

# Mitochondrial Depletion Syndromes in Children and Adults

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**ABSTRACT:** To highlight differences between early-onset and adult mitochondrial depletion syndromes (MDS) concerning etiology and genetic background, pathogenesis, phenotype, clinical presentation and their outcome. MDSs most frequently occur in neonates, infants, or juveniles and more rarely in adolescents or adults. Mutated genes phenotypically presenting with adult-onset MDS include POLG1, TK2, TYMP, RRM2B, or PEO1/twinkle. Adult MDS manifest similarly to early-onset MDS, as myopathy, encephalomyopathy, hepato-cerebral syndrome, or with chronic progressive external ophthalmoplegia (CPEO), fatigue, or only minimal muscular manifestations. Diagnostic work-up or treatment is not at variance from early-onset cases. Histological examination of muscle may be normal but biochemical investigations may reveal multiple respiratory chain defects. The outcome appears to be more favorable in adult than in early-onset forms. Mitochondrial depletion syndromes is not only a condition of neonates, infants, or juveniles but rarely also occurs in adults, presenting with minimal manifestations or manifestations like in the early-onset forms. Outcome of adult-onset MDS appears more favorable than early-onset MDS.

**RÉSUMÉ:** Syndromes de déplétion de l'ADN mitochondrial (SDM) chez les enfants et les adultes. Le but de l'étude était de mettre en évidence les différences entre les SDM débutant tôt dans la vie et ceux qui apparaissent chez l'adulte en ce qui concerne l'étiologie et le contexte génétique, la pathogénèse, le phénotype, le mode de présentation clinique et l'issue. Les SDM surviennent le plus fréquemment chez les nouveau-nés, les nourrissons ou les enfants et rarement chez les adolescents ou les adultes. Le phénotype SDM qui apparaît chez l'adulte est associé à une mutation de certains gènes dont POLG1, TK2, TYMP, RRM2B ou PEO1/twinkle. Les manifestations du SDM de l'adulte sont similaires à celles des plus jeunes, soit une myopathie, une encéphalo-myélopathie, un syndrome hépato-cérébral ou une ophtalmoplégie externe progressive chronique, de la fatigue ou seulement des manifestations musculaires peu marquées. La démarche diagnostique et le traitement sont les mêmes que ceux de la SDM des plus jeunes. L'examen histologique du muscle peut être normal, mais la biochimie peut révéler la présence de multiples défauts de la chaîne respiratoire. L'issue semble être plus favorable chez les adultes que quand la maladie se manifeste tôt dans la vie. Les SDM ne sont pas uniquement des maladies des nouveau-nés, des nourrissons ou des jeunes, mais elles surviennent aussi, mais rarement, chez des adultes. Les manifestations sont alors minimales ou elles peuvent être semblables à celles des formes précoces de la maladie. L'issue des SDM débutant à l'âge adulte semble être plus favorable que dans les formes à début précoce.

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Mitochondrial depletion syndromes (MDSs) are commonly severe, recessively inherited disorders with onset in infancy and early death. Mitochondrial depletion syndromes are characterized by marked tissue reduction of the mitochondrial DNA (mtDNA) copy number due to mutations in nDNA located genes reducing replication of the mtDNA<sup>1</sup>. Since both genomes are involved, MDS are also called disorders of the nuclear-mitochondrial intergenomic signalling<sup>2</sup>. Nuclear genes mutated in MDS include the polymerase gamma 1 (POLG1), deoxyguanosine-kinase (DGUOK), thymidine kinasekinase (TK2), MPV17 mitochondrial inner membrane protein (MPV17), thymidine phosphorylase (TYMP), succinate-CoA ligase ADP-forming, beta subunit (SUCLA2), succinate-CoA ligase, alpha subunit (SUCLG1), ribonucleotide reductase M2 B (TP53 inducible) (RRM2B), and the chromosome 10 open reading frame 2 (C10orf2), also known as PEO1 / *twinkle*<sup>3</sup>. Three main phenotypes of MDS have been described, the myopathic form, the encephalo-myopathic form, and the hepato-cerebral form<sup>4</sup>. Four phenotypic presentations manifest as syndromic mitochondrial disorders, Alpers-Huttenlocher syndrome (AHS), a fetal disorder, mitochondrial neuro-gastro-intestinal encephalo-myopathy (MNGIE), a potentially treatable disease, infantile onset spinocerebellar ataxia (IOSCA), and

mitochondrial recessive ataxia syndrome (MIRAS)<sup>5</sup>. The myopathic form is associated with mutations in TK2 and RRM2B<sup>6</sup>, the encephalo-myopathic form with mutations in SUCLG1, or SUCLA2<sup>6</sup>, and the hepato-cerebral form with mutations in POLG1, PEO1, MPV17, or DGUOK<sup>6</sup>. Since the first description of a MDS mutation in the late nineties<sup>7</sup>, much progress has been achieved concerning the techniques to quantify the amount of mtDNA in various tissues and to identify mutations in the responsible genes. However, in the vast majority of the cases MDS was diagnosed in infants and only rarely in adults<sup>8</sup>. Aim of the present review was to highlight and discuss differences between early-onset and adult-onset MDS concerning etiology and genetic background, pathogenesis, phenotype and clinical presentation, treatment, and outcome of MDS patients.

