Large departure (7-2 kb) of mitochondrial DNA with novel boundaries in a case of progressive external ophthalmoplegia

Chronic progressive external ophthalmoplegia (CPEO) is a well-characterised form of mitochondrial myopathy, it occurs with single or multiple deletions of mitochondrial DNA (mtDNA), or with the 3243 point mutation. Single deletions occur sporadically and are usually not transmitted to offspring, whereas point mutations are transmitted maternally and multiple deletions are inherited in an autosomal way. Single deletions are often flanked by direct repeats of three to 18 base pairs (bp). The "common deletion" was found in one of 10 patients with CPEO in 4-9 kb long and occurs between direct tandem repeats at positions 8470-8482 and 13 447-13 459. A partial duplication of mtDNA can be associated with the deletion and was reported to be specific for Kearns-Sayre syndrome. We describe a young woman with a sporadic CPEO and a large deletion (7-2 kb) of mitochondrial DNA with novel boundaries, flanked by a 14 bp imperfect tandem repeat at positions 8407-8420 and 15 658-15 671. A 28 year old woman was referred to us for investigation of CPEO. At the age of 12 she developed a progressive ptosis of the left eyelid. A contralateral ptosis appeared two years later, with a fluctuating vertical diplopia. Since then, progressive ophthalmoplegia was noticed. Subjectively there was no limb weakness; she did not have nystagmus, or cardiac arrhythmia. Her family history was negative for neuromuscular disorders, diabetes, and hearing impairment. Relatives were not examined.

Neurological examination confirmed bilateral ptosis, and severe limitation of eye movements in all directions during voluntary and reflex movements. Visual acuity was 6/15 in the right eye and 6/10 in the left; fundus was normal. Hearing was not impaired. There was a mild facial paresis affecting predominantly the orbicular palpebral muscles, and moderate paresis of the trapezius and sternocleidomastoid muscles. Muscle strength was slightly reduced proximally and distally in the four limbs. Tendon jerks and detailed sensory testing were normal.

Electromyography showed a full recruitment on submaximal effort with polyphasic potentials in the examined muscles (right arm and right leg) suggesting a myopathy, whereas nerve conduction studies were normal within the normal range. An ECG was normal. Lumbar puncture was not performed. Brain CT was normal. There was a moderate elevation of creatine kinase and lactate dehydrogenase concentrations. Diabetes mellitus was not present.

Quadriiceps muscle biopsy showed an increased variability in the size of fibres; few fibres showed subsarcolemmal aggregates of mitochondria, without a typical ragged red pattern on trichrome-Gomori staining. Few fibres were cytochrome oxidase negative. Duplication was ruled out by digestion of crystalline mitochondrial inclusions. Enzymatic activity of the respiratory chain complexes (I to IV) was within the normal range. When compared with citrate synthase, complex III activity was very low (5% of citrate synthase activity, normal range, 10-53%, n = 25).

Total DNA was extracted from muscle and blood by standard techniques. Southern blot analysis of total muscle DNA digested with PvuII and hybridised with a polymerease chain reaction (PCR) generated trRNALeu (UUR) probe (3130-3558) disclosed an additional band of approximately 9 kb (length, figure, A), suggesting the presence of a large deletion of mitochondrial DNA. The mean (SD) proportion of mutant versus total amount of mtDNA evaluated by scanning densitometry was 51 (4%) (n = 3). The deletion was present in 51% of mitochondrial DNA molecules in deltoid muscle but it was absent from leucocyte mitochondrial DNA. The presence of an associated deletion in a small proportion of total DNA with BamHI, which cuts within the deletion. Hybridisation with the tRNALeu(UUR) probe (figure, A) showed two slower migrating bands, whereas a probe (11 713-13 932) not flanking the deletion only hybridised to the 16-kb band (figure, B). (Double digestion with SnaBI and BglII gave similar results, data not shown, figure, C). Therefore, the deleted mtDNA could not contain the full length wild type sequence, and cannot be duplications, but most likely are a circular deletion monomer (CDM) and circular deletion dimer (CDD). Amplification by PCR with primers flanking the "common deletion" yielded a 5 kb fragment in leucocyte DNA amplified from wild type DNA. Muscle DNA amplification yielded an additional 1 kb fragment (not shown). This fragment was cloned in a pT7 blue vector (NovagenTM); The DNA sequence was determined in an automated sequencer (ALF™) to map precisely the deletion. The deletion was 72 kb and it was flanked by a 14 bp imperfect tandem repeat at positions 8407-8420 and 15 658-15 671. (Figure, C). Surprisingly, sequencing at primer H2 did not prime where expected (13 506-13 255) but hybridised instead at position 16 248-16 255 where there is a perfect homology over eight nucleotides at the 3' end.

