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Diagnostic and Treatment

Approaches to Mitochondriopathies in Children and Adolescents

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1. Introduction

1.1 Substantiation of the need for a guideline for Mitochondriopathies in children and adolescents

There is almost no other comparable area of inborn metabolic disorders where diagnosis and treatment are as little standardised as in the case of "mitochondrial encephalomyopathies". Even the terminology is heterogeneous and requires explanation. With regard to the frequency of mitochondriopathies in children, one must assume a minimum prevalence of 1 – 1.15 in 10,000 (1, 2, 3). One must assume that the disease continues to often be under-diagnosed. They account for a significant proportion of patients attending metabolic or neuro-paediatric centres and, in terms of differential diagnostics, must be considered in the case of many diseases. The symptoms are extremely heterogeneous, while the course of the disease is often extremely arduous with a high mortality rate.
Many patients, and the affected families, suffer greatly. There is a great demand for structured, consensual information. It often takes a great deal of time - interspersed with detours - before a clear diagnosis is reached. There is an urgent need for a rational algorithm in daily clinical life. Even when a diagnosis has been reached, genetic counselling is often difficult while prenatal diagnostics are impossible.
Patients and their parents must have this information to hand. They are often made insecure by different statements regarding diagnosis, prognosis and treatment.

Inadequate diagnostics contribute to the insecurity of those affected, while false diagnoses cannot be ruled out. Diagnostic detours, delays before reaching a diagnosis, false diagnoses and even unsubstantiated treatment attempts all result in significant costs to the health system. The treatment options for these diseases are extremely limited, and often not 'evidence-based'. Non-uniform diagnostic concepts often result in examinations being duplicated, or unnecessary diagnostic steps being taken. There is insufficient data with regard to the prevention of secondary damage in the case of mitochondriopathies. The level of knowledge among doctors regarding mitochondriopathies is often very low, so the selection of patients for diagnostic purposes is very non-specific. In addition, there is a need for an exchange of information beyond national borders regarding mitochondriopathy patients and studies.

This guideline takes account of current knowledge regarding the diagnostics of mitochondrial diseases and/or diagnosis criteria for children (4, 5), which have been modified with reference to the adult criteria (6).

1.2 Target group of the guideline

Target groups: General practitioners, paediatricians, metabolic specialists, neuro-paediatricians, human geneticists and other professions with an interest in mitochondrial diseases; patients and families.

1.3 Aims of the guideline

The aim of the guideline is to define the patient group in respect of which a suspected diagnosis of a mitochondrial disease should be reached, and to develop standards for diagnosis and diagnostic work-ups with the aim of rationalisation and improving efficiency (avoiding errors), as well as improving patient outcomes (prevention and treatment) and informing doctors and patients.

The guideline is intended to help improve the care provided to children and adolescents with mitochondriopathies in terms of diagnosis, treatment and the prevention of secondary damage. The aim is to help

develop a rational algorithm in daily clinical life. The guideline is intended to help avoid diagnostic detours, thus avoiding multiple diagnostics and unnecessary tests. The aim must also be to achieve a focussed patient pre-selection. The introduction of a more rational diagnostic procedure is also associated with a reduction in costs. It is vital to avoid false diagnoses. Improving diagnostic efficiency is also intended to help diagnose previously undiscovered patients.

The guideline includes a clear definition of the term "mitochondriopathy" and provides aids for a systematic classification of mitochondrial diseases. The guideline is intended to inform not only doctors, but also patients. It is intended to make the information easily available on the Internet, and to promote a uniform regional and supra-regional exchange of information. A more uniform knowledge base will facilitate improved patient information. The severe suffering of patients, as well as the insecurity fostered by the absence of uniform procedures, can be significantly reduced by improved diagnostic and treatment quality.
The guideline offers practical diagnostic and treatment assistance not only for paediatricians and specialist facilities, but also for non-specialists.
It is particularly important that treatment (attempts), as well as symptom treatment measures, be rendered uniform and standardised. The aim must be to substantiate them in the context of evidence-based medicine.

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2. Definition and classification

2.1 Definition of the term "mitochondriopathy"

The terms used to designate this group of diseases vary and may be confusing. The term "mitochondrial encephalomyopathy", which is frequently used especially in paediatrics, indicates that the central nervous system and skeletal muscles are often involved. Historically, this term was used in the 1980s, when these diseases in children (1) and adults (2) were first described. We now know that, theoretically, almost all organ systems can be affected by mitochondrial energy metabolic disorders, and this term is thus conceptualised far too narrowly. Other expressions, such as "mitochondrial cytopathies" (3) or "mitochondrio-cytopathies" (4) indicate a systematic affection of the organism, with the possibility of multiple organ systems being involved. Terms such as "respiratory chain defects" and "OCPHOS illnesses" limit this disease group to defects in the final common path of substrate oxidation, the four respiratory chain complexes and mitochondrial ATP synthase. While the term "mitochondriopathy" does not define itself as relating to organ or system involvement, or to a metabolic route, it is (strictly speaking) too general since, as well as oxidative phosphorylation many other intermediate metabolic processes take place in the mitochondrion. Thus, strictly speaking, urea cycle defects and β-oxidation disorders should also be subsumed in the term "mitochondriopathies".

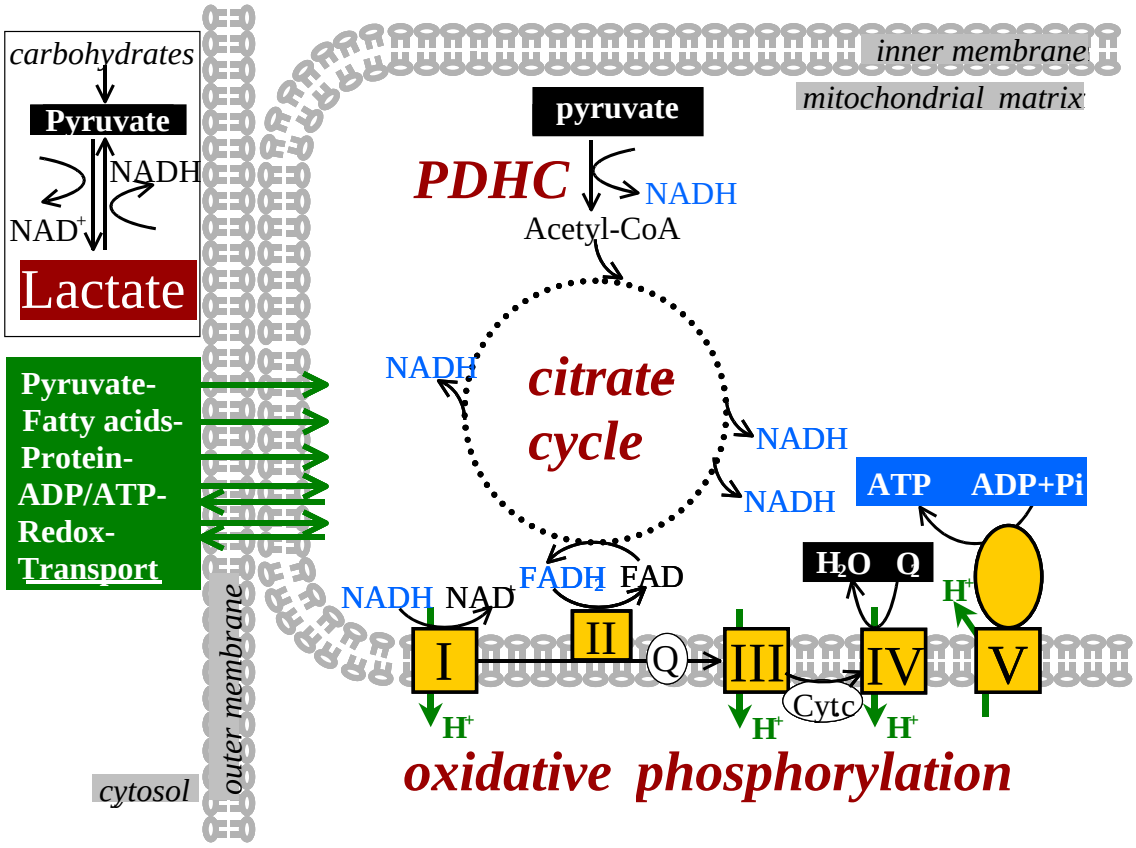
Notwithstanding this, we chose to use the term “mitochondriopathies”, but in this respect we must define the metabolic routes meant with regard to the patients affected.

Mitochondriopathies: affected metabolic routes

We define mitochondriopathies as diseases with a primary disorder in the area of the following metabolic routes:
Disorders in the area of the pyruvate dehydrogenase complex, the citrate cycle and the respiratory chain including ATP synthase (Complex I-V). In this regard, the requisite mitochondrial membrane transport processes (e.g. adenine-nucleotide translocator, voltage-dependent anion channel, aspartate Malat shuttle, phosphate carriers etc (*see Fig. 1*)) must be taken into account. This system encompasses the entire route of the so-called "pyruvate oxidation” process, and is thus delimited from β-oxidation.

A diagnosis of mitochondriopathy may be reached in the case of patients with a verified defect in respect of the pyruvate oxidation metabolic route described above. The verification should be possible on several levels (morphology including enzyme histochemistry, biochemistry, genetics), and the defect should display a clinical relevance.

Fig. 1 Mitochondrial energy metabolism, pyruvate oxidation route



2.2 Classification

The following differentiation must be observed on principle:

a) Primary mitochondriopathies (defects in respect of the pyruvate oxidation route, see Fig. 1)
b) Mitochondriopathies with indirect involvement of OXPHOS (e.g. Barth Syndrome cardiolipin metabolism (5), Mohr-Tranebjaerg Syndrome – protein import (6), Friedrich's Ataxia iron metabolism (7) etc., hereditary spastic paraplegia paraplegin (8))
c) Secondary mitochondrial changes ("not mitochondriopathies in the strict sense")
    - E.g. secondary inhibition of OXPHOS in the context of other metabolic disorders (e.g. glutaraciduria type 1, propion-, methylmalonic acidema etc.) (9).
    - Non-specific mitochondrial adaptation in the case of degeneration such as muscular atrophy, e.g. spinal muscular atrophy (SMA) (10) etc.

Below, the group of mitochondriopathic diseases in divided into three large groups in the context of diagnostics as well as in the flow chart; while this division is arbitrary, it is very logical in terms of clinical procedure. On the one hand, there are *"mitochondrial syndromes"*, i.e. illnesses defined on the basis of a specific combination of symptoms. A molecular-genetic examination generally allows a direct diagnosis. Classic mitochondrial syndromes tend to become apparent in late childhood or adolescence, and the transition to adulthood is blurred. The most common group of diseases in childhood is the group of so-called *"mitochondrial encephalomyopathies"*. Most diseases in this respect relate to isolated or combined respiratory chain defects or defects of the pyruvate hydrogenase complex.
In addition, it has become evident that mitochondrial defects can occur in all organ systems. Therefore, a separate group of "mitochondriopathies with primarily non-neuropathic symptomatics" has been defined.

2.2.1 Mitochondrial syndromes

Below are listed the classic mitochondrial symptoms relevant to children and adolescents. The transition to adult diseases is blurred.