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## History

Alpers-Huttenlocher syndrome was first described in the 1930s without knowing the genetic background at that time<sup>9</sup>. The initial clinical description of MNGIE was reported in 1976<sup>10</sup>. Depletion of mtDNA in humans was first detected in 1991 in two infants with myopathy and liver disease without knowing the underlying mutation at that time<sup>7</sup>. The first nDNA mutation underlying MDS was found in the POLG1 gene and reported in 1999 by Naviaux et al<sup>11</sup>. In the same year the underlying molecular defect of MNGIE was defined by Nishino et al<sup>12</sup>. The first mutation in the DGUOK gene causing MDS was reported in 2001 by Mandel et al<sup>13</sup>. Thymidine-kinase-2-mutations were first detected as cause of MDS in 2001 by Saada et al<sup>14</sup>. SUCLA2-deficiency was first recognised as the cause of encephalo-myopathic MDS in infants by Elpeleg et al in 2005<sup>15</sup>. The first mutation in the MPV17 gene causing MDS was reported in 2006 by Spinazzola et al<sup>16</sup>. Encephalo-myopathic MDS due to SUCLG1-mutations was first described by Ostergaard et al in 2007<sup>17</sup>. MDS due to mutations in the RRM2B gene were first described in 2007 by Bourdon et al<sup>18</sup>. The first mutation in twinkle/PEO1 associated with MDS was reported in 2007 by Sarzi et al<sup>19</sup>.

## Types of MDS

### 1. POLG1 deficiency

#### a) Phenotype

POLG1-mutations causing MDS, present as syndromic or non-syndromic mitochondrial disorder. The most well-known of the syndromic MDS due to POLG1-mutations is AHS, manifesting as fatal brain and liver disease in children or young adults<sup>20</sup>. Alpers-Huttenlocher syndrome additionally presents with intractable seizures, neurodegeneration, and liver disease<sup>9</sup>. Other AHS patients present with psychomotor regression, refractory seizures, stroke-like episodes, hepatopathy, or ataxia<sup>21</sup> or develop failure to thrive, feeding difficulties, various types of infantile epilepsy, psychomotor developmental delay, or muscle hypotonia<sup>22</sup>. In addition to POLG1-mutations, hepatopathy in MDS may be associated with mutations in the DGUOK, MPV17, SUCLG1, or PEO1 / twinkle gene<sup>23</sup>. Juvenile-onset AHS can begin with migraine-like headache and epilepsy and may lead to terminal status epilepticus and hepatic failure<sup>24</sup>. Movement disorder can be a rare clinical manifestation of AHS<sup>25</sup>. In some patients AHS additionally manifests as hypoglycemia, elevated lactate, moderate ketosis, and hepatic failure<sup>26</sup>, another syndromic MDS due to POLG1-mutations is MNGIE. Additionally, a number of non-syndromic phenotypes of POLG1-related MDSs have been described. An infant with MDS due to a POLG1-mutation presented with psychomotor retardation, hypotonia, and abnormal pain perception, resulting in debilitating biting of the thumb, lip, and tongue<sup>27</sup>.

#### b) Onset/outcome

Early-onset: Onset of MDS due to POLG1-mutations is usually in early infancy or childhood<sup>28</sup>. The outcome of AHS is usually fatal in early infancy.

Adult onset: Only some patients with MDS due to POLG1-mutations have been reported in whom the onset of the clinical manifestations was in adulthood<sup>28</sup>. In two patients aged 86 and

50 years (y) with late-onset chronic progressive external ophthalmoplegia (CPEO) and sensory neuropathy due to known POLG1-mutations, mtDNA studies in skeletal muscle showed evidence of multiple deletions and approximately 64% depletion of the mtDNA<sup>29</sup>. In a 58y female compound heterozygous POLG1-mutations resulted in multiple mtDNA deletions and depletion manifesting as multiple system atrophy<sup>30</sup>.

#### c) Genotype

Generally, POLG1-mutations (<http://tools.niehs.nih.gov/polg/>) are associated with an extremely heterogeneous spectrum of phenotypes, ranging from adult-onset CPEO due to multiple mtDNA deletions, to rapidly fatal AHS due to mtDNA depletion (Table 1)<sup>31</sup>. POLG1-mutations leading to MDS include point-mutations and deletions<sup>31</sup>. POLG1-mutations causing AHS are most frequently present in a compound heterozygous form<sup>32</sup>. The most frequent POLG1-mutations causing AHS are the substitutions c.467A>T and c.2243G>C<sup>20,21,33</sup>. In addition to mtDNA depletion, AHS can be also due to multiple mtDNA deletions (Table 1)<sup>21</sup>.

#### d) Instrumental findings

Muscle biopsy shows myopathic changes with cytochrome-c-oxidase (COX)-deficiency<sup>21</sup>. COX-deficiency is uniform and characteristic for severe complex IV deficiency, as in AHS or mitochondrial disorder due to SCO2-mutations. Contrary to uniform COX-deficiency in children, adults or adolescents show complete absence of COX activity exclusively in single fibers (COX-ve fibers). Histological examination of the muscle biopsy can be normal but biochemical investigation may reveal multiple defects of respiratory chain complexes (RCCs), in particular RCCII+RCCIII, and RCCIV<sup>27</sup>. POLG1-mutations often do not alter complex II since complex II contains no mtDNA encoded sub-units<sup>27</sup>. Cerebral imaging can be normal or reveal hypoplasia of the corpus callosum, disturbed myelination of the temporo-occipital area, or hydrocephalus<sup>27</sup>. Neuropathologic investigations reveal lesions in the right striatal area and the inferior colliculi, typical for Leigh syndrome<sup>26</sup>. The biochemical profile most suggestive of a MDS is multiple respiratory chain deficiencies with relative sparing of complex II.

