Clinical similarities and differences of four children with single mitochondrial DNA deletions

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Abstract

Background. Single deletion of mitochondrial DNA (del mtDNA) is a sporadic progressive disorder associated with clusters of symptoms known as Kearns-Sayre syndrome (KSS), chronic progressive ophthalmoplegia (CPEO), isolated myopathy and Pearson marrow-pancreas syndrome. The set of symptoms and signs differs remarkably from patient to patient.

Material and Methods. The aim of the paper is to provide a description of the natural history of the disease in four children with two “common” and two novel mtDNA deletions. Diagnosis was established on the biochemical (plasma lactate concentration), histochemical (muscle biopsy), enzymatic and molecular level.

Results. In early childhood all patients similarly show remarkable growth failure and fatigability. Plasma and cerebrospinal lactate concentrations were always elevated. Differences were seen in the range of multi-organ involvement and the age of its onset. Hypoparathyroidism, diabetes mellitus and suprarenal insufficiency developed earliest at the age of 7 and had a “strange” course. Ptosis, retinitis pigmentosa, deafness, and heart conductive changes developed frequently from late childhood to adolescence. Pearson-like diarrhea was seen transiently only once. Anemia with increased mean corpuscular hemoglobin concentration was found twice. Kidney leakage of magnesium (evoking hypoparathyroidism) and sodium (imitating hypocorticism), and urinary phosphate or potassium loss were found periodically in all children. Delay in the diagnosis ranged from 2 to 14 years.

Conclusion. In our experience, lactate should be measured more commonly to improve early detection of mitochondriopathies. Atypical or “strange” course of progressive chronic disorder associated with hyperlactatemia should especially alert not only neurologists, cardiologists, hematologists but also pediatric endocrinologists and nephrologists.

Key words: mtDNA deletion, children, multi-organ disease, lactic acidemia

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Introduction

Single large-scale mitochondrial deletions present a small subgroup of pathogenic mutations of mtDNA, which cause respiratory chain defects [9, 11, 17, 24, 30]. The disorder occurs sporadically, de novo and leads to a dysfunction of various tissues and organs, mainly these with the highest aerobic requirements, such as the nervous system, skeletal muscles, heart, kidneys and others. Initially mtDNA deletions were described in adults with clearly defined syndromes such as Kearns-Sayre syndrome, chronic progressive ophthalmoplegia and ocular or isolated myopathy [16, 18]. In children they most often cause extremely heterogeneous multigener diseases [14, 19, 34]. As it was written by Smeitink [33] “we have only seen the tip of the clinical spectrum of mitochondrial disorders”. Clinical features and the onset of the disease depend on the percentage of mutated mtDNA (heteroplasmy) and differ from patient to patient [36]. However, there are some features that should alert a physician to the possibility of mitochondrial disorders in order to improve the diagnosis.

The aim of the paper is to provide a description of the clinical course and biochemical findings in four children with single large mtDNA deletions. Two deletions have not been reported previously and two are known as the “common deletion”.

Patients

The clinical picture of four children (two boys and two girls, aged 12, 11, 14 and 4 respectively) with single mtDNA deletions is presented. Natural history of the disease, clinical findings before the diagnosis were thoroughly checked and taken into consideration in each case. Patients 1 and 2 were reported previously [27] and included in our multicentric survey of 180 children with cytochrome c oxidase deficiency [4].

Patient 1, a 12 year old boy, born after normal pregnancy and delivery to healthy unrelated parents, with weight 3150 g, Apgar score 9. First symptoms appeared when he was 1.5 years old. Failure to thrive and periods of vomiting and severe watery diarrhoea during recurrent infections were observed. Anemia had a macrocytic sign. In the next years height increase and weight gain were not satisfactory. His appetite was poor.

At the age of 7 hypoparathyroidism was diagnosed (tetania fits, hypocalcaemia, high normal phosphorus and low PTH). Treatment with oral calcium supplementation and dihydroxyvitamin D was instituted. During this therapy an unusual transient tendency to hypercalcemia was observed and active derivatives of vitamin D had to be withdrawn. Re-examination of parathyroid function revealed severe hypomagnesemia of renal type. In spite of magnesium supplementation, signs of parathyroidism reappeared periodically.

