In conclusion, the usefulness of current PCR based methods of detecting the junction fragment in mtDNA duplications is limited in our experience.

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Pitfalls in the diagnosis of mtDNA mutations

At present, more than 50 mitochondrial (mt) point mutations, mostly involving mt transfer RNAs (tRNA), have been associated with human disease. Many of these are inherited in affected families with a novel mtDNA mutation, consisting of a single nucleotide deletion in tRNA threonine (tRNA\(^{\text{Thr}}\)). Both peripheral blood and fibroblasts were used as a source of DNA.

Family A. The first child, a girl, of non-consanguineous, Turkish parents, is in good health. The second child, a boy, was born after an uneventful pregnancy, but within the first six hours of life he developed severe neurological distress associated with hyperammonemia. He died on the fifth day of life. Post-mortem examination of heart and liver revealed a truncus arteriosus type I. He was severely hypotonic. No deletion of 22q11 was present and metabolic screen was negative. He died at the age of 3 months of a respiratory infection. A myocardi- um and liver biopsy suggested a progressive mitochondrial disease with some mitochondrial alterations. A fourth child, a boy, was admitted to hospital at the age of 3 years for severe cranial trauma. He presented with cere- bral oedema, but had been previously com- pletely asymptomatic. There was no lactic acidosis. Echocardiography showed severe hypertrophic cardiomyopathy resulting in poor circulatory perfusion. The child died of heart failure. A muscle biopsy was normal on light and electron microscopy. A myocardiobioscopy showed swollen and vacuolated mitochondria. The parents refused necropsy on any of these children.

Family B. The proband is a 38 year old male of Italian descent. At the age of 28 he complained of painful cramps and a burning sensation in the feet followed by tingling and numbness in a glove and stocking distribution. Sensory symp- toms spread proximally to involve all four extremities and the trunk. At the age of 32, the patient developed frequent urinary tract infections and impotence owing to absence of penile erection resulting in the necessity for self-catherisation. Muscle strength was normal, except for absent ankle jerks. Sensation was diminished for all modalities, distally more than proximally. Serum creatine kinase levels were 190 IU/l (normal <160). ECG, heart ultrasound, ophthalmological examination including electroretinogram, and serum lactate levels were normal. EMG and nerv conduction studies showed demyelinating sensory neural neuropathy (or neuropathy). Hand and foot sympathetic skin response and the brain and spinal cord MRI studies were normal. Nerve sural biopsy showed marked reduction of myelinated and unmyelinated fibre density. In a triceps muscle biopsy there were subsarcolemmal mitochondrial aggregates, strongly NADH-TR and SDH reactive. Many muscle fibres exhibited patchy loss of cytochrome c oxidase staining. Ultrastruc- turally, aggregates of mitochondria with “parking lot” type I paracrystalline inclusions were abundant. In the patient's mother, severe retinopathy, chronic renal failure, and a predominantly axonal sensorimotor peripheral neuropathy were attributed to diabetes. Interestingly, there was an almost 10 year history of urinary retention owing to detrusor paralysis, which had led to bilateral hydropnephrosis, and which needed regular self-catherisation. A 43 year old brother of the patient presented with urinary tract disease and had urinary retention owing to detrusor paralysis, which was treated by suprapubic catherisation. Neurological examination was normal, except for absent deep tendon reflexes. Electrooculography showed absence of sensory nerve action potentials and sympathetic skin responses. Sural nerve biopsy confirmed the presence of a severe sensory axonal neuropathy or neuropathy. A triceps muscle biopsy showed the presence of subsarcolemmal mitochondrial aggregates and partial cytochrome c oxidase deficiency, and type I paracristalline inclusions in mitochondria. Both brothers suffer from unexplained orthostatic hypotension.

Routine molecular analysis in both families (family A, mother and 2 younger brothers; family B, patient, his mother, and two asymptomatic sibs) had already ruled out the presence of deletions or duplications and common point mutations of MERRF\(^{\text{**}}\), MELAS\(^{\text{**}}\), and NARP\(^{\text{**}}\) mutations. We then investigated the 22 mt tRNA genes and the flanking genomic regions for single base pair substitutions by single strand conformation polymorp- hism analysis (SSCP). An abnormal mobility pattern for the fragment containing the tRNA threonine and proline genes was seen for all investigated members of both families. Nucleotide sequencing of the relevant region revealed a homoplasmic deletion of a T nucleotide in a 5T stretch (including bp 15940 to bp 15944) in the tRNA threonine gene. Apart from the thymine deletion, no other significant differences were observed on the SSCP gel analysis. No albino gene mutations of the index cases in both families. The thymine adenine nucleotide deletion was also absent in more than 70 controls.