**Table 1: Onset of the various types of MDS**

Gene/onset	Infantile	Juvenile	Adolescent	Adult
POLG1	x	x		x
DGUOK	x	x		
TK2	x			x
MPV17	x	x		
TYMP		x	x	x
SUCLA2	x			
SUCGL1	x			
RRM2B	x			x*
PEO1/twinkle	x			x

Infantile: age 0-7y, juvenile: age 8-14y, adolescent: age 15-21y, adult: >21y, \* a single patient with a MNGIE-phenotype

## 2. DGUOK-deficiency

### a) Phenotype

Phenotypically, DGUOK-mutations manifest in two forms, as hepato-cerebral MDS with a neonatal onset<sup>34</sup> or as isolated hepatopathy<sup>35,36</sup>. Patients with the hepato-cerebral form present with failure to thrive, microcephaly, rotatory nystagmus, muscle hypotonia, hepato-splenomegaly, jaundice, or ascites<sup>37</sup>. With progression of the disease patients develop cholestatic liver failure with hypoalbuminemia, portal hypertension, intractable ascites, hypersplenism with thrombocytopenia, and severe coagulopathy<sup>35,37</sup>. A rare complication of DGUOK-deficiency is hepato-cellular carcinoma<sup>38</sup>. Patients with isolated liver disease may additionally develop renal insufficiency<sup>36</sup>.

### b) Onset/outcome

Early-onset: Onset is neonatal<sup>35,37</sup> or in early infancy<sup>34</sup>. Babies affected by the hepato-cerebral form usually die within a few months after birth<sup>37</sup>. Patients affected by the isolated hepatic form have an infantile or juvenile onset<sup>35</sup> and survive into adolescence<sup>39</sup>.

Adult onset: No patients with adult onset of the disease have been reported so far.

### c) Genotype

Deoxyguanosine-kinase-deficiency is transmitted in an autosomal recessive manner<sup>36</sup>. Deoxyguanosine-kinase is one of the two mitochondrial deoxynucleoside salvage pathway enzymes involved in precursor synthesis for mtDNA replication<sup>39</sup>. Deoxyguanosine-kinase catalyses the phosphorylation of purine deoxy-ribonucleosides, the first step of the mitochondrial deoxypurine salvage pathway<sup>40</sup>. One of the point-mutations in the DGUOK gene causing MDS is the transition c.313C>T in exon 3, resulting in a stop codon<sup>37</sup>. Other mutations are the transitions c.34C>T<sup>40</sup> and c.3G>A, the transversion c.494A>T, the insertion c.766\_767insGATT<sup>41</sup>, and the splice site insertion c.444-62C>A<sup>42</sup>. Mutations which cause isolated liver disease include the transition c.137A>G and the transversion c.797T>G<sup>39</sup>. Deoxyguanosine-kinase-deficiency-mutations not only cause MDS but also multiple mtDNA deletions, manifesting as mitochondrial myopathy with or without CPEO, recurrent rhabdomyolysis, hepatopathy, lower motor neuron disease, or mild cognitive impairment (Table 1)<sup>43</sup>. In some cases DGUOK-mutations resulted from maternal uniparental disomy 2<sup>44</sup>.

### d) Instrumental findings

Blood testing shows lactacidosis, hyperbilirubinemia, elevated liver transaminases, coagulopathy, elevated ferritin,  $\alpha$ -fetoprotein, severe preprandial hypoketotic hypoglycemia, hypoalbuminemia, and elevated alanine and tyrosine<sup>35,37,41</sup>. Urinary organic profile shows mild elevation of dicarboxylic acids<sup>37</sup>. Cerebral imaging is usually normal in DGUOK-deficiency but some patients show moderate hyperintensity of the globus pallidus bilaterally and subtentorial abnormal myelination<sup>42</sup>. Liver biopsy shows bile ductular proliferation, cholestasis, micro-vesicular steatosis, and bridging fibrosis leading to micronodular transformation or cirrhosis<sup>35,37,41</sup>. Histological work-up of the pancreas shows islet cell hyperplasia

resulting in hyperinsulinism and severe hypoglycemia<sup>41</sup>. In mtDNA depleted tissues iron overload can be found<sup>41</sup>.

## 3. TK2 deficiency

### a) Phenotype

Thymidine kinasekinase (TK2)-mutations manifest clinically as myopathic form of MDS<sup>45</sup>, more rarely as encephalomyopathy<sup>46-48</sup>, or very rarely as hepato-myopathic form<sup>49</sup>. Patients with the myopathic form present with generalized muscle weakness predominantly of axial and proximal muscles but also affecting facial, ocular, and respiratory muscles<sup>50</sup>. Some of these patients develop normally until 12-14 months-of-age to become symptomatic thereafter<sup>51</sup>. Pediatric patients with the encephalo-myopathic form have a normal early developmental phase, followed by psychomotor regression, seizures, or myopathy<sup>52</sup>. In some infants, MDS manifests with periventricular pseudocysts<sup>53</sup>. Some patients develop severe hypoacusis<sup>54</sup>. Rarely, TK2-mutations initially present phenotypically as spinal muscular atrophy<sup>52,55</sup>. Patients with adult-onset MDS due to TK2-mutations present with slowly progressive myopathy<sup>50</sup>.