Table 1: Mitochondrial syndromes

	Name	Heredity	Cardinal symptoms	Cardinal findings	Diagnosis
MELAS	Mitochondrial encephalomyopathy with lactic acidosis and 'stroke-like' episodes	Maternal	Generally starts in the 2nd decade, stroke-like episodes (often hemianopia), microsomia	In the MRT, stroke-like lesions not bound by the vessel limits	Mutation analysis of the mtDNA Point mutations in MTTL1 MTND6, MTTQ)
MERRF	Mitochondrial encephalomyopathy with 'ragged red fibres'	Maternal	Progressive myoclonus epilepsy	"Ragged red fibres" in themuscle biopsy	Mutation analysis of the mtDNA (point mutations in MTTK, MTTL1)
NARP	Neuropathy, ataxia and retinitis pigmentosa	Maternal	Ataxia, loss of sight	Retinitis pigmentosa, neuropathy. MRT: Symmetrical hyper-intensive signal (T2w) in the basal ganglia	Mutation analysis of the mtDNA (point mutations in MTTK, T8993G/C)
KSS	Kearns-Sayre Syndrome	Sporadic	Ptosis, progressive external ophthalmoplegia, ataxia, retinitis pigmentosa	Increased fluid protein, conduction defects in the ECG, cardiomyopathy, calcification of the basal ganglia, indicative conspicuousness of the white substances	Mutation analysis of the mtDNA (deletions)
Pearson	Pearson-Marrow-Pancreas Syndrome	Sporadic	Anaemia, malabsorption, microsomia	Exocrinal pancreas insufficiency, refractory sideroblastic anaemia Subsequent development of a KSS possible	Mutation analysis of the mtDNA (deletions)
CPEO	Chronic progressive external ophthalmoplegia	Sporadic, autosomal-dominant	Ptosis, progressive external ophthalmoplegia	Ptosis, progressive external ophthalmoplegia	Mutation analysis of the mtDNA (deletions). Nuclear genes are often involved in the case of multiple deletions
LHON	Leber's hereditary optic nerve atrophy	Maternal / spontaneous	Painful loss of sight in young men	Optic nerve atrophy	Mutation analysis of the DNA (point mutations in the complex I-genes - MTND1, MTND6, MTND4, homoplasmic and thus verifiable in the blood)

MNGIE	Mitochondrial neuro-gastrointestinal encephalo-myopathy	Autosomal-recessive	Myopathy, episodes of gastro-intestinal pseudo-obstruction, frequent neuropathy, ptosis and CPEO	MRT: Striatae-like indicative conspicuousness of the white matter (T2w), muscle biopsy: Possible RRF, multiple dele-tions or depletions of the mtDNA	Biochemical diagnostics: Thymidine phosphorylase activity, thymidine in the blood; mutation analysis ECGF1 (22q13.32-qter)
Leigh[1]	M. Leigh, Leigh Syndrome, DD: Leigh-like Syndrom[1]	Autosomal-recessive, maternal, x-chromosomal recessive	Neuro-degenerative disease starting in babyhood or in-fancy, ataxia, brain stem symptoms	In the MRT in T2w, symmetrical hyper-intensive signal in the basal ganglia and the brain stem, typical neuro-pathological findings	Depends on the biochemical findings relating to the respira-tory chain diagnostics
Alpers	M. Alpers-Huttenlocher	Autosomal-recessive	Rapidly progressing neuro-degenerative disease starting in babyhood or infancy, epi-lepsy, liver insufficiency	Microcephaly, cortical atrophy, liver co-involvement, typical histological findings	Depends on the biochemical findings relating to the respira-tory chain diagnostics
Barth	Barth Syndrome	Syndrome, x-chromosomal, recessive	Heart insufficiency, recurring infections, failure to thrive	Granulopenia, dilative cardio-myopathy 3-methylglutacon-aciduria (Type II)	Biochemical diagnostics: tetralinoleyl- cardiolipin in thrombocytes; mutation analysis G4.5 (Xq28)
(Depletion Syndrome)[2]	mtDNA Depletions Syndrome	Autosomal-recessive	Variable (myopathy, hepato-cerebral syndrome)	Varies depending on the basic disease	Quantification of the mtDNA in the affected tissue; mutation analysis depending on the above quantification

1. M. Leigh is, strictly speaking, a neuropathologically-anatomically defined disease. Clinical pictures with suspicious lesion distribution patterns in the central nervous system, based on the MRI finding, may be defined as Leigh-like syndromes if the biochemical or molecular-genetic findings do not make it possible to assign them more specifically.
2. The term 'syndrome', in the case of mtDNA depletions, does not denote a syndromatic clinical picture, and – unlike the other syndromes – cannot be extrapolated from a combination of characteristic symptoms.

2.2.2 Non-syndromatic encephalopathies, myopathies and neuropathies

This group of diseases – often also termed "mitochondrial encephalomyopathies" – is most prevalent in children. It includes all forms of respiratory chain defects and/or defects of the pyruvate hydrogenase complex, with a wide range of encephalomyopathic courses. The spectrum ranges from inborn lactic acidosis which swiftly proves fatal, to Leigh-syndrome-like courses. In this regard, diagnostics tend to be on an enzymatic level. The vast majority of these patients have nuclear mutations.

2.2.3 Mitochondriopathies with primarily non-neurological pathology

Below is a list of the mitochondrial clinical pictures known to date which can impose themselves primarily without neurological/myopathic semeiotics. In the case of certain diseases, neurological/myopathic semeiotics can develop during the course of the illness. We have stated the OMIM numbers where there are entries in the OMIM; these numbers can be used to access the current literature.

Table 2: Mitochondriopathies with primarily non-neurological pathology

*Pearson Syndrome**
#557000 PEARSON MARROW-PANCREAS SYNDROME

Primarily mitochondrial cardiomyopathy
#510000 CARDIOMYOPATHY, IDIOPATHIC DILATED, MITOCHONDRIAL
*212350 CATARACT AND CARDIOMYOPATHY
#252010 COMPLEX I, MITOCHONDRIAL RESPIRATORY CHAIN, DEFICIENCY OF
*590045 TRANSFER RNA, MITOCHONDRIAL, ISOLEUCINE; MTTI
#220110 COMPLEX IV, MITOCHONDRIAL RESPIRATORY CHAIN, DEFICIENCY OF

*Barth Syndrome**
#302060 BARTH SYNDROME; BTHS

Hepatopathy with/without mtDNA depletion
#251880 MITOCHONDRIAL DNA DEPLETION SYNDROME

Fanconi Syndrome and hepatopathy (BCS1L)
#606104 TUBULOPATHY, ENCEPHALOPATHY, AND LIVER FAILURE DUE TO COMPLEX III DEFICIENCY

Chronic diarrhoea with microvillous atrophy
#520100 DIARRHOEA, CHRONIC, WITH VILLOUS ATROPHY

*MNGIE, pseudo-obstruction**
#603041 MITOCHONDRIAL NEUROGASTROINTESTINAL ENCEPHALOPATHY SYNDROME; MNGIE

Hereditary paragangliomas and phaeochromocytoma (Complex II)
168000 PARAGANGLIOMAS, FAMILIAL NONCHROMAFFIN, 1; PGL1
#605373 PARAGANGLIOMAS, FAMILIAL NONCHROMAFFIN, 3; PGL3
#115310 CAROTID BODY TUMORS AND MULTIPLE EXTRAADRENAL PHEOCHROMOCYTOMAS
#171300 PHEOCHROMOCYTOMA

Multiple symmetrical lipomatosis
151800 LIPOMATOSIS, FAMILIAL BENIGN CERVICAL

Non-immunological endocrinopathies
e.g. material diabetes mellitus
*590050 TRANSFER RNA, MITOCHONDRIAL, LEUCINE, 1; MTTL1
hypoparathyroidism
Andrenal cortex insufficiency
Hypothyroidism
Growth hormone deficiency

Rhabdomylosis and myoglobinuria

Andreu et al. 1999 Ann Neurol (Cytb) and other mutations with mtDNA COX structural genes

Primary retinopathy

Retinopathy may be the first symptom to occur in the case of various syndromes passed on by mtDNA

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* Those syndromes (see 2.2.1., Table 1), which need not primarily impose themselves with neurological semeiotics, are listed here again. It is important to emphasis that the phenotype of a syndrome may change during the course of a disease (11), and that overlap syndromes also exist (12).

2.3 Diseases not addressed by this guideline

As mentioned in 2.2, other metabolic processes and their defects, which may also occur intramitochondrially, are not deemed to be mitochondriopathies. Fatty acid oxidation disorders, urea cycle defects, types of organo-aciduria, ketolysis defects etc. We have also not addressed secondary mitochondrial disorders which can, for example, occur as a result of an OXPHOS inhibition due to accumulated metabolites (see 2.2 c)).

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3. Diagnostics in the case of mitochondriopathies in children and adolescents

3.1 Preamble

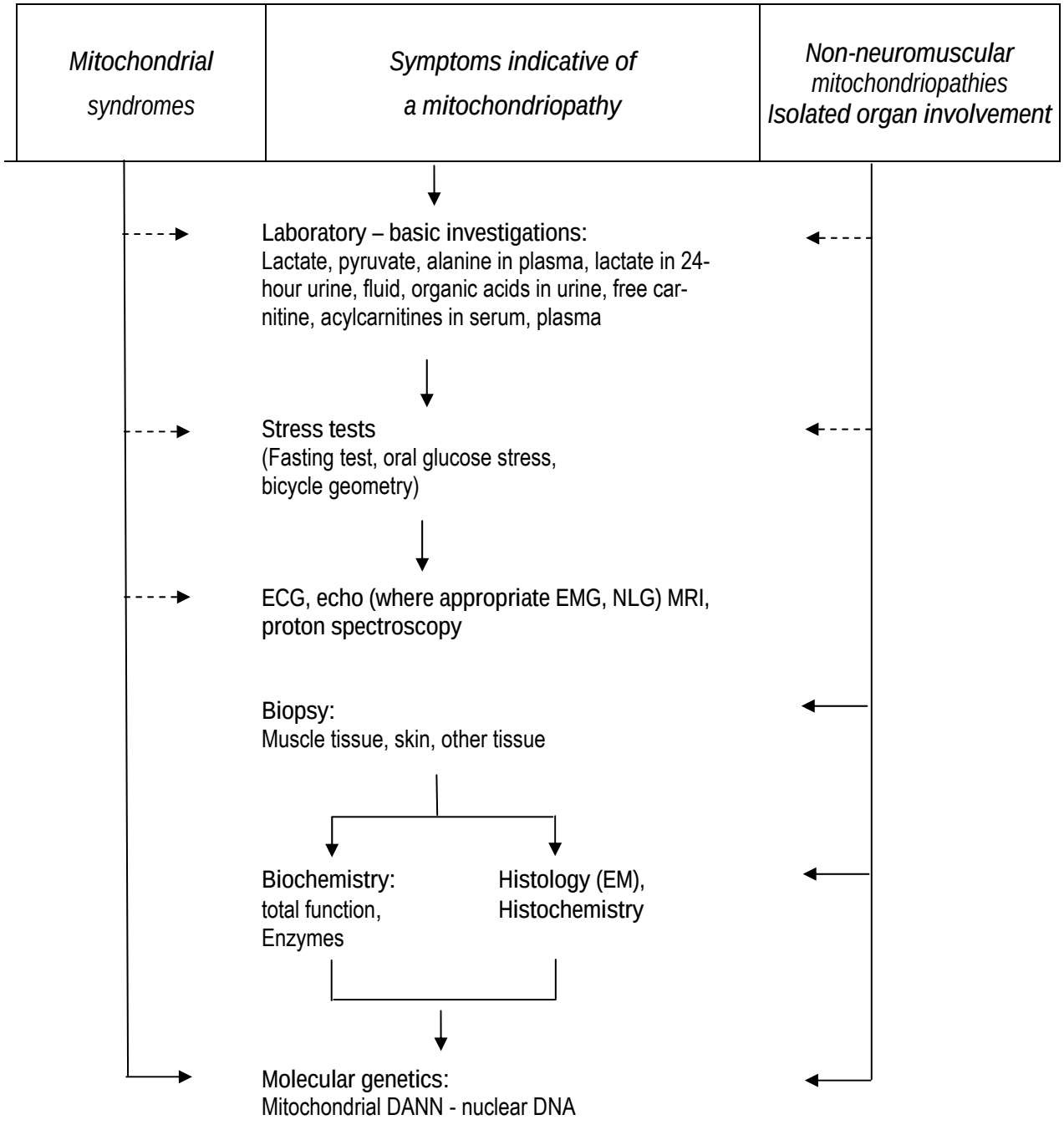
The diagnosis of mitochondriopathies in children and adolescents is a complex process which should most appropriately take place in experienced, dedicated and specially equipped diagnostic centres. A holistic picture must be obtained of symptoms, laboratory findings, neuro-physiological and imaging data, as well as the results of histological/electron microscopic, histochemical, biochemical and molecular-genetic investigations. It should be possible to verify mitochondriopathies on different levels. Muscular tissue, rich in mitochondria, continues to be greatly preferred for diagnostic purposes. Histological and histochemical investigations may support the diagnosis of a mitochondriopathy. However, some phenomena such as COX-negative muscle fibres, or even ragged red fibres, may be non-specific, or may not be present, especially in children. Biochemical analysis must take account of a range of aspects: On the one hand, the purely enzymatic investigations of individual components of the oxidative phosphorylation system should take priority over functional investigations (respiromatry or radiochemical substrate oxidation measurements). This also allows transport process disorders to be detected. A complete biochemical analysis, with a measurement both of the respiratory chain enzymes including the ATP synthase, and the PDHC, is vital. In the case of clinical syndromes, the molecular-genetic investigation can lead directly to a diagnosis. DNA diagnostics for the purpose of localising the defect in the mitochondrial or nuclear genome form the basis for genetic counselling. During the diagnostic process, it is vital to consider different aspects such as secondary mitochondrial changes, age-dependency of enzyme activities, and polymorphisms of the mitochondrial DNA. False diagnoses can only be avoided with the help of standardised, networked and multidisciplinary diagnostics.

