Duplications of Mitochondrial DNA: Implications for Pathogenesis

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Summary: This paper describes the mapping data obtained on two patients in whom there was clear evidence for a rearrangement of mitochondrial DNA, using restriction enzyme analysis of DNA from whole blood and of polymerase chain reaction products. This suggested that a direct tandem duplication was present, and this was confirmed by sequence analysis of the junction fragment between duplicated segments. In each case the gene for cytochrome oxidase subunit I (MTCOX1) was interrupted, creating reading frames which, if transcribed and translated, would result in truncated versions of this peptide.

Heteroplasmy and mosaicism for the abnormal mtDNA population were apparent. Preliminary data also suggest that high-molecular-weight rearrangements of the duplicated region are present in all tissues.

The hypothesis that these duplicated genomes caused the phenotype was investigated by examining the distribution of duplicated genomes in various tissues using Southern hybridization and by RNA analysis. This included Northern blotting and cDNA sequencing.

In order to investigate the origins of the duplicated mtDNAs, their distribution in different cells within a tissue was documented using the polymerase chain reaction.

Both major rearrangements and point mutations in mtDNA have recently been associated with human disease (reviewed by Wallace 1989). The commonest rearrangements are large deletions which occur in sporadic cases of Kearns–Sayre syndrome. Duplications are not common population polymorphisms in man, and have been associated with an abnormal phenotype in plants (Young and Hanson 1987). In this paper, two patients with direct tandem duplications of mitochondrial DNA (mtDNA) and mitochondrial myopathy are described (see Poulton et al 1989a,b). The implications for pathogenesis and relationship with other rearrangements will then be discussed.

Patients
Patient 1 presented with diabetes mellitus and short stature at the age of 7 years. Classical features of Kearns–Sayre syndrome developed, with ptosis, external ophthalmoplegia, retinopathy, ataxia, proximal muscle weakness and deafness. Muscle biopsy showed classical ragged red fibres. She later developed a cardiac conduction
defect and hypokalaemia, probably because of a renal tubular defect. She died suddenly, probably as a result of this combination.

In patient 2, mitochondrial myopathy was diagnosed at the age of 12 years when she had developed progressive ataxia, dysarthria, muscle weakness, external ophthalmoplegia, ptosis and mental retardation. Muscle biopsy early in her illness did not reveal any specific histological abnormality. She subsequently developed a transient hemiparesis following a stroke-like episode.

Both patients are similar to two sibs described by Rotig and co-workers (1991b). There was a duplication of mtDNA in this family, detectable by Southern hybridization in two sibs, and by polymerase chain reaction (PCR: Saiki et al 1985) in the mother. The two children had diabetes, skin abnormalities, a renal tubular defect, mitochondrial myopathy with ragged red fibres, and cerebellar ataxia. Their mother had ptosis, external ophthalmoplegia, and muscle weakness.

**RESTRICTION ENZYMES ANALYSIS**

Figures 1 and 2 illustrate the results of restriction enzyme analysis on these patients, and demonstrate that duplications can be mistaken for deletions if only one digest is performed. Total cellular DNA was extracted from whole blood from the patients and from unaffected relatives. The DNA was electrophoresed, blotted and probed

![Figure 1](image)

**Figure 1** Restriction enzyme analysis and pedigree of two patients with mitochondrial myopathy and their families. Total cellular DNA was extracted from whole blood from the patients, and from unaffected relatives. The DNA was digested with *Bam* H1, electrophoresed, blotted and probed with purified whole human placental mtDNA. This linearizes the genome, and hybridization with total mtDNA reveals a single 16.5 kb band. An extra band 8 kb larger than the normal band was present in patients 1 and 2, but not in unaffected family members, suggesting that either an insertion or a duplication was present. ♂, affected male; ♀, unaffected female; □, unaffected male (no DNA available from father in family 2)