### b) Onset/outcome

Early-onset: The myopathic form presents as an early-onset or adult-onset disease<sup>50</sup>. Patients with the early-onset form usually die within a few months after birth<sup>49,55,56</sup> or survive the first or second decade of life<sup>55,57</sup>. Patients with the encephalomyopathic phenotype die within a few months or years after birth<sup>47,48</sup>.

Adult-onset: Patients with adult-onset MDS due to TK2-mutations present with slowly progressive myopathy<sup>50</sup>, manifesting as generalised muscle weakness of the axial and proximal limb muscles. Facial, ocular, and respiratory muscle weakness was also reported in three other adult patients<sup>58</sup>. TK2-mutations also cause arCPEO with multiple mtDNA deletions (Table 1)<sup>59</sup>. Patients with the adult-onset form have normal life expectancy<sup>53</sup>.

### c) Genotype

TK2 mutations are present in the compound heterozygous form<sup>56</sup> or in the homozygous form<sup>47</sup>. TK2-mutations result in reduction of the mtDNA content down to <10% of normal<sup>60</sup>. Some TK2-mutations do not cause mtDNA depletion<sup>61</sup>. TK2-mutations not only cause MDS but also multiple mtDNA deletions, manifesting as arCPEO in adults (Table 1)<sup>58</sup>.

### d) Instrumental findings

Muscle biopsy shows typical features of mitochondrial myopathy with a mosaic pattern of COX-negative and ragged-red fibers<sup>50</sup>. Biochemical investigations reveal multiple RCC deficiencies<sup>50</sup> or normal activity of RCCs<sup>57</sup>. In accordance with the disease's relatively slow progression, the residual mtDNA content is higher in adult cases than that observed in pediatric cases. This difference could not be explained by the type of TK2-mutations or by the residual TK2 activity<sup>50</sup>. The minimal amount of mtDNA density in single muscle fibers to allow residual COX activity was determined as 0.01 mtDNA/ $\mu\text{m}^3$ <sup>62</sup>.

#### 4. MPV17-deficiency

##### a) Phenotype

Mitochondrial inner membrane protein (MPV17)-mutations are responsible for a hepato-cerebral form of MDS<sup>6</sup>. The phenotype is characterised by recurrent episodes of severe hypoglycemia, hepatopathy evolving towards cirrhosis and liver failure, and growth retardation<sup>6</sup>. Patients present with poor feeding, failure to thrive, diarrhoea, and recurrent vomiting<sup>6</sup>. Within the first few months of life they develop generalised muscle wasting and muscle hypotonia<sup>6</sup>. Common neurological phenotypic features include microcephaly, ataxia, developmental delay, muscle weakness, seizures, ischemic stroke, or dystonia<sup>63-66</sup>. Patients who survive develop polyneuropathy and lesions of the cerebellum and the cerebral cortex<sup>6</sup>. The phenotype follows a two-stage presentation with metabolic dysfunction progressing to hepatic failure as the first stage and neurological involvement as the second stage<sup>64</sup>.

##### b) Onset/outcome

Earl onset: Onset is usually at birth or early infancy<sup>6</sup>. The outcome is generally poor and patients die within a few weeks or months after birth<sup>6,67</sup>. Exceptionally, some patients survive into their late teens<sup>63,68</sup>.

Adult onset: No patients with adult onset of the disease have been reported so far.

##### c) Genotype

Mitochondrial inner membrane protein (MPV17) encodes a small protein of unknown function located on the inner mitochondrial membrane<sup>6</sup>. To date 20 mutations in 29 patients have been described<sup>6,69</sup>. Homozygous, heterozygous or compound heterozygous nonsense or missense mutations or macrodeletions have been reported<sup>64</sup>. Mutations in the MPV17 gene additionally result in multiple mtDNA deletions but normal mtDNA content (Table 1)<sup>70</sup>. Mitochondrial inner membrane protein mutations are also responsible for Navajo neuro-hepatopathy<sup>69</sup>, which is a hepato-cerebral variant of MDS, presenting with hepatopathy, polyneuropathy, corneal anesthesia and scarring, acral mutilation, leukoencephalopathy, failure to thrive, and recurrent metabolic acidosis with intercurrent infections<sup>68</sup>.

##### d) Instrumental findings

Blood chemical investigations show elevated liver transaminases, hyperbilirubinemia, hypoalbuminemia, and coagulopathy<sup>6</sup>. Additionally, plasma amino acids, urine amino acids, and serum lactate may be elevated. Abdominal imaging shows hepatomegaly and nephrolithiasis<sup>6</sup>. Cerebral magnetic resonance imaging (MRI) shows cortical and subcortical hyperintensities involving the cerebellar white matter and the hili of the dentate nuclei<sup>6</sup>. Other patients show hyperintensities in the reticular formation of the lower brain stem and within the reticulospinal tracts<sup>71</sup>. Aminoaciduria in MPV17-mutations is attributed to proximal tubulopathy<sup>69</sup>.