Not all so-called mitochondrial changes are due to a primarily mitochondrial pathology. Biochemical, histological/histochemical and even genetic findings can all be non-specific. It is not justifiable to extrapolate a primary mitochondriopathy from an isolated finding. One must also consider secondary mitochondrial changes in respect of other metabolic disorders (PA, MMA etc), or in respect of non-specific mitochondrial adaptation, for example in the case of neurogenic muscular atrophy (SMA etc.) (2).

Fig. 2 shows a simplified schematic in the form of a diagnosis cascade for mitochondriopathies. While preparing this guideline, a special detailed flow chart was developed as an aid for a structured diagnostic procedure (*see 3.2 and Annex 1*). Based on the classification (2.2), we selected three entry levels on the clinical level. However, in respect of the diagnostic route, we recommend following the procedure specified in the flow chart in all cases.

Fig. 2 Simplified overview diagnostic schematic relating to mitochondriopathies

(See ANNEX 1 – DIAGNOSIS FLOW CHART for details and an extensive algorithm to be observed in a diagnostic context)



3.2 Flow chart

The flowchart provides an overview of all procedures considered necessary in the context of a comprehensive diagnostic process. It serves to structure the diagnostic process, and thus constitutes a guideline governing a systematic diagnostic course of action. The main elements of the flowchart are described in detail in the individual chapters of the guideline. The flowchart may be used as a graphic table of contents of the diagnostic section of the guideline.

3.3. Diagnosis of mitochondriopathies

3.3.1 Anamnesis

Any diagnosis must start with a thorough anamnesis. In this regard, one should enquire about symptoms with specific significance to mitochondriopathies. The symptom questionnaire (see Annex 3) is intended as an aid in this regard. It is also important to take a complete family history. In this case of mild disease courses, pure myopathy forms or symptoms in their initial stages, particular note should be taken of the performance anamnesis.

Family anamnesis
If a mitochondrial disease is suspected, one should pay very careful attention to the family anamnesis and the preparation of a family tree, since - under certain circumstances - particular constellations could render invasive diagnostic procedures redundant. The heterogeneity of mitochondrial diseases is also reflected in the heredities. This means that all heredities (autosomal-dominant, autosomal-recessive, x-chromosomal, maternal) are possible. Following the diagnosis, or during the diagnostic process, genetic counselling should be provided by a centre specialising in human genetics (or a specialist human geneticist).
See Annex 2: Family history questionnaire

3.3.2 Status and symptoms

Patients with mitochondrial diseases display diverse and non-specific clinical symptoms. In this regard, functional impairments can often be verified in the energy-dependent organs such as the brain (mental retardation, lethargy, ataxia, tetra-spasticism, epilepsy) skeletal muscles (muscular hypotonic, ptosis), heart (cardiomyopathy) and eyes (retinitis pigmentosa, optic nerve atrophy). The aim is to allow a carefully selected group of patients to avail of specific morphological, biochemical and molecular diagnostics.
A thorough general paediatric and neurological status should be ascertained during the clinical investigations. Special note should be taken of the following: unusual features relating to metrics (hyposomia, microcephaly), skin (lipomatosis), hair (thin head hair, hypertrichosis, growth disorders, haematomas), eyes (ptosis, cataract), heart (dysrhythmia, valve insufficiency), lungs (respiratory disorders, infections) and abdomen (intestinal motility, liver enlargement, kidney and pancreas changes), lethargy, motoric retardation, muscular hypotonia or hypertonia, stress intolerance, muscular atrophy, spasticity, paresis, ataxia, dystonia, tremors etc.
Standardised questionnaire for ascertaining symptoms where mitochondriopathies are suspected (Annex 3):

a) Aims of the questionnaire
This questionnaire was developed in the context of the development of the guidelines for the diagnosis and treatment of mitochondrial diseases in children.

If, during the diagnostic process relating to a patient, the hypothesis of a mitochondrial disease has arisen, a systematic anamnesis should first be conducted, together with an exploration of symptoms and indications (referred to below as "clinical presentation") relating to the general and neurological status. This should be done before initiating diagnostic measures such as imaging, testing bodily fluids or other procedures. This questionnaire includes all those clinical presentations which the experts who were questioned when developing this guideline considered indicative of a need for further specific diagnostic measures.

The questionnaire is not, strictly speaking, a diagnostic instrument. That means that it does not result in a mitochondrial diagnosis, or quantify the probability of a mitochondrial diagnosis. This is because, although clinical presentations have a high level of sensitivity (e.g., patients with a verified mitochondrial diagnosis generally have one or more of the features listed, or have a characteristic combination of features), the level of specificity is low (i.e., a great many patients with non-mitochondrial diagnoses may also display one or more of the features mentioned).
Therefore, this questionnaire is an aid for recording, systemising and evaluating the clinical presentation found during course of diagnostics in respect of mitochondrial diseases. It is therefore no substitute for clinical expertise or further diagnostic measures.

b) Structure of the questionnaire

The questionnaire has several components which should be processed and/or used in the sequence specified.

- The questionnaire itself:
  Here, 71 clinical presentations, classified according to organs or organ systems, are evaluated to find whether they are present, not present or have not been investigated.
- Evaluation according to importance and implementation of investigations:
  In accordance with expert opinion, the presence of the various clinical presentations may be of varying significance when deciding to proceed further. Therefore, the presentations listed in the questionnaire have been divided into significant and very significant presentations.
  Both the presentations present in respect of a patient, and those which have not been investigated, should be evaluated. The nature and number of uninvestigated presentations, in particular, may indicate features which have yet to be recorded, or may indicate the degree of completeness.
  Evaluation according to syndromes
  The clinical presentations found during the investigation can then be compared with those presentations characteristic of various mitochondrial syndromes. In this regard, too, it should be noted that that due to the lack of presentation specificity, the presence of a presentation pattern should not lead one to conclude that a syndrome is present. The table of syndromes should be viewed as a heuristic structural aid for proceeding further, rather than as a diagnostic algorithm.

c) Development of the questionnaire:

The questionnaire was developed in 4 stages.

1st stage: Members of the Guideline Development Group collated the clinical presentations to be investigated in the context of diagnosing mitochondrial diseases.

2nd stage: The list of clinical presentations was sent to all members of the Guideline Development Group and to external experts*. Each clinical presentation was to be evaluated on a scale from 1 to 4 (under no circumstances = 1; more NO = 2; more YES = 3, in all circumstances = 4) in respect of whether the presence of such a presentation indicated a need for further diagnostic measures. In addition, all participants were requested to add to the list of clinical presentations where appropriate.

3rd stage: All clinical presentations which the participants had evaluated as 1 (under no circumstances) during Stage 2 were removed from the list. All additional presentations mentioned were added.

4th stage: In the course of a second mailing, a total of 80 presentations were again evaluated on a scale from 1 to 4.

5th stage: The arithmetical mean of the evaluations was ascertained for each presentation. Eight presentations were deleted from the list as a result of low evaluations combined with substantive considerations (delayed motoric development, fatigue, sleep apnoeas, thrombocytosis, depression, strabismus, macrocephely, cardiac dysrhythmia). The presentations "diabetes mellitus in respect of the index patient" and "diabetes mellitus – maternal heredity", originally recorded separately, were merged. Thus, the present questionnaire includes a total of 71 clinical presentations.

* We would like to thank the following colleagues for their help in developing the questionnaire:
Prof. Boltshauser (Zürich), Prof. G.F. Hoffmann (Heidelberg), Prof. Korinthenberg (Freiburg), Prof. Krägeloh-Mann (Tübingen),
Dr. M. Lindner (Heidelberg), Dr. Marquart (Oldenburg), Prof. Mayatepek (Düsseldorf), Prof. Rating (Heidelberg), PD Dr. Skladal (Innsbruck), PD Dr. Dr. Zschocke (Heidelberg)

3.3.3 Basic analyses of bodily fluids

The basic diagnostics facilitate the early detection of laboratory changes typical of mitochondrial diseases (e.g. lactate acidosis), and/or allow other diseases to be ruled out at an early stage.

Blood:

- Blood count, liver values, kidney values, electrolytes, glucose, creatine kinase, lactate, pyruvate, amino acids (alanine), acid base balance
- For ruling out common differential diagnoses (ß-oxidations defects, organiciduriae):
- Acylcarnitine (TMS)
- CDG diagnostics

Urine:

- Quick urine analysis (Stix)
- Organic acids

Cerebrospinal fluid:

- Lactate, protein, glucose, where appropriate alanine, cell count / differentiation.,
- Protein differentiation / oligoclonal bands

3.3.4 Imaging and magnetic resonance spectroscopic cerebral diagnostics (MRI/MRS)

3.3.4.1 General

Non-invasive cerebral imaging plays a central role in further diagnostics relating to suspected or certain mitochondriopathies in children. Magnetic Resonance Imaging (MRI) is the preferred imaging investigation method. In children, this method is far preferable to the less sensitive computer tomography, which also involves radiation exposure. The MRI can sensitively image focal damage due to the impaired ATP synthase. Even the dynamics of such damage can be well characterised using specific radiological crite-

ria, especially when incorporating the recent MR diffusion technology. In this regard, the pattern of damage can provide indications for certain disease syndromes, in respect of which the radiological appearance is also extremely diverse (similar to the clinical and biochemical heterogeneity). As well as focal damage, associated or pure atrophies are quite common. Certain special MR Proton Spectroscopy (IH-MRS) or Phosphorous Spectroscopy (still in the development stage) specialist centres can complement MRI. These also facilitate the non-invasive metabolic investigation of specific cerebral regions, and in particular facilitate the verification of pathologically increased cerebral lactate concentrations. An appropriate combination of apparatus allows structural and biochemical MR investigations to be carried out in one investigatory step. This allows valuable differential diagnostic indications to be obtained (especially relating to the delimitation of old hypoxial lesions). It also allows one to ascertain quantifiable course parameters regarding the gravity and the cerebral affection patterns of known mitochondriopathies, which can also be used to monitor treatment in the case of experimental treatment approaches (Krägloh-Mann et al). To exploit this diagnostic information to the full, optimised investigation protocols and standardised evaluations (especially of the MRI) are advisable. Encephalopathic processes are always an indication for imaging diagnostics. In this regard, even experts have different opinions on the need for complementary 1H-MRS tests. As a whole, this depends largely on the individual symptoms and the laboratory results obtained previously. By the same token, a cerebral MRI and 1H-MRS may also be useful in the case of mitochondriopathies without encephalopathic signs, in order to record any sub-clinical cerebral involvement.

3.3.4.2 Recommendations for carrying out and charting examinations

Since examinations generally take at least 1 hour, infants and toddlers must be sedated or anaesthetised to guarantee a motionless examination. Therefore, in the case of a relevant suspected diagnosis, it is advisable to have the examination carried out at a centre offering a combined MRI/MRS test.

On principle, we recommend carrying out an examination very shortly after the occurrence of encephalopathic signs, in order to increase the diagnostic in formativeness as a result of the known dynamics of both MRI and 1H-MRS findings.

Cerebral phosphorous spectroscopy would be a suitable method for directly verifying the phosphorylation potential while resting and during neuronal activity in order to detect a cerebral respiratory chain disorder. With regard to mitochondriopathies, encouraging but isolated case reports are available. This means that it is not possible at present to issue a general recommendation.

Protocol recommendation

- T2 and T1 weighted axial sequences. Important: recording of the entire medulla oblongata
- MR diffusion, including diffusion-weighted images and quantitative diffusivity charts. (Optional: fractional anisotropy charts)
- Lactate-sensitive 1H-MRS in lesions (the best chance of ascertaining lactate is in lesions with reduced diffusivity) and grey matter (cortex or if Leigh Putamen is suspected). Measurement time required: approx. 10 – 15 minutes per region.

