# 3-Methylglutaconic aciduria—lessons from 50 genes and 977 patients

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**Abstract** Elevated urinary excretion of 3-methylglutaconic acid is considered rare in patients suspected of a metabolic disorder. In 3-methylglutaconyl-CoA hydratase deficiency (mutations in *AUH*), it derives from leucine degradation. In all other disorders with 3-methylglutaconic aciduria the origin is unknown, yet mitochondrial dysfunction is thought to be the common denominator. We investigate the biochemical, clinical and genetic data of 388 patients referred to our centre under suspicion of a metabolic disorder showing 3-methylglutaconic aciduria in routine metabolic screening. Furthermore, we investigate 591 patients with 50 different, genetically proven, mitochondrial disorders for

the presence of 3-methylglutaconic aciduria. Three percent of all urine samples of the patients referred showed 3-methylglutaconic aciduria, often in correlation with disorders not reported earlier in association with 3-methylglutaconic aciduria (e.g. organic acidurias, urea cycle disorders, haematological and neuromuscular disorders). In the patient cohort with genetically proven mitochondrial disorders 11 % presented 3-methylglutaconic aciduria. It was more frequently seen in ATPase related disorders, with mitochondrial DNA depletion or deletion, but not in patients with single respiratory chain complex deficiencies. Besides, it was a consistent feature of patients with mutations in *TAZ, SERAC1, OPA3, DNAJC19* and *TMEM70* accounting for mitochondrial membrane related pathology. 3-methylglutaconic aciduria is found quite frequently in patients suspected of a metabolic disorder, and mitochondrial dysfunction is indeed a common denominator. It is only a discriminative feature of patients with mutations in *AUH*, *TAZ, SERAC1, OPA3, DNAJC19 TMEM70.* These conditions should therefore be referred to as inborn errors of metabolism with 3-methylglutaconic aciduria as discriminative feature.

## Introduction

In the urine of healthy individuals the branched-chain organic acid 3-methylglutaconic acid (3-MGA) is found only in traces. Elevated urinary excretion of 3-MGA (3-MGA-uria) was first described in patients with 3-methylglutaconyl-CoA hydratase deficiency (former type I, *AUH*, MIM ID: #250950), a defect of leucin catabolism leading to a late onset leukoencephalop-athy (Wortmann et al 2010b).

Only in 3-methylglutaconyl-CoA hydratase deficiency the origin of 3-MGA is known, therefore it can be considered a "primary 3-MGA-uria". This is in contrast to all other disorders in which 3-MGA-uria is seen, where the pathomechanism underlying this biomarker is still not elucidated. However, 3-MGA-uria is the hallmark of several phenotypically heterogeneous but highly distinctive "3-MGA-urias" which were given Roman numbers randomly (Barth syndrome (for-mer type II, TAZ, MIM ID: #302060), Costeff syndrome (former type III, OPA3, MIM ID: #258501) and DCMA syndrome (former type V, DNAJC19, MIM ID #610198)).

Besides these well defined syndromes, there is a confusing and ever growing subgroup encompassing all "unclassified" patients, designated 3-MGA-uria type IV (MIM ID 25951).

Recently the underlying genetic defects of several of these disorders were elucidated allowing more insight into the underlying pathophysiology. Combined with the findings presented in this paper, we propose a proper pathomechanism based classification and new nomenclature of these "inborn errors of metabolism (IEM) with 3-MGA-uria as discriminative feature" which is presented in a separate article in this issue of JIMD (Wortmann et al JIMD 2013). To prevent confusion we will use the new nomenclature in this article as well. The old and new nomenclature are shown in parallel in Table 1.

After exclusion of the well-defined syndromes described above, each patient with elevated urinary 3-MGA has always been labelled as 3-MGA-uria type IV (MIM ID: 250951). This rapidly growing, clinically heterogeneous group challenges the physician in planning further diagnostic steps and does lead to serious confusion. Does 3-MGA-uria always indicate a mitochondrial disorder? Are there specific mitochondrial disorders one should search for in patients with 3-MGA-uria? Has every patient with an occasionally elevated urinary 3-MGA to be labelled 3-MGA-uria type IV?

In this study we address these questions above with a bi-directional approach by evaluating 977 patients. Group 1 patients ($n$=388) were referred to our hospital in the period 1992–2010 and had increased urinary 3-MGA. We report the clinical, biochemical and genetic data on these patients, which will reflect the diversity of underlying causes of 3-MGA excretion. Group 2 consisted of 589 patients with a vast array of 50 genetically proven mitochondrial disorders in whom organic acid analysis in urine had been performed. The group was included to evaluate the occurrence of 3-MGA-uria in mitochondrial disorders.