The boy was short and slim, his skin was dry. Neurological examination revealed ptosis and motor clumsiness. CT scan of the brain showed mild cerebral atrophy. Ophthalmoscopy showed pigmented degeneration of the retina. ERG was markedly abnormal. USG revealed hyper-
good clinical and biochemical response. Except for urinary magnesium loss the boy did not present any other signs of kidney involvement.

The diagnosis of mitochondrial disorder was established at the age of 11, when the patient was admitted to our pediatric metabolic center. He was short and very slim, hypoactive, with atrophy of muscles and mild hepatopathy (elevated aminotransferases). An increase of plasma lactate (4.8-5.1 mmol/dl) and CSF (4.2 mmol/dl) was found. Multigorgan involvement with growth failure, myopathy, ptosis, hypoparathyroidism, pigmentary retinopathy, deafness and lactic acidosis suggested mitochondrial cytopathy. Parents agreed only to DNA analysis from the blood. Muscle biopsy was not performed. The boy died in the local hospital at age of 12. An autopsy was not performed.

**Patient 3**, a 14 year-old girl, born to healthy nonconsanguineous parents after uneventful pregnancy and delivery. Birth weight was 3100 g, Apgar score 9. Her younger brother is healthy. Her psychomotor development was normal, but tendency to vomiting and failure to thrive were observed from infancy. She was always short.

When the patient was 6 years old hypoactivity and fatigability started to present. Gradually decreased writing skills were observed at school.

At the age of 10 years insulin dependent diabetes mellitus developed. One year later bilateral ptosis and mild gait disturbances appeared. Neurological examination disclosed cerebellar ataxia, bilateral ophthalmoplegia and brisk tendon reflexes with bilateral Babinski and Rossolimo signs. Progressive sensory hypoacussis was also detected. EEG and brain auditory evoked potentials were abnormal. Brain CT showed mild cerebellar and cerebral atrophy. Her intellect was relatively well preserved.

A mitochondrial disorder was diagnosed in our metabolic center when the girl was 14 yrs old. She was short with thin habitus. An increase of lactate in blood (3.0 mmol/dl) and cerebrospinal fluid was found (4.3 mmol/dl) as well as hyperproteinorrhachia (95-110 mg%). Fundoscopy showed pigmentary retinopathy. Heart conduction block was detected. EEG and brain auditory evoked potentials were abnormal. Brain CT showed mild cerebellar and cerebral atrophy. Her intellect was relatively well preserved.

At the age of 18 she was wheelchair bound because of pyramido-cerebellar syndrome as well as muscular atrophy. The course of diabetes mellitus is rather mild, though fluctuating (insulin dose about 0.8 IU/kg daily).

**Patient 4**, a girl 4.5 years old, born after an uneventful pregnancy and delivery with birth weight 3000 g. Apgar score was 9. Her unrelated parents as well as her two older brothers are healthy.

The first symptoms appeared when she was 2 years old. Decreased growth rate, tendency to vomiting, episodes of dehydration and metabolic acidosis were observed. Macrocytic anemia was also found.

At the age of 3 years hepatopathy with increased serum ammonia and transaminases was detected as well as renal tubulopathy with hypokalemia, hypophosphatemia and hyperchloremic acidosis. Neurological examination showed muscle hypotonia and brisk tendon reflexes. Ophthalmologic study revealed a cataract. The girl was short and thin, near cachectic. Plasma lactate concentration was elevated to 5.4 mmol/dl.

The diagnosis of mitochondrial cytopathy was established in our metabolic center when she was 4. The girl died at 5 from multiorgan insufficiency. The autopsy was performed at a regional academic center.

**Methods**

Diagnosis was established on the biochemical (plasma and cerebrospinal lactate concentration), enzymatic, histochemical (muscle biopsy) and molecular level.

Blood was collected after cannulation of a vein, in the morning, after overnight sleep.

Muscle tissue was obtained by biopsy of the vastus lateralis and frozen immediately.

