In conclusion, the usefulness of current PCR based methods of detecting the junction fragment in CMT1A 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. Here we report on two unrelated families with a novel mtDNA mutation, consisting of a single nucleotide deletion in tRNA threonine ($tRNA^{thr}$). 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 hyperammonaemia. He died on the fourth day of life. Urea cycle enzymes were determined in a liver biopsy. The results were normal. The third child, a boy born after an uneventful pregnancy, was dysmorphic with abnormal ears, a small chin, and narrow mouth. He had cyanosis in the first days of life and was found to have a truncus arteriosus type 1. 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 myocardium and liver biopsy were suggestive of 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 cerebral oedema, but had been previously completely 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 myocardial biopsy showed some abnormally large mitochondria. The parents refused necropsy on any of these children.

Family B. The proband is a 38 year old white male of Italian descent. At the age of 28 he complained of painful cramps and a burning sensation in the fingers and toes followed by tingling and numbness in a glove and stocking distribution. Sensory symptoms spread proximally to involve all four extremities and the trunk. At the age of 32, the patient developed dysuria with frequent urinary tract infections and impotence owing to absence of penile erection resulting in the necessity for self-catheterisation. 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 nerve conduction studies showed a pronounced sensory axonal neuropathy (or neuronopathy). Hand and foot sympathetic skin response and the brain and spinal cord MRI studies were normal. Sural nerve 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. Ultrastructurally, aggregates of mitochondria with "parking lot" type I paracristalline 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 hydronephrosis, and which needed regular self-catheterisation. A 43 year old brother of the patient suffers from Crohn's disease and had urinary retention owing to detrusor paralysis, which was treated by suprapubic catheterisation. Neurological examination was normal, except for absent deep tendon reflexes. Electrodiagnostic studies showed absence of sensory nerve action potentials and sympathetic skin responses. Sural nerve biopsy confirmed the presence of a severe sensory axonal neuropathy or neuronopathy. A triceps muscle biopsy showed the presence of subsarcolemmal mitochondrial aggregates, 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 youngest sons; family B, the patient, his brother, and two asymptomatic sibs) had already ruled out the presence of deletions or duplications and common point mutations such as $MERRF^{8344}$, $MELAS^{3243}$, and $NARP^{8993}$ mutations. We then investigated the 22 mt tRNA genes and their flanking sequences for mutations by single strand conformation polymorphism 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 (involving bp 15940 to bp 15944) in the tRNA threonine gene. Apart from the thymine deletion, no other significant differences were observed on the SSCP gels analysing all other tRNA genes of the index cases in both families. The thymine:adenine nucleotide pair deletion was also absent in more than 70 controls.

This deletion mutation does not fulfil the classical criteria commonly used to define pathological 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 $tRNA^{thr}$ gene of both patients and asymptomatic relatives in both families. This is seen both by PCR-SSCP analysis and by direct sequencing. 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 structure of the wild type human mt tRNA molecules is not well known, but the 5T stretch is probably partially located in the TΨC stem of the $tRNA^{thr}$ clover leaf, while the fifth T forms part of the loop.[1] Computer modelling and stability calculations of the mutant $tRNA^{thr}$ TΨC loop, according to the Zuker algorithm, predict a ΔG(TΨC loop) destabilisation with 1.1 Joule/mol. Pathological molecular alterations in tRNA genes are most often single base pair substitutions, but mini rearrangements are not surprising in mtDNA associated diseases. A single nucleotide insertion in the transfer RNA genes for serine[2] and tryptophan[3] has been described. However, to our knowledge, only one patient with a heteroplasmic single nucleotide deletion in a tRNA gene has been reported previously.[4]

The pathological role of the $tRNA^{thr}$ deletion mutation is unclear. Several possibilities 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 suggests a role for a nuclear gene(s) or 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.