Technical notes on ascertaining lactate using 1H-MRS:

PRESS or STEAM as single voxel or spectroscopic imaging. TR > 1.5 sec, TE = 144 ms for reliable detection of lactate (signal inversion, cave alanine, amino acids), TE = 30-35 ms will generate a higher signal-to-noise ratio (S/N) and give a good spectrum quality (low line width, at least resolution of the lactate double peak at 1.33 ppm), facilitating the detection of other metabolites.
Crucial: lactate separation of lipids.
PRESS is superior to STEAM with regard to the S/N ratio, and more resistant to unfavourable parameter settings (TM-dependency of the J modulation can lead to a loss of signal at TE =144 ms). Due to its limited S/N ratio under clinical examination conditions, 1H-MRS is not a sensitive detection method.

Metabolites become detectable from a concentration of approx. 1mmol/l in relatively large volume units (approx. 3-8 ml). In this regard, the measuring process is generally repeated 128 times. This means that large-scale, severe increases in lactate concentration can be reliably detected. Lactate increases limited to smaller lesions, or slight lactate increases, may not be detected.

3.3.4.3 Morphological findings in the case of mitochondriopathies in toddlers

Analogous to the heterogeneous clinical phenotype, the radiological appearance is extremely variable. Therefore, the rule in this case too is that almost any cerebral region can be affected, and that even a normal finding does not rule out the presence of a mitochondriopathy. There are, however, specific patterns of damage which are diagnostically indicative with regard to a non-mitochondriopathic (e.g. hypoxic-ischemic) or, indeed, mitochondriopathic genesis. Furthermore, there are predilection patterns which support a diagnostic sub-specification relating to specific mitochondrial syndromes, such as Leigh syndrome, MELAS or KSS.

Frequent radiological findings in the case of mitochondrial diseases are as follows:

- 1. Affection of the grey matter, especially the deep cores.
- 2. Mixed form with an affection of the white and grey matter.
- 3. Affection of the white matter (delimited from the classic leucodystrophy).
- 4. Atrophy

As is the case with other metabolic diseases, a bilateral and often symmetrical affection is characteristic. Exception: unilateral infarctions in the case of MELAS. The calcifications described as typical in CTs cannot be reliably detected by MR tomography. Nevertheless, this is not a sufficient indication for also carrying out a CT examination, particularly in children. Disposition variants may be associated with mitochondriopathies, particularly cerebral hypoplasia and a corpus callosum deficiency, the latter being described primarily in the case of a pyruvate hydrogenase deficiency.
The most common but non-specific spectroscopic finding is the reduction of N-acetyl-aspartate (resonance at 2.0 ppm) as an indication of neuronal damage. A detection of increased lactate (doublett at 1.33 ppm, see above) is characteristic. Although an increased lactate concentration indicates a focally or globally impaired cerebral metabolism, it is not, in itself (in the acute stage) pathognomonic for a mitochondrial disease, but a continuing lactate peak in the chronic stage. In terms of differential diagnostics, ischemic, hypoxic, inflammatory or other metabolic damage must be ruled out. It is helpful, in terms of differential diagnostics, to consider the extent of the lactate concentration with reference to the entire metabolite profile. Follow-up examinations may be required. The verification of pathological lactate increases in cerebral regions not subject to morphological changes is particularly supportive of a diagnosis. In particular cases massive pyruvate or succinate increases can be detected spectroscopically, indicating the specific damage.

3.3.4.4 Selection of typical patterns of findings in the case of syndromes

Leigh Syndrome

- Bilateral striatum necrosis (not obligatory, very rate in the case of a SURF1 mutation)
- Infratentorial lesions (peri-adequate grey matter, tectum, nucleus dentatus, nucleus subthalamicus – especially in the case of SURF1)
- Bilateral pallidum lesions
- Leucoencephalopathy (in rare cases, even isolated)
- Lactate increases in lesions (obligatory in fresh cytotoxic lesions, and often persisting for years

MELAS

- Focal or multi-focal, cortical/sub-cortical infarction-type lesions (early increase in diffusivity), generally not corresponding to the vascular supply regions
- Combined affection of the white and grey matter (basal ganglia).
- Atrophy (cerebral and cerebellar)
- Protracted lactate detection in lesions, but also in unaffected regions
- • Rare: cortical laminar necrosis, haemorrhages

KSS

- Bilateral medullary layer hyperintensities (even cerebellar)
- Bilateral thalamic lesions (substantia nigra, globus pallidus)
- Variable lactate increase

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3.3.5 Expanded diagnostics

The aim of expanded diagnostics is to record not only the involvement of the neuromuscular system, but also of other organ systems, and/or to rule out other clinical pictures which could cause an encephalo-myopathy. These investigations need not be carried out on principle, but rather as dictated by clinical suspicion.
Neuro-psychological testing is appropriate if the central nervous system is also involved.

3.3.5.1 Apparatus-based diagnostics

○	Ergometry:	Bicycle (see 3.3.5.2 b) or treadmill ergometry Anaerobic threshold value, exercise intolerance?
○	Electrocardiogram:	Hypertrophy indications, cardiac dysrhythmia
○	Echo cardiography:	Cardiac insufficiency, hypertrophy
○	Abdominal ultrasound:	
	• Liver: Consistency, size?	Consistency, size?
	• Intestinal motility	
○	EEG: potentials typical of epilepsy	
○	Electro-physiology (EMG*, NLG*, AEP, VEP, SEP etc.):	E.g. involvement of the peripheral nervous system?
○	Audiometry:	E.g. inner ear hearing difficulties?
○	Ophthalmological examination including a sight test and funduscopy	Loss of visual acuity, eye motility restriction, reti-nopathy, retinitis pigmentosa, cataract, ptosis
○	Lung function:	Vital capacity restriction?

* he position of the EMG and NLFG is described in even more detail below.

3.3.5.2 Stress tests

a) Biochemical tests:

Oral glucose stress: 1.75 g/ kg wt glucose, blood samples taken after 0, 15, 30, 45, 60, 120, 180 min.
Reduction of glucose, lactate, pyruvate, ß-hydroxy butyric acid (ß-OHB), aceto acetate (AA).
i.v. pyruvate stress: 500 mg/kg wt pyruvate i.v.
i.v. alanine stress: 300 mg kg wt i.v. measurement of glucose, lactate, alanine
To be determined after fasting and after eating/ postprandial (1 hour after eating)
Lactate/pyruvate (L/P) and ß-OHB/AA ratio
L/P > 25 and ß-OHB/AA ratio > 3.5 mainly in: -respiratory chain defect
L/Pratio ≤ 10 – mainly in: PDHC defect
High L/P, normal ß-OHB/AA ratio: citrate cycle defect and pyruvate carboxylase defect?
Questionable: L/P ratio in cerebrospinal fluid

b) Bicycle ergometry

Presupposes child-suited adaptation as follows: lower minimal resistance, lower width, appropriate seat height, shorter length of the peddles; the aim is exercise to capacity. Staged intensification, at least two minutes each. Total duration of the examination: 6 – 12 minutes. Commencement with a half watt/kg wt, increasing every two minutes by a half watt/kg/ wt.
Abandonment criteria: exhaustion of the child, heart frequency exceeding 185/min.

Evaluation:
Maximum oxygen uptake, VO_2max (spiroergometry)
Watt/kg body weight

Anaerobic threshold (watt or oxygen uptake)
Normal figure approx. 3-4 watt/kg/wt
PWC 170= Physical working capacity 170
Output in watt at a heart frequency of 170 beats/minute
In the case of all children where it was not possible to exercise to capacity.

3.3.5.3 Laboratory

- Blood:
  - Hormone constellation, e.g. in the case of microsomia, pubertas tarda, hypoparathyroidism, hypothyrosis etc.
  - Vitamins (thiamine etc.), trace elements (Cu, Fe, Zn etc.)
  - Bone marrow smear if pancytopenia is suspected
  - Autoimmune system diagnostics, e.g. polymyositis
- Cerebrospinal fluid
  - Neurotransmitters or neurotransmitter disorders
- Urine
  - Protein differentiation E.g. tubulopathy

3.3.5.4 Position of electro-physiological examinations (EMG and NLG)

EMG
Electromyography is not indicative in the diagnostics of mitochondriopathies, and is rarely used in children when addressing this issue. No systematic studies relating to children are available. Most published data originates from case descriptions or from patients with progressive external ophthalmoplegia. The following points should be viewed as significant:

a) As a whole, a wide variability of findings
b) not necessarily pathological EMG findings
c) Varying EMG findings depending on the muscle examined
d) Normal, as well as myopathic and neurogenic, alterations are possible.
e) EMG sensitivity in the case of childhood mitochondrial encephalomyopathies tends to be quite low, although clear findings can be made in individual cases.

NLG

Motoric neurography
When compared with electromyography, this does not involve systematic investigations relating to motoric neurography in children. However, there are patients with both axonal and demyelinising damage in the context of mitochondrial diseases. Thus, mitochondrial diseases form part of the differential diagnosis of polyneuropathy in children. In this regard, of course, age-adapted standard values must be used.
Although no prospective studies are available, one should consider to what extent standard monitoring, especially during the follow-up examinations, might be appropriate. Analogous to other diseases, one suggestion would be to examine an upper extremity nerve (e.g. n. medianus) and a lower extremity nerve (preferably n. peronaeus). It is unlikely that recording F-waves would provide any significant additional information.

Sensory neurography
As a whole, at least as shown by the data relating to progressive external ophthalmoplegia, sensory neurography is more sensitive than motoric neurography. However, especially in early infancy, consid-

erable technical problems should be anticipated.
One proposal for minimal monitoring would be as follows:

a) N. medianus
b) N. suralis or the more sensitive n. peroneus

Available neurography data
No major study is available relating to the frequency of unusual neurographic features in respect of childhood mitochondrial diseases. However, case reports repeatedly mention a slowing-down or loss of the SNAPs.

3.3.6 Muscle biopsy, histology and bio-chemistry

Selection of the tissue and preservation of the samples taken:
The selection of the biopsied tissue is vital when verifying respiratory chain defects. Wherever possible, the clinically affected tissue (tissue specificity of the respiratory chain enzymes) should be selected and subjected to morphological and biochemical diagnostic processes!

3.3.6.1 Muscle biopsy

3.3.6.1.1 Selection based on clinically leading organ systems

Neuromuscular symptoms: Muscle biopsy
Where possible, all biopsies should provide material both for the light microscopic and ultrastructural diagnostics, and for the further biochemical and molecular-genetic diagnostics. The muscle biopsy should be executed following consultation with the examining laboratory, taking into account all the laboratory's requirements: coordination is vital! Special note should be taken of the following: Sampling point (e.g. m. vastus lateralis), suitable anaesthetic (especially in the case of local anaesthetic), requisite amount of pure muscle tissue (depends on the laboratory and the intended analyses: 200 to 600 mg; a minimum amount of pure muscle tissue ranging in size between a grain of rice and a coffee bean is required; most of this will be needed for the biochemical tests).

- Selection of the biopsy point:
  If a diffuse mitochondrial function impairment is suspected, the biopsy point should be selected in accordance with the most favourable technical conditions in the laboratory. However, in the case of diseases where individual fibre alterations are being sought (such as progressive external ophthalmoplegia), an attempt should be made to find to find the most affected muscle (where possible), using clinical criteria (myalgia, weakness). Imaging processes contribute little to the biopsy point selection.

- Collection technique:
  On principle, there are two possible collection methods: needle biopsy and open biopsy. Each centre has its own preference with regard to the method used. However, given the relatively large amount of tissue involved, the open biopsy method often appears to be preferable.

- Muscle preservation for histology, electron microscopy:
  The material should be prepared for subsequent shipping as follows
  - for electron microscopic diagnostics, immersed in 6.25% glutaraldehyde for 2-3 hours at 4 degrees centigrade, and then rinsed three times in 6.84% saccharose, each rinse lasting 1 hour.
  - for histological diagnostics, frozen in liquid nitrogen.

- Muscle preservation for biochemical tests:

Material intended solely for measuring the respiratory chain enzymes and the PDHC must, immediately following collection, be deep frozen, preferably in liquid nitrogen (cryo-pipette) in a native state, and then stored in nitrogen at -80°C until being shipped.