#### 5. TYMP deficiency

##### a) Phenotype

Thymidine phosphorylase-mutations manifest clinically as MNGIE, which is clinically characterised by ptosis, ophthalmoparesis, gastro-intestinal dysmotility, cachexia, neuropathy, myopathy, and leucoencephalopathy<sup>70,72</sup>. Gastrointestinal manifestations include diarrhoea, abdominal pain, nausea or vomiting, abdominal cramps, weight loss, borborygmi, failure to thrive, intestinal pseudoobstruction, bloating, or intestinal invagination<sup>70</sup> why most patients develop severe intestinal pseudo-obstruction<sup>73</sup>. Ocular manifestations include ptosis, ophthalmoparesis, eye wandering, or loss of vision<sup>70</sup>. CNS manifestations include leucoencephalopathy, but it remains asymptomatic in 80% of the cases. In the remaining patients it manifests as cognitive impairment, dementia, seizures, or headache<sup>70</sup>. Peripheral nervous system (PNS) manifestations include demyelinating polyneuropathy and myopathy, the latter in about one quarter of the patients. Initial manifestations other than gastrointestinal and ocular include neuropathy, hypoacusis, dry mouth, tinnitus, or myopathy and exercise intolerance<sup>70</sup>. Initial manifestations are often polyneuropathy or CPEO. An additional manifestation is endocrine or exocrine pancreas insufficiency, diverticulosis, hypertriglyceridemia, short stature, cardiomyopathy, or course bronze skin<sup>70</sup>. Most patients present with the complete manifestations of the syndrome and only some with incomplete expression of the phenotype<sup>73</sup>. In the early stages MNGIE can be misdiagnosed as hereditary neuropathy, eating disorder, coeliac disease, inflammatory bowel disease, or Whipple disease<sup>70</sup>.

##### b) Onset/outcome

Early onset: Onset is typically before age 30y (mean: 18y)<sup>70</sup>. However, the majority of patients report their first symptoms before age 12y<sup>70</sup>. An early-onset and adult-onset MNGIE type are differentiated<sup>74,75</sup>. Though there are indications that allogeneic hematopoietic stem cell transplantation is beneficial in at least some MNGIE patients, it is still associated with a high mortality<sup>70</sup>. Early onset does not correlate with short life expectancy<sup>70</sup>.

Adult onset: Patients with adult-onset MNGIE present with similar manifestations as patients with the early-onset form. Contrary to patients with early-onset MNGIE, patients with late-onset MNGIE can develop rapidly progressive disease<sup>70</sup>. Mean age at death in these patients is 35y<sup>70</sup>. The later the onset of adult MNGIE the longer the patients survive<sup>76</sup>.

##### c) Genotype

Among TYMP-mutations causing MNGIE, point-mutations are the most common type of splice-site mutations<sup>70</sup>. To date, over 30 different mutations have been reported<sup>77</sup>. TYMP-mutations are transmitted via an autosomal-recessive trait of inheritance and not only cause MDS but also multiple mtDNA deletions (Table 1)<sup>73</sup>. TYMP-mutations result in complete abolition or severe reduction of the TYMP activity<sup>73</sup>.



**d) Instrumental findings**

Rapid tests to diagnose MNGIE include determination of the TYMP activity, which is decreased in MNGIE patients, and determination of the thymidine levels, which are increased in MNGIE<sup>73</sup>. In the early-onset form, TYMP-mutations cause thymine phosphorylase activity reduction to <10% of normal<sup>74</sup>. In late-onset MNGIE the activity of the thymine phosphorylase is 10-15%<sup>70</sup>. CSF protein can be slightly elevated<sup>70</sup>. White matter lesions in MNGIE are patchy initially, eventually becoming diffuse or confluent<sup>70</sup>. In some patients severe hypokalemia occurs<sup>70</sup>. Some patients present with lactacidosis<sup>70</sup>. Nerve conduction studies reveal demyelinating polyneuropathy in most patients. Muscle biopsy shows COX-negative fibers and, more rarely, ragged-red fibers. Biochemical investigations reveal deficient RCCIV, RCCI and RCCIV, or RCCI+III+IV activities<sup>70</sup>. However, MNGIE patients without involvement of skeletal muscle have been also reported<sup>78</sup>.

**6. SUCLA2-deficiency****a) Phenotype**

SUCLA2-related MDS is a rare disorder of infancy clinically characterised by neonatal or infantile-onset severe muscle weakness, muscle hypotonia, muscle wasting, resulting in failure to achieve independent ambulation, progressive kypho-scoliosis, dystonia, hyperkinesia with athetoid or choreiform movements, epilepsy (infantile spasms, generalised convulsions), growth retardation, or severe hypoacusis<sup>79,80</sup>. This compilation of manifestations represents the Leigh-like phenotype.

**b) Onset/outcome**

Early onset: Onset of clinical manifestations is at birth or within the first few months thereafter<sup>4,80</sup>. The outcome is poor with early lethality<sup>80</sup>.

Adult onset: No patients with adult onset of the disease have been reported so far.

**c) Genotype**

SUCLA2 deficiency is due to mutations in the SUCLA2 gene encoding the ADP-binding specific  $\beta$ -subunit of the tricarboxylic acid (TCA)-cycle enzyme succinyl-CoA synthetase. SUCLA2 is related to SUCLA1 in that SUCLA1 encodes the catalytic  $\alpha$ -subunit of the TCA-cycle enzyme succinyl-CoA synthetase. Mutations in the SUCLA2 gene reported to cause MDS include the point mutations c.352G>A, c.850C>T, c.534+1G>A<sup>79</sup>, and c.308C>A<sup>81</sup>. SUCLA2-associated MDS is most prevalent on the Faroe islands with a mutant allele frequency of 2%<sup>79</sup>.