Secondly, other, as yet unidentified, mutations in the mt genome of the patients could have a modulatory effect on the phenotypic expression of the thymine deletion. PCR-SSCP analysis of all other tRNA genes did not show additional mtDNA variations, but the complete sequence of the mt genome was not determined. Therefore, the presence of an additional heteroplasmic mtDNA mutation cannot be excluded.

Thirdly, the mt genome is known to be highly polymorphic. Therefore, the deletion could be classified as a very uncommon genetic polymorphism, and its presence in the two families suffering from mt disorders is no more than a striking coincidence.

We screened, along with the two families, more than 70 people (both patients and controls) for mutations in the same DNA fragment. Five different nucleotide substitutions, at positions 15904 C to T, 15907 A to G, 15924 A to G, 15928 G to A in $tRNA^{thr}$ (all previously reported), and 16017 T to C in $tRNA^{pro}$, were identified.[5 6] All sequence variations were homoplasmic and observed both in patients and control mtDNA. The $tRNA^{thr}$ gene also harbours several nucleotide alterations responsible for mt encephalomyopathies: a G to A transition at nucleotide 15915, an A to G point mutation at nucleotide 15923, and a G to A mutation at bp 15927.[5 7] This particular DNA fragment seems to represent a "hot spot" for DNA variations in the mt genome.

This report illustrates the difficulties in diagnosing mt disorders. More pedigrees with this deletion mutation will need to be characterised to understand the real significance of this DNA aberration. In addition, micro-rearrangements involving tRNA genes should be considered as possible causes for oxidative phosphorylation diseases, even if they are homoplasmic in both patients and unaffected relatives.

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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. This 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.

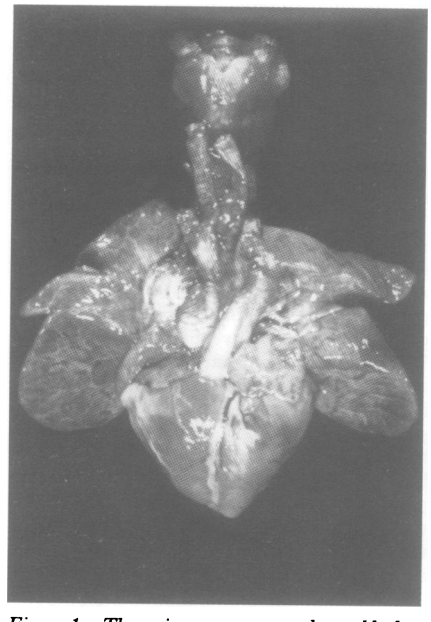

Figure 1 Thoracic organs removed as a block. Severe pulmonary hypoplasia is illustrated by the fact that the inferior surfaces of the lungs are not in line with the apex of the heart.

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 bronchiolar level with almost complete absence of mature alveoli and disproportionately numerous bronchi and bronchioli (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 aspect with a pale matrix. The diaphragm and phrenic nerves 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. Her

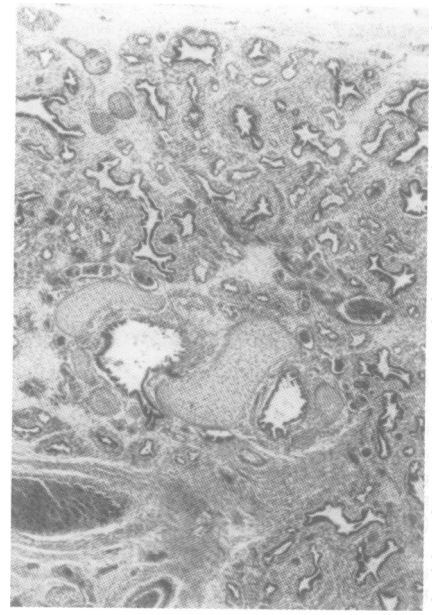

Figure 2 The peripheral lung tissue contains virtually no alveoli, but consists merely of bronchioles. The bronchial cartilage plates are unusually prominent. Note the immature and cellular ("dysplastic") aspect of the cartilaginous tissue (H&E).