If additional functional tests (see II.2.1 below) are to be carried out, the muscle biopsate should immediately be transmitted to the analysing laboratory using the water-ice refrigeration process and, if appropriate, a special shipping buffer.
If logistically possible, it would be desirable to produce a myoblastic and fibroblastic culture simultaneously.

Other biopsy points: liver, cardiac muscle, kidney
The investigating laboratory should be consulted first. It must have reference figures to hand and be experienced! Preservation in liquid native N2.

Additional tissue: fibroblasts, leucocytes
Defects may sometimes also be expressed in fibroblasts and leucocytes. Therefore fibroblasts, in particular, must always be produced in the context of a muscle biopsy! Culture conditions: addition of 200 µM uridine, 2.5 mM pyruvate.

Tissue which is not to be biopsied: central nervous system, ocular muscle, auditory and optic nerves, pancreas etc.
The defect may also sometimes be detected in other tissues (e.g. muscle, fibroblasts). In some cases (e.g. CPEO, LHON, Pearson Syndrome, diabetes), a direct molecular-genetic verification may be most successful (CAVE: tissue specificity even in the case of mitochondrial DNA mutations!).

3.3.6.1.2 Selection of samples to be preserved post mortem

On principle, if a mitochondriopathy is suspected the same principles apply post mortem regarding the selection of samples to be preserved: clinically affected tissue must, if possible, be obtained for subsequent diagnostics. If possible, samples should be collected 1-2 hours post mortem and preserved as described. In this case, too, a skin biopsy should always be taken, and a fibroblast culture should be produced!

3.3.6.1.3 Sample dispatch, pre-analysis

In the case of all tissue samples and cultured cells, it is vital that the laboratory be informed in advance of the dispatch. Especially in the case of biochemical tests, the interpretability of the results obtained depends largely on whether the analysed samples were collected, stored and shipped in accordance with the conditions outlined above (faultless refrigeration chain!). See table 3.
To facilitate a concluding interpretation of the analysis results in a clinical context, a summary of the clinical and clinical-chemical findings should be sent to the biochemical laboratory.

Table 3: Collection and shipping conditions for tissue biopsies

Material	Planned test	Treatment following collection	Storage	Shipping	Possible shipping time frame
Muscle	Biochemistry with functional tests	Refrigerated, but the material must not freeze	-	Cooled on water ice	A few hours
	Biochemistry without functional tests	Immediately shock-frozen in N_2 nitrogen	Unlimited intermediate storage in nitrogen	On dry ice	Until the following day (depending on the amount of dry ice)
Muscle	Histology	1) refrigerated but not frozen or: 2) one piece frozen in N_2 + One piece unfrozen in glutaraldehyde	- Indefinite in N_2 + days at +4°C	Immediate Possible with a time lag	Hours
	Production of a myoblast culture	Sterile in a special culture medium		Normal post	1-2 days
Other tissue (e.g. liver, heart, kidney)	Biochemistry, possibly histology	Immediately shock-frozen in N2 nitrogen	Indefinite intermediate storage in nitrogen	On dry ice	Until the following day (depending on the amount of dry ice)
Skin punch	Production of a fibroblast culture	Sterile in a culture medium, not frozen	(possibly a few days)	Unrefrigerated	Following day if possible
Fibroblasts	Biochemistry, genetics	Culture flask with confluent fibroblasts		Completely filled with culture medium, unrefrigerated	1-2 days
EDTA blood (3-10 ml depending on age)	Genetic tests on the leucocyte DNA	EDTA motovettes, not frozen		Normal post, unrefrigerated	1-2 days

3.3.6.2 Diagnostics in respect of tissue samples

3.3.6.2.1 Histology

- Light microscopy

If possible, all biopsies should be subjected to a light microscope examination. Not only should this record any alterations which might indicate a mitochondrial myopathy, but it should also rule out other diseases which could result in a secondary pathology of the mitochondria. In addition, one must assess the extent to which a lipomatosis-myosclerotic conversion has taken place, and whether artificial alterations are present. At a minimum, all biopsies should be examined using the following stains.
HE, van Gieson, trichrome
Succinate dehydrogenase
Cytochrom-c-oxidase reaction
Lipid staining (Sudan Black, Oilred-O)

Light microscopy evaluation criteria
The following are indications for a mitochondrial disease:
- Ragged red fibers
- Diffuse mitochondria proliferation
- Focal, sub-sarcolemmal mitochondria proliferation
- Alteration in the enzyme-histochemical presentation of the COX

While individual fibre necroses are rarely found in mitochondrial diseases, their presence does not rule such diseases out.

As the term implies, 'ragged red fibres' refers to fibres with increased red colouring in the trichrome stain, and a ragged appearance. However, in individual cases, these changes are found much more clearly in the succinate dehydrogenase reaction, NADH-tetrazolium-reductase- reaction and the lipid stain. These fibres are not specific to mitochondriopathies. Thus, for examples, 'ragged red fibres' are described in respect of inclusion myocitis.
A diffuse mitochondria proliferation is primarily shown in the succinate dehydrogenase reaction and the NADH reductase reaction. A diffuse mitochondria proliferation is an etiologically non-specific indication of a mitochondrial function impairment.

Alteration of the COX
On principle, a few patterns can be differentiated, facilitating differential diagnostic conclusions to a limited extent.

a) Reduced activity:
   Individual fibre defects in diffusely arranged 'ragged red fibres' primarily indicate a heteroplasmatic mutation of the mtDNA with impairment in the synthesis of mitochondrial proteins. Mutations may be found in the XOX I – II – III gene.

b) A diffuse weakening tends rather to indicate a homoplastic mutation. A weakening which includes the vessels can be found in some patients with Leigh Syndrome.

c) Diffuse weakening - when counting out the neuromuscular spindles and the vessels - can be found both in the benign myopathy, and in the fatal infantile myopathy.

d) Increased activity: an increased COX activity in the vicinity of 'ragged red fibres' can be found both in respect of the MELAS Syndrome and in respect of other mitochondrial diseases which do not affect COX.

Important: The absence of histological pathological findings does not rule out a mitochondriocytopathy (even a myopathy), while pathological findings may be of a secondary nature. Therefore, any decision regarding the need for, and usefulness of, further mitochondrial diagnostic measures should not be made based on the morphological findings as the deciding criteria, but must rather be reached in a clinical and biochemical context.

- Electron microscopy

In general, ultrastructural alterations precede the occurrence of light microscopic alterations in the case of childhood mitochondrial diseases. Here too, however, the absence of ultrastructural alterations does not mili-

tate against the assumption of a mitochondrial disease, especially in the first few months of a baby's life. Ultrastructural criteria are as follows:

a) Proliferation of mitochondria
b) Enlargement of the mitochondria
c) Alterations of the shape of the mitochondria
d) Alterations of the cristae
e) Paracrystalline inclusions
f) Osmophile inclusions

The nature of the alterations does not allow any differential diagnostic conclusions. Due to the absence of standard values, it is difficult to evaluate quantitative parameters such as the size of the mitochondria, the number of cristae and similar.

3.3.6.2.2 Biochemistry

3.3.6.2.2.1 Functional examination

of unfrozen, native tissue (measurement within a maximum of 2-4 h)

The functional examination represents a screening process for the mitochondrial energy metabolism. It also records transport defects relating to substrates or ions (e.g. adenine nucleoid translocators) and the ATP synthase. Defects in the citrate cycle can be isolated.
Two complementary methods are used in routine diagnostics. These are respirometry in respect of permeabilised tissue (e.g. muscle fibre, fibroblasts or leucocytes) or isolated mitochondria, and radio-chemical substrate oxidation measurement.

3.3.6.2.2.2 Enzymes of the oxidative phosphorylation and pyruvate dehydrogenase complex

The respiratory chain complexes I –IV and the ATPases are quantified individually using spectrophotometry, as well as the pyruvate dehydrogenase complex and the citrate synthase as a mitochondrial marker enzyme. These measurements can be carried out using tissue samples which were frozen immediately following collection (faultless refrigeration chain at a minimum of -80°C until analysis).
In respect of both processes, reference is made to the amount of sample material used (often the protein content). In order to isolate non-specific deviations, which occur especially in the case of premature or newborn babies, but can also occur in the case of tissue degeneration, correlation in respect of mitochondrial marker enzymes (e.g. citrate synthase) is vital.

3.3.6.2.2.3 Quality of the laboratory making the findings

Given the frequently fundamental and directional nature of biochemical diagnostics for the diagnosis of mitochondrial cytopathies, there are high demands in respect of the quality of the investigating laboratory. The validity of the findings made must be verified using standard values, standardisation processes, and the laboratory's experience of the tissue types being examined. Direct cooperation between biochemists and clinicians would appear to be vital for the concluding interpretation of the results obtained.

3.3.6.2.2.4 Specific biochemical diagnostics in respect of Barth syndrome and MNGIE

In respect of patients with Barth Syndrome, it has been shown that the concentration in respect of ***tetralinoleyl- cardiolipin in thrombocytes*** is reduced in comparison to the controls (1). If the presence of a Barth Syndrome is suspected, an analysis of this biochemical parameter should be carried out prior to the molecular-genetic examination of the G4.5 gene.

In respect of patients with a suspected diagnosis of MNGIE (mitochondrial neuro-gastrointestinal encephalomyopathy), it is possible to determine the concentration of deoxyuridine and thymidine in the plasma, as well as the activity of the thymidine phosphorylase in the leucocytes. The deoxyrudine and thymidine concentrations are raised, while the activity of the thymidine phosphorylase is lowered. Increased deoxyuridine and thymidine concentrations can also be measured in the urine (2, 3, 4).

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3.3.7 Genetics, including pre-natal diagnostics

3.3.7.1 Introduction:

In the case of genetic diagnostics, one must first ask in which genome the defect should be sought. Sometimes, the constellation of enzyme activities may offer a pointer, for example if all mitochondrially coded enzymes are affected. On principle, however, both the mitochondrial and the nuclear DNA may be affected. Structural, transport, assembly or anchoring genes may be defective, and there may also be an impairment of the inter-genome interaction. Especially with regard to newly identified mitochondrial mutations, there is a question of pathological relevance. In this regard, it is important to isolate them from polymorphisms. In the case of PDHC defects, especially the common E1α defects, a variable X-inactivation must be considered both in respect of the clinical characteristics and in respect of the enzyme investigation. In the case of nuclear mutations, given the large number of candidate genes, molecular diagnostics are particularly difficult and time-consuming, and therefore limited in terms of routine diagnostics.
Issue of genetic counselling: the heredity process in respect of mitochondriopathies is very heterogeneous, and various heredity patterns are known: both maternal heredity and Mendel heredity (in this case X-linked, autosomal dominant, autosomal recessive and sporadic). Maternal heredity is typical for mtDNA mutations. Nevertheless, the majority of mtDNA mutations are sporadic, so the absence of maternal heredity does not rule out an mtDNA mutation. At the moment, clear counselling is only possible for a minority of all patients with mitochondriopathies. Although, in the case of mtDNA mutations, maternal heredity is of overwhelming relevance, paternal heredity of mtDNA in the skeletal muscles has been described in respect of a patient with mitochondrial myopathy, and must be considered in the case of sporadic mutations (1).

3.3.7.2 Pre-natal diagnostics

Pre-natal diagnosis is only possible in rare cases. It is almost impossible to carry out a reliable prenatal diagnosis in the case of mitochondrial DNA mutations because the percentage of heteroplasmia in respect of a specific patient can vary greatly in the different tissues (2). Currently, pre-natal diagnostics are limited to just a few centres (3). The defect must be multi-systematic, and must also be expressed in the fibroblasts.

When collecting muscle tissue, a fibroblast and a myoblast culture must also be produced to eliminate the need for further subsequent biopsies. Up to now, fibroblasts have proven useful in enzymatic prenatal diagnostics. If there is a proven expression of a respiratory chain defect in the fibroblasts, this allows one to consider a biochemical examination of chorionic villi or amniocytes in the case of a subsequent pregnancy. In many cases, however, neither the fibroblasts nor the chorionic villi express the enzymatic defect, and are therefore not of any diagnostic use. In all cases, in the case of a known gene defect, genetic verification is superior to enzymatic determination.