**d) Instrumental findings**

Methyl-malonic acid is mildly or moderately elevated in the urine of these patients<sup>81</sup>. Methyl-malonic acid can be also elevated in the serum. Compared to methylmalonic aciduria due to mutations in the methylmalonic mutase, elevation of methylmalonic acid in SUCLA2 deficiency is mild to moderate. There may be lactacidosis and increased C3-carnitine or C4-dicarboxylic-carnitine<sup>80</sup>. Urinary excretion of C4-dicarboxylic-carnitine is markedly elevated<sup>80</sup>. Cerebral imaging shows diffuse

atrophy, lesions in the putamen and caudate nuclei, or delayed myelination, similar to findings in Leigh syndrome<sup>79,80</sup>.

**7. SUCLG1-deficiency****a) Phenotype**

SUCLG1-deficiency is clinically characterised by intra-uterine growth retardation (dysmaturity), hepatomegaly, muscle hypotonia, respiratory insufficiency due to acidosis, and severe hypothermia<sup>17</sup>. Respiratory insufficiency is usually so severe that patients require ventilatory support<sup>17</sup>.

**b) Onset/outcome**

Early onset: Onset is congenital<sup>17</sup> and the outcome poor with death within a few days after birth<sup>4,17</sup>. Occasionally, patients survive into adolescence<sup>82</sup>.

Adult onset: No patients with adult onset of the disease have been reported so far.

**c) Genotype**

SUCLG1 encodes the alpha-subunit of the succinate-CoA ligase<sup>83</sup>. Deletions and missense mutations causing MDS have been reported<sup>82-86</sup>.

**d) Instrumental findings**

There is severe neonatal lactacidosis but also elevation of pyruvate<sup>17</sup>. Some patients develop hypoglycemia<sup>17</sup>. Urine screening shows elevated levels of lactate and pyruvate, mildly or moderately elevated excretion of methyl-malonate and methylcitrate, and slightly elevated excretion of the Krebs cycle intermediates fumarate, malate, citrate, and 2-oxoglutarate<sup>17</sup>. Plasma and urine amino acid determination reveals highly elevated taurine and glycine and moderately elevated lysine and alanine<sup>17</sup>. Electroencephalography (EEG) can show focal paroxysmal activity, sharp waves, and triphasic potentials bilaterally<sup>17</sup>. Post-mortem morphology of the skeletal muscle shows intracellular lipid accumulation exclusively<sup>17</sup>. Liver histology shows microvesicular steatosis and sinusoidal dilatation<sup>17</sup>. Activity of RCCI+III+IV can be decreased in muscle and liver<sup>17,83,86</sup>.

**8. RRM2B deficiency****a) Phenotype**

Clinical manifestations start at birth or shortly afterwards and include failure to thrive, congenital deafness, muscle weakness, axial hypotonia, diarrhoea, proximal tubulopathy, seizures, lactacidosis, respiratory distress, and intractable status epilepticus<sup>18,21</sup>. In a single adult patient RRM2B-mutations manifested as MNGIE-phenotype<sup>87</sup>.

**b) Onset/outcome**

Early onset: Onset of clinical manifestations is congenital or shortly after birth with rapidly progressive course and death within a few weeks or months later<sup>18,21</sup>. Less severe phenotypes have been also reported in some patients who survived until age three years<sup>88</sup>.

Adult onset: A single patient with onset at age 30y who developed a MNGIE phenotype due to a RRM2B-mutation has been reported<sup>87</sup>. Typical findings indicating adult onset RRM2B deficiency include bulbar dysfunction, hearing loss, and gastrointestinal dysfunction (gastrointestinal dysmotility, borborygmi, early satiety, diarrhea, constipation, vomiting, weight loss)<sup>89</sup>.

### c) Genotype

The phenotype is caused by nonsense, missense, splice-site, or in-frame deletions in the RRM2B gene<sup>18</sup>. These mutations lead to mtDNA depletion to 1% of the normal content<sup>18</sup>. RRM2B-mutations not only cause mtDNA depletion but also multiple mtDNA deletions, resulting in a KSS-phenotype (Table 1)<sup>90</sup>.

### d) Instrumental findings

Blood chemical investigations show mild to severe lactacidosis<sup>18</sup>. Lactate can be also elevated in the cerebrospinal fluid (CSF)<sup>91</sup>. Histological investigations of the muscle biopsy shows COX-negative fibers and ragged-red muscle fibers<sup>18</sup>. Biochemical investigations of the muscle homogenate reveal decreased malate and glutamate oxidation, isolated RCCIV deficiency, combined RCCI+III+IV deficiency<sup>18</sup>, or combined RCCI+III+IV+V deficiency<sup>92</sup>. Magnetic resonance imaging of the cerebrum shows mild hypomyelination<sup>92</sup>. Electro-encephalogram shows generally increased slow wave activity<sup>91</sup>. In addition to mtDNA depletion, RRM2B-mutations also cause multiple mtDNA deletions in adults (Table 1)<sup>90</sup>. Urinary organic acids show combined keto-acidosis and lactic acidosis<sup>91</sup>.