## Material and methods

### Urinary organic acid analysis

Urinary organic acid analysis was performed by gas chromatography/mass spectrometry (GC-MS) on a HP 6890 Gas Chromatograph (Agilent, Amstelveen, The Netherlands). 3-MGA was quantified on the basis of an in house synthesized 3-MGA model compound. For quantification purposes flame ionisation detection with standard calibration curve

**Table 1** New and old nomenclature

| | New nomenclature (affected gene) | Old nomenclature |
|---|---|---|
| Inborn errors with 3-MGA-uria as discriminative feature | | 3-MGA-uria |
| Primary 3-MGA-uria | 3-methylglutaconyl-CoA hydratase deficiency *(AUH)* | 3-MGA-uria type I |
| Secondary 3-MGA-uria | TAZ defect or Barth syndrome *(TAZ)* | 3-MGA-uria type II |
| | OPA3 defect or Costeff syndrome *(OPA3)* | 3-MGA-uria type III |
| | SERAC1 defect or MEGDEL syndrome *(SERAC1)* | 3-MGA-uria type IV |
| | DNAJC19 defect or DCMA syndrome *(DNAJC19)* | 3-MGA-uria type V |
| | TMEM70 defect *(TMEM70)* | 3-MGA-uria type IV |
| | Not otherwise specified (NOS) 3-MGA-uria | 3-MGA-uria type IV |

was used. The reference range for urinary 3-MGA was 0–20 mmol/mol creatinine and has been established on a healthy subject population in our laboratory. In selected cases additionally $^{1}$H-NMR spectroscopy on a Bruker DRX 500 spectrometer was performed (Engelke et al 2006).

Group 1: diagnoses and sampling circumstances in patients with 3-MGA-uria; inclusion and exclusion criteria

The Radboud University Nijmegen Medical Centre (RUMC) is a tertiary academic referral centre in the Netherlands with a focus on mitochondrial disorders. The database of the Laboratory of Genetic, Endocrine and Metabolic Diseases (LGEM) at the RUMC was searched for all patients with elevated urinary 3-MGA excretion of > 20 mmol/mol creatinine in the period 1992–2010. The charts of these patients were reviewed for the clinical circumstances at the time of sampling and for the diagnoses. Inclusion criteria: i) referral of the patient to RUMC under suspicion of a metabolic disorder (patients from whom only urine was received for analysis were not included), ii) routine metabolic screening completed (urinary organic acid analysis; serum lactate, amino acid and carnitine profile analysis, transferrin isoelectric focussing, and upon indication urine oligosaccharide analysis). Exclusion criteria: i) multi-organ-failure or ii) total parenteral nutrition at the moment of sampling.

Group 2: patients with genetically proven mitochondrial disorders

We aimed to investigate the excretion of 3-MGA in patients with genetically proven pathogenic mutations causing a mitochondrial disorder. As such we have considered patients with pathogenic mutations in nuclear or mitochondrial genes involved in the biogenesis and assembly of oxidative phosphorylation system (OXPHOS) complexes, mitochondrial nucleotide synthesis and transport, mitochondrial DNA replication and translation, mitochondrial protein processing and quality control, mitochondrial protein import, mitochondrial membrane biogenesis and maintenance, pyruvate metabolism and tricarboxylic acid (TCA) cycle. We have reviewed the clinical and laboratory files of patients under treatment in our hospital or under the care of one of our co-authors. Furthermore, we have searched the PubMed database for papers describing patients with genetically proven mitochondrial disorders of whom the results of urinary organic acid analysis were included in the paper.

## Results

Group 1: diagnoses and sampling circumstances in 388 patients with 3-MGA-uria

Searching the database of the LGEM at the RUMC revealed 20991 urinary organic acid profiles measured between 1992 and 2010. In 647 (3 %) samples of 388 patients 3-MGA-uria was detected. Of these patients 69 did not fulfil the inclusion criteria, another 92 met the exclusion criteria (see 2.2), consequently 227 patients were eligible for further investigations.

The results are summarized in Table 2, and more details about the diagnosed disorders and the urinary organic acid results can be found in the supplementary data. Sixty one patients were diagnosed with a classical metabolic disorder, 43 patients with other, non-metabolic disorders (see Table 2A, B). A subgroup of 23 patients were diagnosed with an inborn errors of metabolism with 3-MGA as dis-criminative feature (see Table 2C).

Of the remaining 100 patients (see Table 2D), 49 patients were diagnosed with a mitochondrial disorder. Of them 18 had a genetically proven diagnosis and in the other 31 patients single or multiple OXPHOS enzyme deficiencies were found in muscle. Patients in whom a decreased ATP-production was found without evidence for single or multiple OXPHOS enzyme deficiency were considered as "possible" mitochondrial disorder, and therefore not included in this group. Ten patients had (mostly isolated) 3-MGA-uria during a single hypoglycaemic episode throughout a febrile illness, mostly of gastroenterological origin, in early childhood. Despite extensive metabolic investigation no underlying metabolic disease was detected in these patients and they are all doing well during follow up of up to 15 years. In