This deletion mutation does not fulfil the classical criteria commonly used to define patholog- ical base pair changes in the mt genome. The mutation is homoplasmic and heteroplasmy is a very common feature of pathological mtDNA mutations. The T:A base pair deletion is present in the tRNA\(^{\text{Thr}}\) gene of both patients and asymptomatic relatives in both families. It was detected by PCR-SSCP analysis and by direct sequenc- ing. Despite these observations, the nature of the mutation, namely a base pair deletion in a tRNA gene, might suggest a link with disease.

The secondary structural model of the wild type human mt tRNA molecules is not well known, but the 5T stretch is probably partially located in the tRFC stem of the tRNA\(^{\text{Thr}}\) clover leaf, while the fifth T forms part of the loop.1 Computer modelling and stability calculations of the mutant tRNA\(^{\text{Thr}}\) tRF loop, according to the Zuker algorithm, predict a A:G (tRF loop) destabilisation with 1.1 Joule/mol. Pathological molecular altera- tions in tRNA genes are most often single base pair substitutions, but mini rearrange- ments are not surprising in mtDNA associat- ed diseases. A single nucleotide insertion in the transfer RNA genes for serine and tryptophan has been described. However, to our knowledge, only one patient with a heteroplasmic single nucleotide deletion in a tRNA gene has been reported previously.2 Pathological base pair "DNA" deletion mutation is unclear. Several possi- bilities can be considered. The homoplasmic deletion might represent a mutation with variable penetrance, so the deletion is not disease causing per se. This particular point of view is supported by the finding of an environmental factor in modifying the expression of the mtDNA mutation. This has already been proposed for the mutations causing Leber's hereditary optic neuropathy (LHON). More than 50% of the maternal relatives (often females) of symptomatic LHON patients do not suffer from blindness (or other neurological problems), even with homoplasmic mutation levels in their blood mtDNA.
Severe primary pulmonary hypoplasia (“acinar dysplasia”) in sibs: a genetically determined mesodermal defect?

We report on two sisters who died neonatally from severe pulmonary hypoplasia without obvious cause. It was not associated with any other pathological condition, except mild growth retardation. Histological examination of the lungs showed greatly reduced alveolar parenchyma, with almost complete absence of mature alveoli. The amount of interstitial connective tissue was increased and the bronchial cartilage plates appeared dysplastic. The form of severe pulmonary hypoplasia is very unusual; so far only two similar cases have been reported as pulmonary “acinar dysplasia”. We present the first familial occurrence of this condition and suggest autosomal recessive inheritance.

Case 1 was the first child of a young, healthy, and non-consanguineous couple. She was born at 40 weeks after an uneventful pregnancy. Clinically, there was no oligo- or polyhydramnios and the mother experienced normal fetal movements. No maternal medication or exposure to potentially teratogenic agents were recorded. Immediately after birth, the infant developed severe respiratory distress. Chest x-rays and CT scans showed marked pulmonary hypoplasia, complicated by bilateral pneumothoraces. No other radiological abnormalities could be documented and the skeletal structures were normal. Despite intensive treatment, she died on the second day.

Clinical examination showed no external abnormalities. Body length (49.5 cm) and foot length (8.0 cm) were normal, but body weight (2860 g) and head circumference (32.5 cm) were between the 10th and 25th centile. The karyotype was normal.

The only gross abnormality found at necropsy was pulmonary hypoplasia (fig 1), with a combined lung weight of 20.2 g (normal 56±15) and a lung weight/body weight ratio of 0.007 (normal >0.012). The lungs were normally lobed, but the lobular markings were accentuated owing to thickened interlobular septa. Histology of the lungs showed extreme hypoplasia beyond the bronchial level with almost complete absence of mature alveoli and disproportionately numerous bronchi and bronchioles (fig 2). The bronchial branching pattern was normal. The peripheral lung parenchyma consisted merely of bronchioles and few immature canalicular structures, separated by an increased amount of interstitial connective tissue. The loosely textured and cellular interstitial tissue contained numerous, small, thin-walled, and dilated blood vessels and foci of extramedullary haematopoiesis. The bronchial cartilage plates appeared unduly large and numerous and displayed a persistent immature cellular layer with a mesenchymal Trehlar-Mair and chondrogenic lamellar cartilaginous structure. Peripheral neurones were normal. The brain and spinal cord were normal both grossly and microscopically. Skeletal muscle biopsies showed no abnormalities.

Case 2 was born at 40 weeks as the product of the mother’s third pregnancy. A normal amount of amniotic fluid was documented clinically and confirmed by ultrasound. Here.

Figure 1 Thoracic organs removed as a block. Severe pulmonary hypoplasia is illustrated by the fact that the inferior surfaces of the lungs are not in line with the apex of the heart.

Figure 2 The peripheral lung tissue contains virtually no alveoli, but consists merely of bronchioles. The bronchial cartilage plates are unusually prominent. Note the immature and cellular (“dysplastic”) aspect of the cartilaginous tissue (H&E).