## 9. PEO1 / Twinkle deficiency

### a) Phenotype

Mitochondrial depletion syndromes due to twinkle-mutations manifest as encephalopathy, hepato-encephalopathy, or CPEO. In the first description of a twinkle-mutation causing mtDNA depletion, two siblings with the hepato-cerebral form of MDS were presented<sup>93</sup>. The phenotype was characterised by severe, early-onset encephalopathy, liver involvement, hypotonia, athetosis, ophthalmoparesis, hearing impairment, sensory neuropathy, intractable epilepsy, and ataxia<sup>94</sup>. In adults, the most common manifestation of MDS due to twinkle-mutations is adCPEO, characterised by isolated affection of external eye muscles<sup>94</sup>. Twinkle deficiency also manifests as infantile-onset spinocerebellar ataxia (IOSCA) or as mitochondrial recessive ataxia syndrome (MIRAS)<sup>5</sup>, why IOSCA and MIRAS should be regarded as subtypes of MDS<sup>5</sup>. More rare manifestations in IOSCA include refractory status epilepticus, epilepsia partialis continua, migraine-like headache, and psychiatric abnormalities<sup>95</sup>. The initial status epilepticus occurs between 15 and 34y of age<sup>95</sup>.

### b) Onset/outcome

Early onset: Twinkle-mutations usually cause early-onset MDS<sup>94</sup>.

Adult onset: Rarely, adult-onset MDS due to twinkle-mutations has been reported<sup>8</sup>. The most common adult-onset

manifestation of twinkle-mutations is adCPEO<sup>94</sup>. Other initial manifestations of adult-onset MDS due to twinkle-mutations are epilepsy, migraine-like headache, and psychiatric abnormalities<sup>94</sup>.

### c) Genotype

Mutations in the twinkle gene most frequently cause multiple mtDNA deletions (Table 1)<sup>93,96</sup>. Recently, however, it has been shown that certain twinkle-mutations also cause mtDNA depletion, clinically manifesting as encephalopathy or hepato-encephalopathy<sup>93,95</sup>. These phenotypes resemble those of POLG1-mutations causing MDS (AHS)<sup>95</sup>. Accordingly, mtDNA depletion is most prevalent in liver and only mild in the skeletal muscle<sup>94</sup>. Twinkle-mutations causing mtDNA depletion occur in the homozygous or compound heterozygous form<sup>95</sup>.

### d) Instrumental findings

Serum transaminases can be elevated<sup>94</sup>. Cerebral MRI shows focal stroke-like lesions, which vary between small cortical lesions to large hemispheric edematous lesions<sup>95</sup>. Neuropathological investigations show laminar cortical necrosis or hippocampal damage<sup>95</sup>.

## Other causes of mtDNA depletion

In addition to MDS due to mutations in any of the nine genes, mtDNA depletion experimentally also occurs if OPA1 variants are silenced<sup>81,96</sup>. A further candidate gene that could be responsible for MDS is GFER<sup>81</sup> or DNA2. The mtDNA content can be reduced in HIV patients under nucleoside reverse transcriptase inhibitors (NRTIs)<sup>97</sup>. Highly active anti-retroviral therapy (HAART) leads to mtDNA depletion and lipotrophy through direct interference with POLG1. Additionally, HAART causes oxidative stress by increasing reactive oxidative species (ROS) production, which is buffered by the antioxidative capacity of mitochondria and up-regulation of the mitochondrial protease LON<sup>98</sup>. HIV patients on HAART and lipotrophy have mtDNA depletion in fat<sup>99</sup>.

## MDS in adults

In the majority of the cases, MDS is a condition of early infancy or juvenile age and has a poor prognosis. The reason why MDS is highly prevalent in the early ages is unclear. One reason could be that due to the poor prognosis in most of the MDSs, affected patients do not survive into adulthood. Mutated genes associated with early-onset MDS include POLG1, DGUOK, TK2, MPV17, TYMP, SUCLA2, SUCLG1, RRM2B, and PEO1 / twinkle. A typical infantile-onset MDS is IOSCA<sup>5</sup>. Thus, all genes involved in MDS present with the early-onset form but some of them (POLG1, TK2, TYMP, RRM2B, and PEO1) also present with adult-onset subtypes. Conditions, which represent adult-onset MDS include AHS due to POLG1-mutations occurring in young adults<sup>20</sup>, adult-onset MDS due to TK2-mutations presenting with slowly progressive myopathy<sup>50</sup> and normal life expectancy<sup>54</sup>, adult-onset MNGIE due to TYMP-mutations<sup>74,100</sup>, adult-onset MDS due to RRM2B-mutations, and adult-onset MDS due to PEO1 twinkle-mutations<sup>8,101</sup>. Why some of the MDSs survive into adulthood or have their onset in adulthood, is unknown. It can be speculated, however, that some

MDSs result in biochemical defects which are compatible with survival into adulthood or that the amount of mtDNA depletion has a progressive course and manifests clinically not before a certain cut-off is undercut. The phenotype of adult-onset MDS can vary greatly from that in early-onset MDS. While MDS due to POLG1 mutations frequently manifests as severe multisystem disease, MDS due to POLG1 mutations in adults presents as CPEO and sensory neuropathy<sup>29</sup> or as multiple system atrophy<sup>30</sup>. Patients with very late-onset MNGIE may present without neuropathy<sup>75</sup>. Adult patients carrying RRM2B mutations present with ophthalmoplegia, ptosis, gastrointestinal dysmotility, cachexia, peripheral neuropathy, and brain magnetic resonance imaging changes<sup>87</sup>. Overall, among MDS with adult onset, the clinical presentation may vary compared to early onset MDS but final conclusions on this matter can be drawn only after further studies on larger cohorts.