Autopsy is available only in one case.

**Histopathology and histochemistry**

Frozen muscle sections were stained with hematoxylin-eosin, modified Gomori trichrome, oil red O. Histochemical reactions for NADH-dehydrogenase, succinate dehydrogenase (SDH), cytochrome oxidase (COX), and myosin ATP-ase after preincubation at pH 4.3; pH 4.6 and pH 9.4 – stainings were performed.

**Enzymatic study**

Respiratory chain enzyme activity in muscle homogenates was assayed using the spectrophotometric method as described previously [20].

**Molecular investigation**

DNA isolated from leukocytes or from muscle biopsy was digested with single endonucleases BamHI or PvuI according to the manufacturer’s recommendation. Deletions were detected by the Southern method utilizing a probe obtained by using PCR and Bio-X-Act polymerase (Bioline) to amplify a 15649bp fragment (571-16220) using XL 1 and X12 starters [25].

Long PCR was used to amplify appropriate fragments, which were then analysed to determine where the deletion was localised by digestion with restriction endonucleases HpaI, Aval, EcoRI and SnaBI. The results of these digestions were used to design primers to amplify small regions containing the boundaries of the deletions. These products were used for sequencing in the Laboratory of DNA Sequencing and Oligonucleotide Synthesis in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw.

**Results**

**Patients’ data and clinical features**

All patients had a marked increase in lactate concentrations in plasma as well as in cerebrospinal fluid (CSF) when
examined for the first time in our metabolic center. The estimation of lactate levels has not been performed previously during long-term diagnostic process at many endocrinologic, neurologic and pediatric clinics. There was a long interval between when the first symptoms appeared and when the diagnosis of mitochondrial disorder was finally established. The diagnosis delay ranged from 4 to 14 years. Only one patient was still alive at the age of 18 years. Three others died at the age of 13, 12, and 5 respectively. None of the patients' had any family history of mitochondrial disorders.

Details of the patients clinical signs and symptoms are given together in the table 1. Despite of variable presentation there are a number of similarities.

As it can be noted, all our patients from infancy/early childhood presented with a different level of failure to thrive and growth retardation. They were short and with slim habitus. In addition the parents reported hypoactivity, fatigability and poor appetite. All those patients had multiorgan involvement.

The signs of muscle involvement presenting as hypotonia and/or muscle weakness were observed in all children. Disturbances in the gastrointestinal system with tendency to vomiting and sometimes diarrhoea were observed in three children from the beginning of the disease. Slightly elevated aminotransferases as signs of mild hepatopathy were noted in all cases.

Visual problems were detected in all the patients. Three out of four had retinal pigmentary degeneration; one had cataract. Additionally a corneal clouding was seen in one case.

Endocrine system involvement was found in three children. There was hypoparathyroidism in two children, diabetes mellitus in two and adrenal cortex insufficiency in one of them. Polyendocrinopathy was diagnosed in one child. Endocrinological symptoms were treated as an independent entity for a long time (as well as cardiac conduction disorders). The clinical course of endocrinological insufficiencies did not fulfill the criteria of classical condition. In all cases, a "strange", fluctuating course of the disease was observed. Hypersensitivity to routine endocrinological therapy was noted.

Two affected children had hematologic problems such as macrocytic anemia, and one of them also had thrombocytopenia. Increased mean corpuscular hemoglobin concentration value (MCHC) was evident in two cases. Cerebral dysfunction was revealed in all cases as unspecific signs and symptoms. Two patients were assessed as developmentally delayed. Decreased writing skills and mild cognitive decline was observed in one case, but psychomotor sluggishness was seen in all children. None of them had epileptic jerks. Mild cerebral atrophy was detected on CT in three cases, hyperproteinorhachia in one, ptosis and ophthalmoplegia and ataxia in three. Brisk reflexes or lack of tendon reflexes were found in all children. Peripheral neuropathy was detected in one case, after many years of the disease. Progressive sensory hearing loss was detected in three children at different ages, however, the youngest affected child was not examined by audiometry.