As a whole, the following procedure should be applied:
If there is a significant respiratory chain defect in the fibroblasts of an affected child in the family, one could attempt an enzymatic examination of the chorionic or amniotic cells in the case of a subsequent pregnancy.

- In the case of a reliably verified pathogenic mutation in nuclear genes, a prenatal diagnosis is possible following a contamination control of the chorionic villi (absence of maternal tissue).
- In the case of a reliably verified pathogenic mutation in the mitochondrial DNA (in respect of the mother or affected children, or other family members), a prenatal diagnosis is generally not advisable, since one cannot make any predictions regarding the mutation distribution, nor can one assess the risks. This does not appear to apply to the mutation at position 8993 of the mitochondrial DNA, which displays a more regular distribution. In exceptional cases, this examination can take place in consultation with the relevant laboratory.

3.3.7.3 Molecular genetics in the case of mitochondrial diseases

Table 4: Molecular genetics in the case of mitochondrial diseases

Erkrankung	Biochemie/Histologie	Betroffenes Gen
MELAS	Complex I, III, IV deficiency; histology Generally typical mitochondriopathy	MtDNA tRNA LeucinUUR
MERRF	Complex I, III, IV deficiency; histology Generally typical mitochondriopathy	MtDNA tRNA Lysin
KSS	Complex I, III, IV deficiency; histology Generally typical mitochondriopathy	MtDNA deletion
Pearson Syndrome	Complex I, III, IV deficiency; histology Alterations not obligatory	MtDNA deletion
CPEO	Sporadic Complex I, III, IV deficiency; histology Generally typical mitochondriopathy	MtDNA deletion
Autsomal dominant or autosomal recessive CPEO	Complex I, III, IV deficiency; histology-Generally typical mitochondriopathy	C10orf2 (Twinkle), ANT1, POLG, secondary: multiple deletions of the mtDNA
NARP maternal	Complex X deficiency	MtDNA, ATPase6
MNGIE	Thymidine phosphorylase deficiency, Complex I, III, IV deficiency; histology Generally typical mitochondriopathy	ECGF1 secondary: multiple deletions and/or depletions of the mtDNA
Dystonia deafness	No indications	TIMM8A (DDP1)
Amish microcephaly	No indications	SLC25A19 (deoxynucleotide transport)
MtDNA Depletion Syndrome (liver form)	Complex I, III, IV deficiency (not obligatory); histologically generally typical mitochondriopathies in the liver	DGUOK (deoxyguanosinkinase)
MTDNA Depletion Syndrome (muscular form)	Complex I, III, IV, V deficiency; histology: generally typical mitochondriopathy	TK (thymidine kinase)
Friedrich's Ataxia	Complex I, II, III, aconitase deficiency	FRDA (X25)
Autosomal recessive hereditary spastic paraplegia (SPG7)	No indications; histological indication of mitochondriopathy	Paraplegine (SPG7)
Autosomal dominant hereditary spastic paraplegia (SPG13)	No indications	Hsp60
Barth Syndrome	Variable combinable deficiencies	G4.5 (Tafazzin) (cardiolipin remodelling)
Autosomal dominant atrophy of the optic nerve, Type 1	No indications	OPA1

Mitochondrial myopathy and sideroblastic anaemia MLASA	No indications	PUS (pseudouridine synthase 1)
X-chromosomal inherited sideroblastic anaemia and ataxia XLSA/A	No indications	ABC7
Leigh Syndrome or Leigh-like Syndrome	Complex 1 deficiency. Histologically, often no typical indications	NDUFV1,NDUFV2, NDUFS1, NDUFS2,NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8
	Complex II deficiency; often no typical histological indications	SDHA
	Complex III deficiency; histological indication not reliable.	HUMQPC, BCS1L
	Complex IV deficiency; histochemical Complex IV deficiency	SCO1, SCO2, SURF1, COX10,COX15, LRPPRC
	Complex V deficiency, often no typical histological indications	ATP12 Mt ATPase6
	PDH deficiency. often no typical histological indications	PDHA1, PDX1
Stress intolerance, myoglobinuria	Complex II or IV deficiency; often typical histological mitochondriopathy.	MtCOX I – III; MtCYB

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3.3.8 Parental information, education

Broaching the subject of a suspected diagnosis is difficult in respect of mitochondriopathies, since the possible clinical spectrum is broad. Therefore, one should only make general statements during an initial discussion. Broaching the topic of a final diagnosis, as well as its range of symptoms and prognosis, should be left to a specialist.
The following points should be addressed during the parental consultation:

- What are mitochondriopathies?
- What are the prognoses for the diseases?
- What treatment options are there?
- How high is the risk of recurrence?

It is vital to avoid early stigmatisation. It is not certainly not justifiable to extrapolate a primary mitochondriopathy from an isolated finding.
An explanatory meeting with parents should only be held once the diagnosis has been reliably secured.
Diagnostic standardisation is of fundamental significance to avoid false or partial diagnoses, and excessive or insufficient diagnostic measures. Optimally thorough diagnostics are only possible using a multi-disciplinary approach, via an internationally recognised centre of excellence which has centralised and accumulated the relevant know-how relating to the diagnostics of mitochondrial diseases.
See *Appendix 4*: speaking notes for a parental consultation

4. Treatment of mitochondriopathies in children and adolescents

4.1 Preamble

The treatment of mitochondriopathies is very limited. It is currently not possible to cure this group of diseases. In many cases, treatment is limited to purely symptomatic measures. Individual reports, or a few studies including therapeutic effects, have only been published for a few substance groups.
Due to the absence of sufficient data, it is currently not possible to issue valid treatment recommendations with the character of a guideline.

Table 5: Problems when evaluating treatment

Problems when evaluating treatment affects
• Heterogeneity of the genotypes and phenotypes • Unpredictability of the clinical course • Undulating or remitting course of the illness • Pre-existing and irreversible tissue damage at the time of diagnosis and/or when commencing treatment • Training and placebo effects • Inadequate follow-up, no long-term evaluations

4.2. Possible therapeutic approach levels

The principle options for treating mitochondriopathies exist on various therapeutic approach levels (3)

a) Influencing the intermediary metabolism by:
   - Stimulating the residual enzyme activity using co-factors
   - Treating with electron transporters or antioxidants
   - Reducing toxic metabolites
   - Antioxidative membrane protection
   - Replenishing the energy storage pool
   - Supplementation in the case of secondary deficiencies
   - Ketogenic diet

b) Gene therapy
   To date, there have been various approaches involving a so-called “mitochondrial DNA treatment”, such as the selective replication inhibition of mutated DNA (6), and the infection of mitochondria with mitochondrial DNA using the mitochondrial protein import system (5, 14). An important approach also involves the selective reduction of mutated DNA by importing restriction enzymes into the mitochondria (6). Recent approaches have also involved the use of mitochiondriotopic cationic vesicles (DQA somas) (7).

c) Symptomatic treatment
   The symptomatic treatment is non-specific for mitochondriopathies, but accounts for a significant portion of medical practice in respect of these patients. Such treatment must be adapted to the individual and carried out by an experienced team. At present, it is not possible to issue any treatment recommendations with the character of a guideline.

4.3 Substances for which there is some evidence in the literature relating to effectiveness of the treatment

In general, there is a lack of large-scale randomised prospective studies relating to the treatment of mitochondriopathies. The literature includes numerous individual reports, or reports on a few patients. In this regard, there are well-researched recent overview articles (1, 2). The following substances are reported to have a therapeutic effect: Co-enzyme Q_{10} , idebenone, thiamine, ketogenic diet, riboflavin, L-carnitine, creatine and aerobic endurance training (*see Table 6*).

Table 6: Substances described as clinically effective in the literature (see overview works 1, 2)

Diagnosis	Substance (ref.)
Co-enzym Q_{10}-defect	Quinone (Co-enzym Q, Idebenone)
Friedreich-Ataxia +/- CMP	Idebenone +/- Vit E
PDHC E1 defect	Ketogenic diet (11), thiamine
Complex I defect, mitochondrial myopathy	Riboflavin
Mitochondriopathies regardless of the biochemical defect, with secondary carnitine deficiency	L-carnitine
Mitochondrial myopathy, regardless of the biochemical defect	Creatine (10) Aerobic endurance training (13)

4.4 Substances for which there are anecdotal reports or case reports relating to a positive effect in respect of mitochondriopathies

These substances are listed below (Table 7 etc.) With regard to the dosage, we refer to the recommendations published elsewhere (1, 2, 3, 15).

Table 7: Substances for which the literature contains reports on a case-by-case basis of positive treatment effects in respect of mitochondriopathies

Substance	Area of application
Alpha lipoic acid	PDHc E3 Leigh Syndrome
Ascorbic acid (vitamin C)	All respiratory chain defects (antioxidants)
Co-enzyme Q10	All mitochondrial cytopathies
Dichloroacetate	Severe / chronic lactate acidosis
Idebenone	LHON, mitochondrial cardiomyopathy
L-carnitine	All mitochondriopathies
Riboflavin (vitamin B2)	All respiratory chain defects (antioxidants)
Succinate	Complex 1 deficiency
Vitamin K3	All respiratory chain defects (antioxidants)

4.5 Treatment recommendations

In respect of the diagnosis groups specified in *Table 6,* we recommend the corresponding substance groups listed in the table. In respect of dosage, we refer to the appropriate reference (1, 2, 3).
In respect of other clinical pictures, the substances listed in Table 7 can be used in the context of a therapeutic attempt. Here, too, we refer to the treatment recommendations published elsewhere with regard to dosage (1, 2, 3, 15).

4.6 Symptomatic treatment

Symptomatic treatment accounts for the major part of medical practice in respect of patients with mitochondriopathies. Some examples are listed below.

a) Acidosis correction
b) Treatment of attacks (anti-convulsants), stroke-like episodes (cortisone), spasticity (botulinum toxin), dystonia (L-dopa); implementation of adequate physiotherapy, ergotherapy, logopaedy etc.
c) Sufficient calorie supply; where appropriate feeding through a PEG tube
d) Avoidance of stress factors such as valproinic acid without L-carnitine, tetracycline, chloramphenicol, long-term use of propofol; early detection and timely substitution in the case of endocrinal involvement such as diabetes mellitus, hypoparathyroidism
e) Interval treatment in the case of MELAS using salicylates, NSAR
f) Hearing aids, cochlear implantation in the case of inner ear co-involvement
g) Ptosis operation (blepharoplasty)

The symptomatic treatment is non-specific and, as with all other neuro-degenerative diseases, must be carried out by an appropriately trained multidisciplinary team at experienced neuro-paediatric and/or metabolic centres.

4.7 New experimental treatment approaches

- Mitochondrial neuro-gastrointestinal encephalopathy (MNGIE): thymidine reduction (16)
- SCO2 deficiency: copper supplementation
- MELAS L-arganine application – increase in transient NO level (17)
- Complex 1 deficiency: ketogenic diet (18)

4.8 Outlook

There is an urgent need for a standardisation of both the diagnostic and the treatment measures. To date, there have been no large-scale prospective randomised studies. Multi centre studies with precisely defined patient groups, and a precisely specified follow-up, would be desirable.

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5. Questionnaire design methodology

In an initial step, the guideline group first collated symptoms and signs deemed to be indications of a mitochondrial disease. The list of items was first sent to all members of the guideline group, as well as experts in paediatric neurology. Using a four-step scale, all items were evaluated to assess whether they should be recorded: under no circumstances (rating 1) or in all circumstances rating 4). It was also possible to suggest additional items for the questionnaire. All items with a median rating of less than 2.5 were excluded from the questionnaire, while additionally mentioned items were included. In a second step, the revised version was again sent to all experts who received the first mailing, with a request for a renewed evaluation of the items included. Here, too, the median item rating was calculated. Items with a median rating of less than 2.5 were excluded. In both steps, the statistical analysis was validated by a substantive vote of the expert members of the guideline group.

6. Procedure for reaching a consensus

First meeting of the guideline group, July 19th – 20th 2002:
Constitutive meeting; specification of the work programme and the working groups

Second meeting of the guideline group, January 17th – 19th 2003:
Report by the working groups and discussion of the results

Third meeting of the guideline group, June 27th – 29th 2003:
Finalisation and harmonising of the working groups' contributions

Approval by the Working Group on Paediatric Metabolic Disorders:
The guideline was submitted to the board of the Working Group on Paediatric Metabolic Disorders.
The guideline was sent electronically to all members of the Working Group on Paediatric Metabolic Disorders.
Final editing of the guidelines.