### Diagnosis

The diagnosis of MDS is based on the clinical presentation, blood chemical investigations, instrumental investigations, verification of the mtDNA depletion in muscle, liver, or cerebrum, and detection of the mutation underlying the mtDNA depletion. Differential diagnoses that have to be excluded are hemochromatosis in the hepatic form or hepato-cerebral form of MDS<sup>35</sup>.

### Techniques to detect mtDNA depletion

The most frequently applied technique to reveal mtDNA depletion is quantitative (real-time) polymerase chain reaction (PCR)<sup>3,27,56</sup>. mtDNA depletion is said to be best detected in tissues such as muscle, brain, or liver, but not in blood or skin fibroblasts<sup>3</sup>. This is why those tissues, which are predominantly affected, should be biopsied (Table 2). Comparative genomic hybridization (CGH) or high-density single-nucleotide polymorphism (SNP) array analysis are only rarely applied and may reveal MDS due to uniparental isodisomy<sup>102</sup>.

### Treatment

#### a) Non-invasive

There is no treatment available for MDS. Only symptomatic measures can be recommended in case of epilepsy, cognitive impairment, movement disorder, migraine-like headache, stroke-like episode, myopathy, failure to thrive, or liver disease<sup>103</sup>. In patients with myopathy or encephalo-myopathy, physical therapy could be beneficial to promote mobility and prevent contractures. Mechanical assistance with a wheelchair will guarantee mobility, bracing may delay kyphoscoliosis<sup>80</sup>. Muscle relaxants can be beneficial in case of dystonia or hyperkinesia<sup>80</sup>. Antiepileptic drugs are essential to treat concomitant epilepsy. Patients with cholestasis profit from formulas with enriched medium-chain-triglyceride content and fractional meals with enteral nutrition at night<sup>36</sup>. Severe hypoglycemic episodes in MPV17-deficiency can be prevented by corn-starch-based meals<sup>6</sup>. Regular glucose intake at short intervals can also slow progression of liver dysfunction in MPV17-associated MDS<sup>63</sup>. In patients with liver involvement, drugs with liver toxicity, such as valproate, isoniazid, acetaminophen, and others should be absolutely avoided<sup>36</sup>. Promising results with dAMP / dGMP supplementation have been reported in myotubes carrying TYMP-mutations, DGUOK-mutations, or POLG1-mutations<sup>103</sup>. *In vitro* supplementation with dAMP / dGMP resulted in a significant increase in the mtDNA copy number in myotubes from patients with DGUOK-mutations<sup>103</sup>. In POLG1-mutations the improvement of mtDNA depletion is mild and non-significant<sup>103</sup>. Patients with liver failure, ascites, and coagulopathy require regular transfusions of fresh frozen plasma, vitamin K, ursodeoxycholic acid, and spironolactone<sup>37</sup>.

#### b) Invasive

Intermittent positive pressure ventilation may be necessary in case of respiratory failure<sup>80</sup>. Gastrostomy may be necessary to guarantee sufficient intake of calories and liquids<sup>80</sup>. Patients with sensorineural hearing loss will benefit from implantation of a cochlear device<sup>80</sup>. Liver transplantation is a therapeutic option in

**Table 2: Manifestations of MDS**

Mutated gene	Syndrome	Clinical manifestation	mtDNA depletion	Multiple deletions	Reference
POLG1	HC, AHS	Brain, liver	x	x	[21,28]
DGUOK	HC	Brain, liver	x	x	[25,43]
TK2	M	Muscle	x	x	[45,59]
MPV17	HC	Cerebrum, liver	x	x	[6]
TYMP	MNGIE	Nerve, muscle, GI, brain	x	x	[73]
SUCLA2	EM	Brain, muscle	x		[17]
SUCLG1	EM	Brain, muscle	x		[17]
RRM2B	M	Muscle	x	x	[90]
PEO1/twinkle	HC, IOSCA, MIRAS	Brain, liver	x	x	[5,95]

HC: hepato-cerebral form, EM: encephalo-myopathic form, M: myopathic form, GI: gastro-intestinal, AHS: Alpers Huttenlocher syndrome, MNGIE: mitochondrial neuro-gastro-intestinal encephalopathy, IOSCA: infantile onset spinocerebellar ataxia, MIRAS: mitochondrial recessive ataxia syndrome



patients with the isolated hepatic form of MDS due to DGUOK-mutations<sup>35</sup>, MPV17-mutations<sup>63,67</sup>, or in patients with hepato-cerebral MDS. However, transplantation is controversial in the hepato-cerebral form if encephalopathy is a strong phenotypic component. Allogeneic hematopoietic stem cell transplantation is a promising therapeutic option in MNGIE<sup>72</sup>.

## CONCLUSIONS

Mitochondrial depletion syndromes most frequently occur in neonates, infants, or juveniles, but rarely in adolescents or adults. Mutated genes phenotypically presenting with adult-onset MDS include POLG1, TK2, TYMP, RRM2B, and PEO1. In adults, MDS manifest either, like early-onset MDS, as myopathy, encephalo-myopathy, or hepato-cerebral syndrome, or with a phenotype at variance from that of the early-onset form. In adults, MDS also manifests with only minimal muscular manifestations. If histological examination of the muscle is normal but biochemical investigations reveal multiple RCC defects, particularly sparing complex II, MDS should be suspected and appropriate genes analysed for mutations in genes associated with MDS. From the few reported adult cases it can be concluded that the outcome appears to be more favorable than in the early-onset forms.

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