Cardiac involvement was observed in two children. There were heart conduction disturbances. In one child a pacemaker was implanted because of MAS attacks. Amongst the signs of kidney involvement ultrasound examination revealed hyperechogenicity of kidney in one child. The major finding was dysfunction of tubules. In one girl a proximal tubulopathy with hypokalemia, hyperchloremic acidosis and hyperphosphaturia was severe. Renal loss of magnesium with hypomagnesemia (and secondary hypoparathyroidism) was detected twice, and episodes of increased sodium excretion lead to hyponatremia and hypovolemia with necessity of intravenous treatment.

Enzymatic study
Increased activity of citrate synthase (CS) was found in two out of three cases as well as a combined decrease in the activity of respiratory chain complexes assessed as CS ratio (table 2). In one case enzymatic study of the muscle tissue did not reveal any respiratory chain involvement. Repeated enzyme measurement performed in another case (from the same muscle biopsy) revealed markedly different data.

Morphological study
Results of pathological investigation of the skeletal muscle are shown in table 2 and figures 1, 2 and 3A,C (see legend). Muscle RRFs were found in two, and cytochrome oxidase deficiency in the muscle (diffuse or mosaic) in two out of three examined samples.

Autopsy of patient 4 revealed the following pathological findings: Liver – mixed macro and microvesicular liver steatosis involving 70-80 percent of the parenchyma, severe cholestasis and slight portal fibrosis (Figure 3, D). Lungs – diffuse atelectasis with edematous fluid and hyaline membranes within alveoli and alveolar ducts. Pancreas – widespread fibrosis supplanting pancreatic parenchyma, large foci of necrosis (Figure 3, B). Kidneys – rare cortical microcysts and occasional granular deposits within collecting tubules. Stomach – focal mucosal myotic ulceration. Heart – mild diffuse fibrosis.

Molecular investigation
The results of molecular findings are summarised in table 2 with results of other investigations. The heteroplasmy of mutated mtDNA in the muscle tissue was assessed as very high in all three examined samples.

Discussion
The frequency of sporadic events of mitochondrial deletion in an individual has not been established in the general population. It seems that the single mtDNA deletions are rare and represent only a minority of the patients with mutations in mitochondrial DNA. Only six cases with mtDNA deletions (17%) were identified in the group of 36 Polish patients with
<table>
<thead>
<tr>
<th>Major symptomatology</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth and weight at the time of diagnosis cm/kg ±SD</td>
<td>122.3 cm – 3.84 SD (12 y), 31 kg – 1.38 SD</td>
<td>96.3 cm – 4.22 SD (6 ½ y), 13 kg – 3.57 SD</td>
<td>144.5 cm – 1.98 SD (13 ½ y), 33.5 kg – 1.55 SD</td>
<td>80 cm – 2.84 SD (2 ½ y), 10 kg – 2.50 SD</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>1 ½ years</td>
<td>Infancy</td>
<td>Infancy</td>
<td>About 2 years</td>
</tr>
<tr>
<td>Neurological/muscular signs</td>
<td>Ptosis, adiophokinesis, areflexia, Babyinski sign (12 y)</td>
<td>Hypotonia, Babyinski sign EMG myogenic features (8 y)</td>
<td>Hypotonia (10 y), Ptosis, Babyinski sign (11 y)</td>
<td>Peripheral neuropathy (16 y)</td>
</tr>
<tr>
<td>Visual problems</td>
<td>Retinitis pigmentosa (8 y), Corneal clouding (12 y)</td>
<td>Corneal clouding (5 y), Retinitis pigmentosa (5 y)</td>
<td>Retinitis pigmentosa (13 ½ y)</td>
<td>Cataract (2 y)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>12 y</td>
<td>8 y</td>
<td>13 ½ y</td>
<td>Not done</td>
</tr>
<tr>
<td>Cardiac involvement</td>
<td>Heart block III° Pacemaker (8 ½ y)</td>
<td>No</td>
<td>Conduction impairment (13 ½ y)</td>
<td>Mild diffuse fibrosis at autopsy (5 y)</td>
</tr>
<tr>
<td>Gastrointestinal problems</td>
<td>Vomiting during infection Watery diarrhoea (14 mo-2 y)</td>
<td>No</td>
<td>Vomiting during infection (2-4 y)</td>
<td>Vomiting (2 ½ y)</td>
</tr>
<tr>
<td>Endocrinological problems</td>
<td>Hypoparathyroidism (7 y), Hypocorticism (7 y) Diabetes mellitus (9 y)</td>
<td>Hypoparathyroidism (6 y)</td>
<td>Diabetes mellitus (11 y)</td>
<td></td>
</tr>
<tr>
<td>Macrocytic anaemia (MCV%)*</td>
<td>95.2% (at 7 y), E 2.8 Hb 7.8 g%</td>
<td>No</td>
<td>No</td>
<td>105% – 115% (2 ½ y), E 1.8 Hb 6.9 g%</td>
</tr>
<tr>
<td>Kidney involvement</td>
<td>Urinary magnesium leakage (7 y) Hypernatriuria (7 y)</td>
<td>Hyperechogeneity (7 y)</td>
<td>Hyperechogeneity (11 y)</td>
<td>Tubulopathy: hypophosphatemia (0.49 mmol/l), hypokalemia (2.6 mmol/l); kidney potassium loss (2 ½ y)</td>
</tr>
<tr>
<td>Liver involvement*</td>
<td>↑AspAT 176; AIAT 127 (9 y)</td>
<td>↑AspAT 59; AIAT 75 (8 y)</td>
<td>No</td>
<td>Mild hepatomegaly ↑AspAT 165; AIAT 200 (2 ½ y)</td>
</tr>
<tr>
<td>Others</td>
<td>Increased fetal Hb (21%)</td>
<td>Conjunctivitis, photophobia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* AspAT, AIAT normal value <50 IU; MCV% normal value 85%
Table 2