March 2005:
The guideline was adopted by the Annual Conference of the Working Group on Paediatric Metabolic Disorders.

Approval by related specialist associations:
Association for Neuropaediatrics

6.1 Group definition

General Paediatrics:	PD Dr. Das, PD Dr. Freisinger, Prof. Dr.Gärtner, Dr. Mayrhofer, PD Dr. Schülke, Prof. Dr. Sperl, Prof. Dr. Stöckler, Prof. Dr. Zeman (guest), Dr. von Kleist, Prof. Dr. Wilichowski
Neuro-paediatrics:	PD Dr. Das, Prof. Dr. Gärtner, Dr. Mayrhofer, PD Dr.Schülke, Prof Dr. Stöckler, Dr. von Kleist, Prof. Dr. Wilichowski, Dr. Wolf
Metabolics:	PD Dr. Das, Prof. Dr. Gärtner, PD Dr. Freisinger, Prof. Dr. Sperl, Prof. Dr. Stöckler, Prof. Dr. Zeman (guest)
Neuro imaging and spectroscopy:	PD Dr. Auer, Dr. Mayrhofer
Neurology:	Prof. Dr. Schröder, Dr. Müller-Felber
Biochemistry:	PD Dr. Jaksch, Dr. Mayr, Dr. von Kleist,
Molecular genetics:	PD Dr. Jaksch, Dr. Mayr, PD Dr. Schülke, Prof. Dr. Wilichowski
Clinical genetics:	PD Dr. Freisinger, Prof. Dr. Wilichowski
Histology & morphology:	Dr. Müller-Felber, Prof. Dr. Schröder
Neuro-physiology:	Dr. Müller-Felber, Prof. Dr. Schröder, PD Dr. Schülke

6.2 Reference to existing guidelines

No.	Source	Topic	Address
1	Guidelines on inherited lactate acido-sis AWMF-Reg.-No.: 027/012	AMWF guidelines	http://www.uni-duesseldorf.de/WWW/AWMF/ll/ll_list.htm
2	German Association for neurology	Mito. guidelines	http://www.dgn.org/leitl/mitochon.pdf
3	Neuropediatrics guidelines	AMWF guidelines	http://www.uni-duesseldorf.de/WWW/AWMF/ll/pneur-18.htm
4	Paediatrics and juvenile medicine guidelines (Schülke)	Mitochondrial diseases	

6.3 Other important sources on mitochondriopathies with the character of a guideline

No.	Source	Topic	Address
1	EFNS, Guidelines for the molecular diagnosis of inherited neurologic dis-eases. Second of two parts.	Neurology-Mitochondriopathies European guidelines	http://www.efns.org/docs/gl_10.htm
2	United mitochondrial disease founda-tion	Organisation	http://www.umdf.org/
3	Mitochondrial and Metabolic Disorders - a primary care physician's guide	Essays on this group of issues	http://biochemgen.ucsd.edu/mmdc/ep-toc.htm
4	The Children's Mitochondrial Disease Network	Organisation	http://www.emdn-mitonet.co.uk/
5	The Mitochondrial Research Society	Organisation	http://www.mitoresearch.org/
6	Muscle Biopsy and the Pathology of Skeletal Muscle	Primarly Histology	http://www.emedicine.com/neuro/topic230.htm

7. Sponsor

The company SHS, Heilbronn

8. Coordination with other associations

Neuropediatric Association (Prof. Dr. Jutta Gärtner) Neurology (Prof. Dr. Schröder)

9. Editorial committee

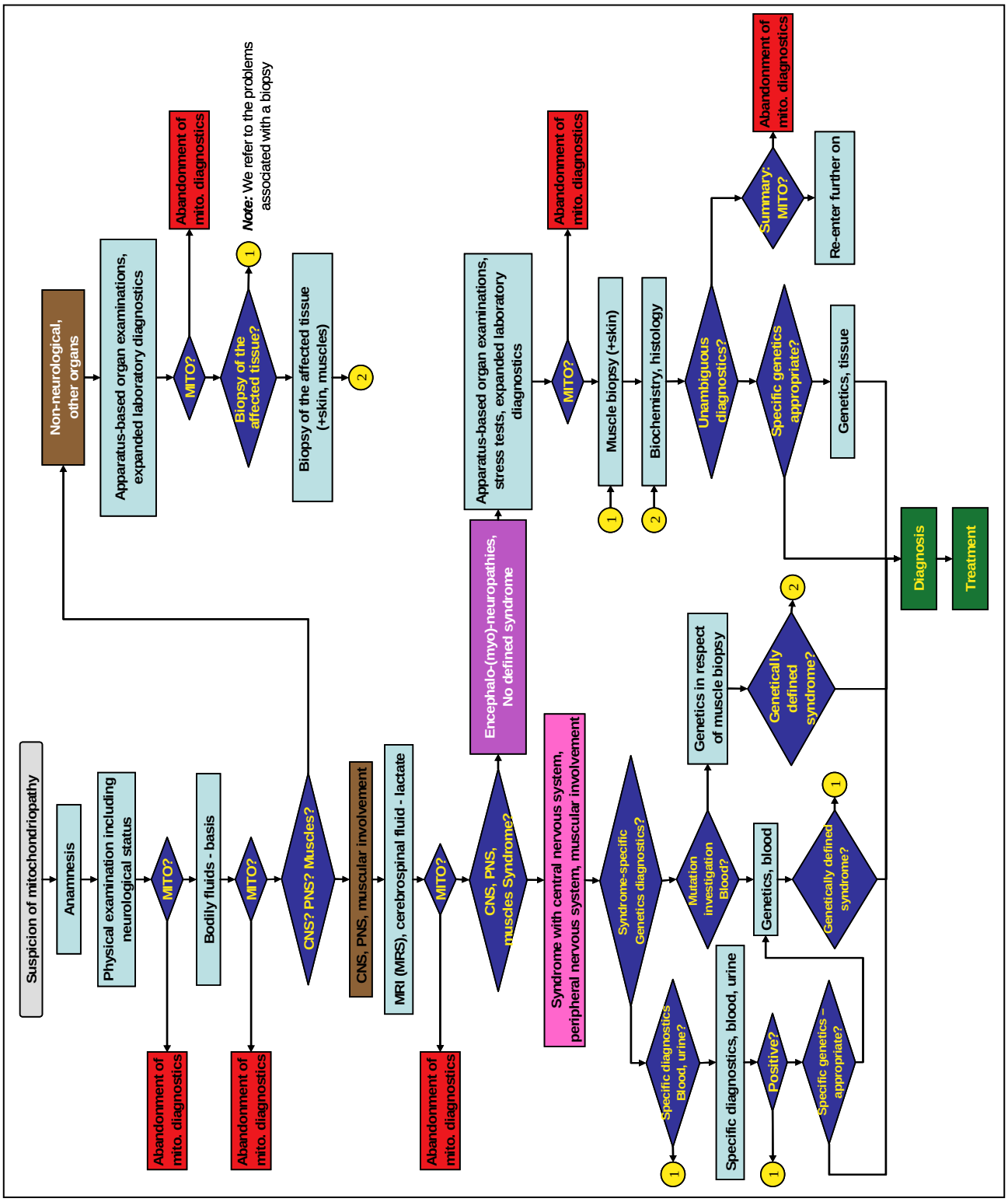
Editorial Committee:
Univ. Prof. Dr. Wolfgang Sperl, PD Dr. Peter Freisinger, Dr. Hans Mayr, PD Dr. Peter Burgard
Management of the Working Group and Final Editing of the Guidelines:
Univ. Prof. Dr. Wolfgang Sperl

10. Term of applicability

Review planned in March 2008

11. Appendices

Appendix 1 Flow chart of recommended diagnostic steps



Instructions on using the flow chart

- Arrow directions: Yes / No
  On principle, arrows pointing down should be read as “Yes”, while arrows pointing to the side should be read as “No".
- One can enter at any point
- The flow chart allows the systematics of available findings to be assessed
- The individual modules are described in the following chapters of the guideline, and stored in the ‘Tools’.
- Mito? = Probing the suspected diagnosis of mitochondriopathy, where appropriate in a clinical case conference with all the diagnostic disciplines / specialists involved.

Appendix 2 Family anamnesis

Date:				
Doctor in charge of the consultation:				
Participants in the consultation:				
Index patient:	Surname:	First name:		
	Date of birth:			
Reason for presentation				
Suspected diagnosis				
Illness commencement				
Are inherited diseases present in the family?			YES☐	NO☐
If YES, in respect of whom?				
Which?				
When did they arise?				
Course?				
Ethic background of the family				
Blood relationship of the parents			YES☐	NO☐
Degree of relationship				
Have there been abortions in the family?			YES☐	NO☐
Do or did family members have a similar diseases in the family?			YES☐	NO☐
If YES, in respect of whom?				
Which?				
When did they arise?				
Course?				
Are there or were there neurological diseases in the family?			YES☐	NO☐
Enquire specifically about:				
Muscle diseases			YES☐	NO☐
Movement impairments			YES☐	NO☐
Epilepsy			YES☐	NO☐
Disabilities			YES☐	NO☐
Stroke			YES☐	NO☐
Migraine			YES☐	NO☐
Other:			YES☐	NO☐
If YES, which?				
In respect of whom?	At what age?			
Are there or were there endocrinologial diseases in the family?			YES☐	NO☐
Enquire specifically about:				
Diabetes mellitus			YES☐	NO☐
other endocrinal diseases			YES☐	NO☐
If YES, which?				
In respect of whom?	At what age?			
Is there or where there, hearing impairment in the family?			YES☐	NO☐
Is there or where there, visual impairment in the family?			YES☐	NO☐
If YES, which?				
In respect of whom?	At what age?			
Have diagnostics already been carried out elsewhere?				
In respect of the index patient			YES☐	NO☐
In respect of family members			YES☐	NO☐
If YES, where? (Findings should be enquired about or obtained)				

Appendix 3 Standardised questionnaire for ascertaining symptoms where mitochondriopathies are suspected

Questionnaire on mitochondrial symptoms and signs

Guideline group of the Working Group on Paediatric Metabolic Disorders (APS)

Please note: This questionnaire is an aid for systemising the clinical symptoms and signs recorded in a diagnostic context in respect of mitochondrial diseases. The questionnaire as such is not a diagnostic instrument in a strict sense, i.e. it will not provide a diagnosis.

Surname:	*First name:*	*Date of birth:*
Clinik:	*Examining physician:*	*Examination date:*

Item No	*Description*	*YES*	*NO*	*Not examined*	*0=important 1=very important*	Remarks
ZNS:						
1	Psychomotoric developmental delay				0	
2	Loss of abilities				1	
3	Incremental occurrence of ≥ 2 neurological symptoms				1	
4	Stroke-like episodes				1	
5	Episodes of unexplained coma				1	
6	Muscular hypotonia				0	
7	Muscular hypertonia				0	
8	Pyramidal signs				0	
9	Extra-pyramidal signs				0	
10	Cerebellaresigns				0	
11	Signs of brain stern involvement				1	
12	Myocloniae				0	
13	Epilepsies				0	
14	Migraine-like headaches				0	
15	Microcephaly				0	
Muscles:						
16	Rhabdomyolysis				1	
17	Stress intolerance				1	
18	Weakness				0	
19	Facies myopathica				0	
20	Muscular atrophy				0	
21	Myalgie				0	
Heart:						
22	Cardiomyopathy				1	
23	Conduction impairments				0	
24	Pre-excitation syndrome				0	

Eyes:						
25	CPEO				1	
26	Ptosis				1	
27	Retinal pigment degeneration				1	
28	Optic nerve atrophy				0	
29	Cataract				0	
30	Reduction in visual acuity				0	
31	Field of vision losses				0	

