited loss of one adenine in the homopolymeric A tract at 16 180–16 183. These results independently confirm the previously reported length variant (Bendall and Sykes 1995), in which shorter polyadenosine tracts were also observed. There was no paternal leakage of mtDNA in the offspring of these two males or any other family members, as expected (Hutchinson et al. 1974).

Discussion

Differences in the efficiency of replication of the normal and mutant mtDNA genomes may result in significantly different proportions of mutant mtDNA among related individuals harboring the same mutation (Yoneda et al. 1992). A marked shift in genotype may also be explained by a "genetic bottleneck" due to transmission of only a few mtDNA species during meiosis (Ashley et al. 1989; Hauswirth and Laipis 1982; Howell et al. 1992). In studies of single human oocytes from in vitro fertilization procedures, we and others have noted such shifts in mtDNA (Gocke et al. 1994; Blok et al. 1997; Marchington et al. 1997). Twins and their first-degree relatives similarly can exhibit variable mtDNA inheritance patterns (Bendall et al. 1996). Although the mechanism of segregation in humans is unknown, yeast mutants defective in mitochondrial resolvase exhibit nonrandom mtDNA transmission due to aggregation of these molecules (Lockshon et al. 1995).

The marked genotype shift in the offspring of UP-072 can be simulated using the Wright-Fisher model of random segregation (in contrast to a Bayesian approach; Bendall et al. 1996). In this model, loss of a variant from a single heteroplasmic cell lineage is analogous to extinction of a monogenic trait in a population undergoing random genetic drift (Hartl and Clark 1989). Like Bendall et al. (1996), we assume that one genetic bottleneck occurred, that mtDNA replication after the bottleneck was faithful, and that somatic and germ-line levels of heteroplasmy were similar. To determine N_e, the effective population size or segregating unit of mtDNA molecules, we estimate that 500–1000 cell divisions have occurred since the variant was lost. This is based on the number of elapsed generations since the mature maternal leukocytes and oocytes developed from the conceptus (assuming an average mammalian mitotic cycle of 30 days (Young and Hay 1995). Applying the Wright-Fisher model (Kimura and Ohta 1969), N_e ranges from 252 to 505 in each of these three individuals. This value is consistent with data from the mouse model (Jenuth et al. 1976; $76 < N_e < 867$), but exceeds other estimates for humans (Bendall et al. 1996; Parsons et al. 1997). If fewer than 500 cell divisions take place prior to fixation of the mtDNA genotype, however, N_e is less than 252. Therefore, the marked shift in mtDNA genotype seen in CEPH 1329 either occurred

Table 2 Putative neutral, heteroplasmic mtDNA variants in humans without evidence of mitochondrial disease

Reference	Heteroplasmic site	Number of individuals	Comment
Wilson et al. 1997	16355 C + T	3	Percentage varied among tissues and individuals
Comas et al. 1995	16293 A + G, 16311 T + C	1	
Gill et al. 1994; Ivanov et al. 1996	19169 T + C	2	
Bendall et al. 1996	16262 C + T and 16293 A + G (twin pair 1); 16192 C + T (twin pair 2); 16222 C + T (twin pair 3); 16239 C + T (twin pair 4); 16189 T + C; 16192 C + T; 16256 C + T; 16311 T + C	1 (a twin from pair 1 was homoplasmic); 2 (twin pair 2); 11 (a twin from pair 3 was homoplasmic); 10 (a son of a twin from pair 4 was homoplasmic); 1; 1; 1	16184–16193 also heteroplasmic in twin pair 2; total of 473 unrelated individuals screened for heteroplasmy
Parsons et al. 1997	16092 C + T; 16256 T + C; 309.1 C	2; 1; 2	327 "generational events" screened
Jazin et al. 1996	37 positions, multiple substitutions	3	Age-related somatic substitutions
Marchington et al. 1997	303–315	2 (multiple tissues), 5 (multiple oocytes)	Length polymorphism; suggests 16184–16193 length polymorphism is regenerated in each individual
Bendall and Sykes 1995	16184–16193	~300	Length polymorphism
Hauswirth and Clayton 1985	303–315	1	Cell line
Mumm et al. 1997	295 A + G	18	
Monnat et al. 1985	9731–9732 C(del), 9727–9732 CT(del)	1	Leukemia cells
Monnat and Reay 1986	94 G + A	1	Liver
Current study	16184–16193;	2; 1	Length polymorphism
	15945 C + T		96 mother-offspring pairs screened

nonrandomly (the result of selective amplification of certain mtDNA variants) or was the result of segregation of a limited maternal subpopulation of mtDNA. mtDNA studies in oocytes (Blok et al. 1997) indicate that the latter explanation is more likely.

Based on the results of this and previous studies, heteroplasmy in normal individuals may not be rare (Table 2). Our finding of 2.5% heteroplasmy is probably conservative, since only a portion of the mitochondrial genome was surveyed (11%) and the SSCP technique may not reveal all of the nucleotide mismatches that may be present. The frequency of heteroplasmy and segregation to homoplasmy was 3.1% in another set of normal pedigrees (Parsons et al. 1997).

Undetected mtDNA genotype shifts and heteroplasmy may conceivably impact the interpretation of mtDNA genotypes in population studies, in forensic analyses, and in counseling for mitochondrial disorders. Complete shifts in genotype would affect determination of genetic relatedness at the individual or population level. We and others (Parsons et al. 1997) suggest that a single mtDNA sequence difference should not be used to exclude relatedness between individuals.

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