Spectrophotometric, morphological, histochemical and molecular findings in muscle biopsy of four children with single mtDNA deletion

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Muscle enzyme activity (nmol/min/mg protein)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate synthase (CS) (reference value 123.3 ± 26.8)</td>
<td>176.6/228.0</td>
<td>Nd</td>
<td>177.0/147.0</td>
<td>62.5</td>
</tr>
<tr>
<td>Complex I (% CS) (reference value 13.1 ± 4.9)</td>
<td>11.4/11.4</td>
<td>Nd</td>
<td>10.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Complex II (% CS) (reference value 7.6 ± 1.9)</td>
<td>3.1/3.9</td>
<td>Nd</td>
<td>6.2</td>
<td>4.3</td>
</tr>
<tr>
<td>Complex II+III(% CS) (reference value 10.0 ± 2.5)</td>
<td>1.0/3.6</td>
<td>Nd</td>
<td>10.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Complex III (%CS) (control value 69.2 ± 24.3)</td>
<td>52.4/22.7</td>
<td>Nd</td>
<td>56.6</td>
<td>53.8</td>
</tr>
<tr>
<td>Complex IV (%CS) (reference value 26.4 ± 7.4)</td>
<td>6.0/17.9</td>
<td>Nd</td>
<td>5.0</td>
<td>28.6</td>
</tr>
<tr>
<td><strong>Morphological investigation of muscle tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gomori Trichrome staining</td>
<td>Individual fibers with very subtle mitochondrial accumulation (RRFs)</td>
<td>Nd</td>
<td>Distinct RRFs</td>
<td>Mild perimysial fibrosis</td>
</tr>
<tr>
<td>Cytochrome c oxidase activity</td>
<td>Universally negative reaction</td>
<td>Nd</td>
<td>Generally weak with mosaic deficit</td>
<td>Reaction positive, although uneven</td>
</tr>
<tr>
<td>Accumulation of lipids within muscle fibers</td>
<td>Mild</td>
<td>Nd</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>Ultrastructure of mitochondria</td>
<td>Increased number of mitochondria, circular cristae, lipid deposits</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td><strong>Molecular investigation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extent of mtDNA deletion</td>
<td>8470–13466</td>
<td>7485 – 15529</td>
<td>8470 – 1346</td>
<td>10892 – 16073</td>
</tr>
<tr>
<td>Size of mtDNA deletion (bp)</td>
<td>4977 (common deletion)</td>
<td>8035</td>
<td>4977 (common deletion)</td>
<td>5182</td>
</tr>
<tr>
<td>Repeated sequences</td>
<td>No</td>
<td>10bp</td>
<td>13bp</td>
<td>9bp</td>
</tr>
</tbody>
</table>