The phenotype of the patient suggested a mitochondrial myopathy. Analysis of DNA showed a large deletion of mitochondrial DNA with novel boundaries. The deletions are described with similar phenotypes and not with healthy controls, strongly sug-
gesting that the deletion is responsible for the myopathy. There was no associated duplication, in accordance with Poulton et al,1 who showed that duplications are characteristic of Kearns-Sayre syndrome. The deletion was flanked by a 14 bp imperfect tandem repeat at positions 8407-8420 and 15 658-15 671. It encompassed nine genes encoding subunits of the complexes I, III, IV, and V of the respiratory chain, as well as six tRNA genes. So far no deletion has been mapped with these boundaries.5 There is a correlation between the percentage of deleted mtDNA and the severity of the myopathy: a recent study6 showed 31 (26)% of mutant DNA in unaffected muscles and up to 95% of deleted muscles in affected muscles. With 51% of mutant versus total mtDNA in a very mildly affected muscle our results are in accordance with the above survey. The assay for enzymatic activity of the respiratory chain was normal showing that a large deletion, with a proportion of 51% of mutant mtDNA in the biopsied muscle is compatible with normal respiratory chain activity.

Histologically signs of a mild myopathy were apparent, with few cytochrome oxidase negative fibres. There were no ragged red fibres, consistent with the fact that these may be absent in established mitochondrial encephalomyopathies.1

The mitochondrial DNA deletion was not present in leukocytes, showing the need for a muscle biopsy to prove the genetic defect.

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5 Human mitochondrial Genome database. The Human Genome Database Project, Department of Genetics and Molecular Medicine, Emory University, Atlanta, GA, USA. World Wide Web (http://www.gen.emory.edu/mitomap. html), 1995.

Pure word deafness after resection of a tectal plate glioma with preservation of wave V of brain stem auditory evoked potentials

Pure word deafness from brainstem lesions is uncommon because of the bilateral representation of the auditory system within the brainstem.2 The unique involvement of the inferior colliculi in cases of word deafness in which other anatomically close structures of the auditory system had been spared has only been reported twice.

It is generally accepted that the inferior colliculus is the generator of wave V of brainstem auditory evoked potentials (BAEPs) and that its bilateral destruction will inevitably lead to abnormalities in their recording.3 Bilateral destruction of the inferior colliculi with preserved wave V of BAEP in humans has not been described to our knowledge.

Here we report a patient in whom a circumscriptive lesion of both inferior colliculi has led to the isolated neurological deficit of pure word deafness with repeatedly documented preservation of wave V of BAEPs.

This 36 year old Turkish patient developed progressive signs of raised intracranial pressure at the age of 28, was diagnosed as having obstructive hydrocephalus, and was shunted. On CT one year before admission a pineal region mass was described for the first time. This showed pronounced enhancement on MRI and occupied the dorsal midbrain with exophytic growth. His neurological examination was normal apart from a reduced visual acuity. Speech audiogram, pure tone audiogram, and BAEPs were normal.

The lesion was attacked via a infratentorial-supracerebellar approach. The inferior colliculi could not be identified during surgery. Complete removal was documented on postoperative MRI as well as the destruction of both inferior colliculi (figure). Histology disclosed a pilocytic astrocytoma grade I.
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