Other organ systems						
Growth:						
32	Microsomia < 3th percentile				0	
33	Dystrophia (weight < 3th percentile or percentile-transcendent weight development)				0	
34	Hypotrophic newborn or premature baby				0	
Peripheral nervous system:						
35	Neuropathy				0	
Gastrointestinal tract:						
36	Pseudo-obstruction				0	
37	Caclic vormiting				0	
38	Chronic-recurrent diarrhoe > 3 weeks				0	
39	Exocrinal pankreas insufficiency				0	
Liver:						
40	Acute liver failure				0	
41	chronic liver insufficiency				0	
42	VPA-induced liver failure				1	
Endocrine system:						
43	Pubertas tarda				0	
44	Hypothyreoidism				0	
45	Hypoparathyreoidism				0	
46	Diabetes mellitus Typ II				0	
Hearing:						
47	Sensory-neural hearing impairment				0	
48	Ototoxicity of specific medications				0	
Skin:						
49	Symmetrical lipomatosis				0	
50	Hypertrichosis				0	
51	Hair growth disorders				0	
Facial dysmorphia signs						
52	Similar to foetal alcohol syndrome (fullness in the anatomical snuffbox, narrow vermilion border, high forehead, flat nose root)				0	
Kidneys:						
53	Fanconi-Syndrome				0	
54	Other tubulopathies				0	
55	Kidney insufficiency				0	

Hematopoiesis system:						
56	Hypo regenerative anaemia				0	
57	Panzytopenia				0	
58	Neutropenia				0	
General:						
59	Simultaneous involvement of independent organ systems (e.g. combination of symptoms in the central nervous system, muscles, eyes, heart)				1	
60	Incremental course				1	
61	Progressive course				1	
62	Association with infections?				0	
63	Maternal HELLP-syndrome				0	
64	Diabetes mellitus Typ II (maternale heredity)				1	
65	Microsomia				1	
66	Hearing impairment				1	
67	Psychiatric episodes				0	
68	Miscariages				0	
69	Infertility				0	
70	SIDSy				0	
If there are other children, please also complete this questionnaire for those children.						
71	Does the family include children with similar / identical clinical presentations??				1	
Enter additional symptoms here						
72						
73						
74						
75						
76						
77						
78						
79						
80						
81						

Evaluation Part 1				
Very important symptoms	Total of those present		Total symptoms not examined	
Important symptoms	Total of those present		Total symptoms not examined	

Evaluation Part 2

Tick the symptoms present here - * Grey-shaded fields are not included in the questionnaire

Mitochondrial syndromes

	NARP		MELAS		MERRF		Pearson		MNGIE	
Typical symptoms	Neuropathy		Stroke-like episodes		Myocloniae		exocrinal pancreas insufficiency		Intestinal Pseudoobstruction	
	Cerebellar signs		Facultative: Microsomia		Epilepsy		Pancytopenia		Neuropathy	
	Retinal pigment impairment		Facultative: Pubertas tarda		Progressive course of disease				Weakness	
			Facultative: Diabetes mellitus Type II		Ragged red fibres *				MR alterations *	
			Facultative: Hearing impairment							
			Lactate increase *							

	KSS		Barth		Leigh		Alpers		Mohr-Tranebjaerg	
Typical symptoms	CPEO		Neutropenia		Psychomotoric retardation		Psychomotoric retardation		Extra-pyramidal symptoms	
	Progressive course of disease		Cardiomyopathy		Progressive course of disease		Progressive course of disease		Hearing impairment	
	Ataxia		Gender = male		Facultative: Loss of acquired abilities		Facultative: Loss of acquired abilities		Facultative: Psycho-motoric retardation	
	Conduction system impairment				Brain stern symptoms		Epilepsy		Facultative:Progressive disease	
	Increased cerebrospinal fluid protein*				Facultative: Cerebellar symp-toms		Microzephaly		Gender = male	
					Typical MY alterations *		Typical EEG alterations *			
							Laboratory chemistry: liver involvement *			

Questionnaire instructions

Aims of the questionnaire

This questionnaire was developed in the context of the development of the guidelines for the diagnosis and treatment of mitochondrial diseases in children
(http://www.uniduesseldorf.de/WWW/AWMF/ll/ll_list.htm; http://aps-med.de).
If, during the diagnostic process relating to a patient, the hypothesis of a mitochondrial disease has arisen, a systematic anamnesis should first be conducted, together with an exploration of symptoms and indications (referred to below as "clinical presentation") relating to the general and neurological status. This should be done before initiating diagnostic measures such as imaging, testing bodily fluids or other procedures. This questionnaire includes all those clinical presentations which the experts who were questioned when developing this guideline (see Chapter 3.3.2) considered indicative of a need for further specific diagnostic measures.
The questionnaire is not, strictly speaking, a diagnostic instrument. That means that it does not result in a mitochondrial diagnosis, or quantify the probability of a mitochondrial diagnosis. This is because, although clinical presentations have a high level of sensitivity (e.g., patients with a verified mitochondrial diagnosis generally have one or more of the features listed, or have a characteristic combination of features), the level of specificity is low (i.e., a great many patients with non-mitochondrial diagnoses may also display one or more of the features mentioned).
Therefore, this questionnaire is an aid for recording, systemising and evaluating the clinical presentation found during the course of diagnostics in respect of mitochondrial diseases. It is therefore no substitute for clinical expertise or further diagnostic measures.

Structure of the questionnaire

The questionnaire has several components which should be processed and/or used in the sequence specified.

1. The questionnaire itself:
Here, 71 clinical presentations, classified according to organs or organ systems, are evaluated to find whether they are present, not present or have not been examined.

2. Evaluation according to importance and implementation of examinations:
In accordance with expert opinion, the presence of the various clinical presentations may be of varying significance when deciding to proceed further. Therefore, the presentations listed in the questionnaire have been divided into important and very important presentations. Both the presentations present in respect of a patient, and those which have not been investigated, should be evaluated. The nature and number of unexamined presentations, in particular, may indicate features which have yet to be recorded, or may indicate the degree of completeness.

3. Evaluation according to syndromes
The clinical presentations found during the investigation can then be compared with those presentations characteristic of various mitochondrial syndromes. In this regard, too, it should be noted that that due to the lack of presentation specificity, the presence of a presentation pattern should not lead one to conclude that a syndrome is present. The table of syndromes should be viewed as a heuristic structural aid for proceeding further, rather than as a diagnostic algorithm.

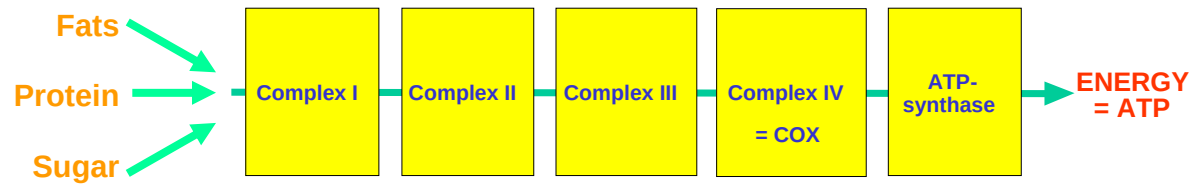
Appendix 4 Code of practice for discussions with parents

The following text can serve as speaking notes for a parental consultation:

What are mitochondriopathies?

Mitochondriopathies are inborn disorders of the mitochondriae. These are present in almost every cell in the body. They are known as the cells' 'power plants', since they provide 90% of the energy for the organism. One of the tasks of the mitochondriae is to convert the energy released by the degradation of nutrients (sugar, protein, fat) into a storage form (ATP), and to make it available to the organism. Disorders in this process result in an energy deficiency. The respiratory chain is the central component of the mitochondriae. It gets its name from the fact that so-called cell respiration takes place in the respiratory chain, and it can only function with oxygen.

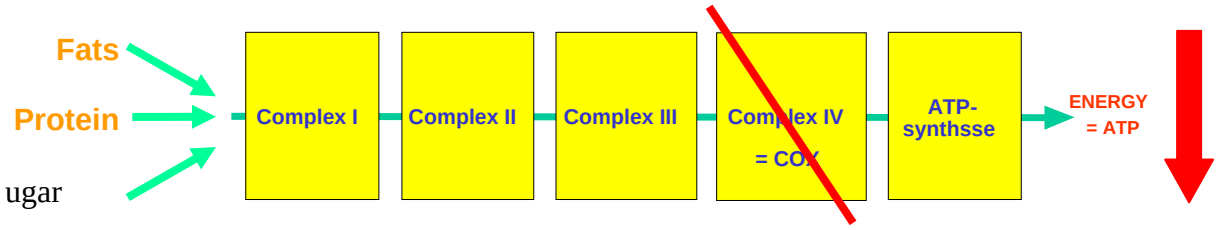
Fig. 3 The energy obtained from nutrients is converted into the ATP form of energy storage within the mitochondriae, and made available to the cells.



Respiratory chain

All respiratory chain disorders result in an energy deficiency which may be more or less pronounced.

***Fig. 4:** Example: disorder in Complex IV of the respiratory chain (cytochrome-C-oxidase) will result in an energy deficiency*



Respiratory chain

The following disorders are known:

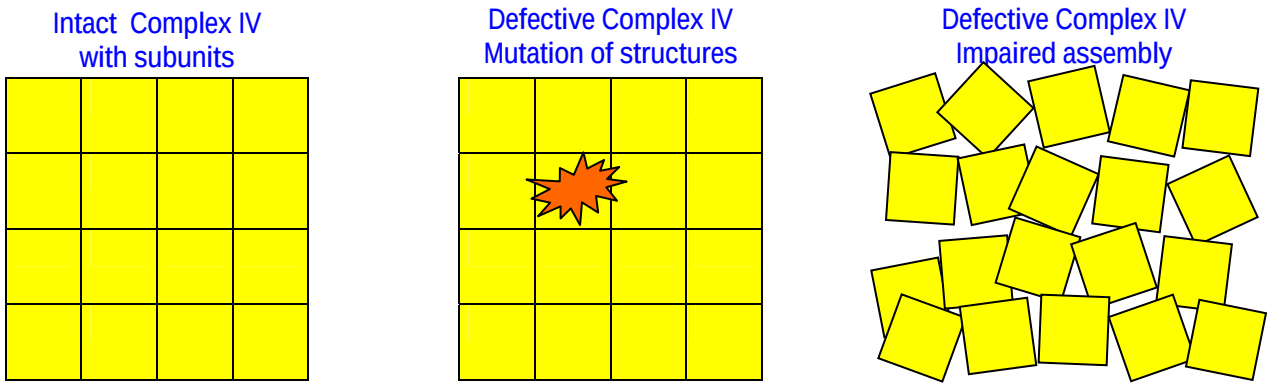
- Disorders of individual respiratory chain complexes
- Disorders in a combined form

The causes of these disorders are as follows:

- Changes in the structure of the subunits
- Disorders relating to the composition of the subunits

The variety of these disorders also explains the diverse disease courses. The actual cause is only known for some mitochondrial diseases.

***Fig. 5**: Complex IV consists of different subunits. Mutations in individual subunits, or anomalies in the assembly of the subunits, result in an impaired function of Complex IV*



All organs may be affected. Organs with high energy requirements – such as muscles, heart and brain, as well as the liver and kidneys – are particularly involved. Which organs are affected depends on the underlying defect. The degree to which the organ involvement is pronounced can vary greatly. This means that the consequences also vary.

Typical signs of illness are as follows:

- Muscles: muscle weakness, impaired gait, pain
- Heart: cardiac muscle weakness, dysrhythmia
- Brain: impaired mental and motoric development, seizures, migraine, visual and hearing impairments
- Other: impaired growth, diabetes and much more.

Prognostics

The prognosis depends on the cause, and may vary from clinical picture to clinical picture. There are diseases which may be fatal in the case of young infants, while - in the case of other diseases - symptoms only occur in adulthood.

Treatment

Up to now, there is no basic treatment for mitochondriopathies.
The aim of treatment is to improve the symptoms and to slow down the progress of the disease. The effect of a treatment varies from clinical picture to clinical picture and from patient to patient. Treatment must be individually adapted.

Treatment components:

- Avoidance of stress situations
- Avoidance of prolonged fasts
- Special diet
- High-dosage treatment with vitamins and ‘co-factors’

Recurrence risk for further pregnancies

All heredities are known in respect of mitochondrial diseases. Therefore, the risk of recurrence can only be assessed following the diagnostic conclusion. Counselling concerning the recurrence risk should then be carried out by a genetic counsellor or a doctor specialising in human genetics.

12. Abbreviations

LL	Guidelines
OXPHOS	Oxidative Phosphorylation
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
NAD	Nicotine amide adenine dinucleotide
FAD	Flavin adenine dinucleotid
PDHC	Pyruvate hydrogenase complex
AWMF	Working Group of the Scientific Medical Specialist Associations