Nd – not done

RRFs in the muscle [21]. The prevalence of 1.6 per 100 000 adults was assessed for a region of Finland [28]. The largest multicenter survey in the world included about 250 cases with single large mtDNA deletions [10]. In the group of 136 Japanese patients with various clinical forms of the disease a presence of relationship between the age of the onset and length of the deletion (and deleted tRNAs number) was found [36].

Mitochondrial disorders are still under-diagnosed not only in Poland but also in other countries [13, 15, 33]. This is true both for adults and for children. This is among others the consequence of the fact that the clinical data
available in most reports unfortunately only provide fragmentary information concerning the course of the disease and the treatment. Not infrequently, molecular methodology was the focus of the authors’ attention. Our intention is to pay more attention to good clinical description of our pediatric patients carrying single mtDNA deletions to contribute to improving the knowledge about the natural history of the disease.

Careful review of older reports on myopathic adults with KSS and CPEO syndromes reveals a number of similarities with more recent descriptions of children with mitochondrial deletions [13-15, 17, 18, 24, 34]. These similarities seemed to be remarkable in spite of significant differences of mitochondrial disorders considering the age of onset, initial symptoms, sequence of organ involvement, severity, progression and outcome.

Among well-recognised syndromes such as KSS, CPEO, Pearson, Wolfram, DIDMOAD, it is useful to design a list of various single signs and symptoms more than occasionally associated with mtDNA deletions. Almost all organs
and tissues may be involved [1, 2, 5, 11, 13, 15, 19, 22, 24, 32]. Many adults with diagnosis of isolated myopathy and with symptoms of KSS and PEO were ill in childhood. They also had additional symptoms (as for example an endocrine disorder) which were not diagnosed as a part of a syndrome and treated as an unrelated disease.

The classical acronyms frequently are “overlapping” or “incomplete”. In our own material, two cases might be referred to as pancreas-narow syndrome of Pearson (case 1 and case 4), as well as KSS (case 1 and 3) or DIDMOAD (case 1, 2, 3). But our aim is not to compare the clinical and biochemical features found by us with these described earlier in the literature. For practical use, it seems to be more important to analyze the natural history of each “mitochondrial” symptom alone, than to again classify cases as “incomplete previously well defined syndrome” or “the syndrome plus”.

All our patients from the infancy and early childhood were short and with thin habitus. They had a tendency to vomit, some suffered from macrocytic anemia and had gradually disclosing multiorgan involvement. Each physician in his practice should be alert to the possibility of mitochondrial cytopathy when he/she meets such a child. An important approach is to routinely measure lactate concentration (in plasma, urine, and/or cerebrospinal fluid). Hyperlactatemia is a very good marker of mitochondrial dysfunction, especially in older children not exposed to the known hypertoxic circumstances as cardiac or respiratory failure [11, 13-16]. However, normal lactate concentration does not exclude a mitochondrial disorder.

Endocrinological abnormalities of various kind and severity seem to be especially common among patients with mtDNA deletions [6, 8, 31]. They were also observed in our patients. However, these findings did not fit into any pattern of known endocrinopathies. Some hypothetic mechanisms may be considered as an explanation.

Delayed and/or decreased peripheral hormone secretion due to low energy supply may contribute to the clinical presentation. But in our patients there are some data suggesting preserved or even exaggerated receptor response to hormone stimuli. Significant elevation of urinary cAMP excretion was observed in patient 1 during hypocalcaemia, in spite of relatively low PTH concentration. Patient 1 and 2 presenting with hypoparathyroidism both showed increased responses (sensitivity) to active derivatives of vitamin D. Patient 1 was also hypersensitive to insulin and prednisone. Unexpectedly low doses of these drugs were fully effective and easily caused an overdose reaction. Hypersensitivity of the receptors or their increased numbers may explain this phenomenon.

Increased receptor response to hormonal stimuli also seem to be evident in our patients. Poor effector reaction (or post receptor event?) may lead to endocrine insufficiency (as in diabetes or hypoparathyroidism).

Finally, dysfunction of kidney tubular resorption may contribute to abnormal endocrinological findings. Increased urinary magnesium loss with hypomagnesemia may lead to hypoparathyroidism (in patient 1 and 2) and sodium loss with hyponatremia may imitate hypocorticism (as in patient 1).

A cataract has been reported previously [2, 18, 24, 30] but in our knowledge a corneal clouding has not been reported in association with mt DNA deletions. It might be caused by past inflammation but may also represent a novel “mitochondrial” sign. Increased fetal hemoglobin concentration detected in one of the patients was not measured systematically in mitochondrial disorders and its significance remains to be established.

Mitochondrial DNA deletions usually lead to a severe combined respiratory chain (RC) defect. We have found the deficit only in two out of three examined cases. The youngest patient had normal RC complex activity in the muscle homogenate, and her muscle histochemistry did not reveal any RRFs or cytochrome oxidase deficit. Such possibility of false negative results (and a discrepancy between subsequent tests, as in one of our cases) should be remembered in the differential diagnosis of mitochondrialopathy. However, it was recently suggested that the measuring of RC activity in muscle homogenate (as we did) is better for detection of the RC complex deficiency, in comparison with mitochondria-enriched specimens [7].

Two out of four deletions found in our patients have not been reported previously in the literature. In all four the end points of the deletions were mapped in the area in which deletions occur the most frequently, that is between the replication initiation sites. In all cases the deleted area contained three tRNA genes (serine, leucine and histidine), which is in agreement with reports that all pathogenic mtDNA deletions encompass at least one tRNA gene.

In three cases repetition of sequences on the deletion boundaries provides support for the theory of slipped mispairing with an intermediate formation of a triple helix as a mechanism for the occurrence of deletions. This theory assumes that during the H strand synthesis a dissociation of the complex take place after DR1 synthesis, a hairpin is formed by the double-stranded template and the complex reattaches and continues synthesis in DR2. This mechanism is due to the possibility of a formation of a triple helix and seems possible in the case of a strong predominance of pyrimidine bases on the template in the area of the repeats [29]. A higher T and C nucleotide content was only noted in one case (patient 4), but not in the case of other three deletions. Other mechanisms have been proposed for making mtDNA deletions, however, both recombination [23] and cleavage by topoisomerase II [3] also assume the presence of repeated sequences at the deletion boundaries. For one of the analysed mtDNAs no repeated sequences were found at the boundaries whereas for other the three such repetitions of several nucleotides were indeed present.

In all our patients there was a long interval between when the first symptoms appeared and when the diagnosis of mitochondrial cytopathy was established. Not only in Poland the patients are diagnosed very late [11, 13]. In the time before definitive diagnosis was established they had...
many unnecessary and exhaustive examinations, which could have worsened their health. In mitochondrial disorders every energy-consuming event could irreversibly worsen the patients’ status. Our own experience suggests that episodes of hyperventilation with deep hypocapnia might also injure the brain [26]. If postmitotic cells of the nervous system and muscle are destroyed the pathological changes are impossible to reverse. Even though there is no specific therapy for mitochondrial disorders, the correct diagnosis is very important to the patient and the family. It can put to an end to inconvenient and unpleasant examinations and give the possibility of suitable supporting care for the affected patient as well as to give the family accurate genetic counseling. As we know single large mitochondrial deletions are sporadic, the risk for the next child of the proband’s parents is low, the same as in the general population [12, 35]. An affected male will not transmit the disease caused by mutation of mtDNA. The risk for offspring of affected females is 5%, but prenatal diagnosis is not possible because of uncertainty of predicting the clinical effect of mutation [12].

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