**Table 2** Diagnosis and sampling circumstances 227 patients with 3-MGA-uria

| Diagnosis | n | Frequency | Max. 3-MGA (mean)* | Other metabolites? |
|---|---|---|---|---|
| A: Classical metabolic disorders | 61 | | | |
| Fatty acid oxidation disorder (FAOD) | 22 | P/D | 42 (26) | Y |
| Methylmalonic aciduria (MMA) | 4 | P/D | 55 (39) | Y |
| Propionic aciduria (PA) | 6 | P/D | 94 (42) | Y |
| Glycogen storage disorder (GSD) | 18 | P/D | 82 (44) | Y |
| Urea cycle disorder (UCD) | 6 | P/D | 152 (52) | Y |
| Other metabolic disorder | 5 | SE | 47 (34) | Y |
| B: Other non-metabolic disorders | 43 | | | |
| Hematological disorder | 4 | R, SE | 35 (27) | N,Y |
| Neuromuscular disorder (see also Table 3) | 13 | R, SE | 50 (28) | N,Y |
| Genetic syndrome/ chromosomal abnormality | 15 | R, SE | 46 (27) | N,Y |
| Apparently life-threatening event (ALTE)/ sudden infant death syndrome (SIDS) | 6 | SE | 48 (33) | N |
| other | 5 | SE | 43 (34) | N,Y |
| C: Inborn errors with 3-MGA-uria as discriminative feature | 23 | | | |
| 3-methylglutaconyl-CoA hydratase deficiency *(AUH)* | 3 | R | 142 (120)*** | N** |
| TAZ defect or Barth syndrome *(TAZ)* | 1 | R | 97 (55) | N |
| OPA3 defect or Costeff syndrome *(OPA3)* | 1 | R | 43 (43) | N |
| TMEM7def*ect (TMEM70)* | 6 | R | 121 (83) | N |
| SERAC1 defect or MEGDEL syndrome *(SERAC1)* | 9 | R | 196 (103) | N |
| NOS 3-MGA-uria | 3 | R | 75 (47) | N |
| D: remaining patients | 100 | | | |
| Mt disorder | 49 | R,SE | 60 (29) | N,Y |
| Hypoglycemia | 10 | SE | 42 (26) | N,Y |
| Ongoing investigations | 41 | R,SE | 70 (32) | N,Y |

Diagnosis and sampling circumstances in 227 patients with 3-MGA-uria. Gene names *in italics*, 3-MGA-uria = 3-Methylglutaconic aciduria, * mmol/mol creatinine, **with exception of 3-Hydroxy-isovaleric aciduria, *** in literature values up to 1000 mmol/mol creatine are reported; *P/D* upon presentation or deterioration, *R* repetitively, *SE* single episode

41 patients with isolated or combined presentation of multi system disorder, psychomotor retardation, leukoencephalopathy, syndromal appearance, myopathy, spastic paraparesis, cataract, neurodegenerative disease, polyneuropathy and/or movement disorders the investigations are ongoing. From the latter group 11 patients showed decreased ATP production without OXPHOS complex deficiencies in a fresh muscle biopsy, three had biochemically and histologically normal biopsies, the remaining 30 did not undergo muscle biopsy.

Group 2: 3-MGA-uria patients with genetically proven mitochondrial disorders

For a total of 591 patients carrying pathogenic mutations in 50 nuclear genes or the mitochondrial DNA we could retrieve the urinary organic acid results. This encompasses 202 patients from our centre or under the care of one of our co-authors and 389 patients from the literature. The data are summarized in Table 4.

## Discussion

3-MGA-uria is a rather common finding in patients suspected of a metabolic disorder

3-MGA-uria was thought to be a rare finding in patients suspected of a metabolic disorder. Unexpectedly, we observed it in nearly 3 % of all samples received for urinary organic acid analysis at our centre in the last 18 years. 3-MGA-uria was frequently seen in association with several metabolic disorders, such as organic acidurias, glycogen storage disorders (GSD), fatty acid oxidation disorders (FAODs), urea cycle disorders.

3-MGA-uria is mostly correlated with mitochondrial dysfunction

A relation with mitochondrial dysfunction undoubtedly accounts for most patients with 3-MGA–uria. Ten percent of all 3-MGA-uria patients were diagnosed with FAODs

(see Table 2A), in fact primary mitochondrial disorders. 3-MGA-uria was also found frequently in patients presenting with a metabolic crisis due to an organic aciduria. Propionyl-CoA has been shown to non-competitively inhib-it pyruvate dehydrogenase complex (PDHc, (Schwab et al 2006)), also multiple OXPHOS deficiency in different tis-sues was detected in organic aciduria patients (de Keyzer et al 2009).

Mitochondrial dysfunction also may play a role in urea cycle disorder patients showing 3-MGA-uria. It is proven in rodents, that hyperammonemia inhibits the TCA cycle enzyme α-ketoglutarate-dehydrogenase and activates the *N*-methyl *D*-aspartate (NMDA) receptor leading to disturbed calcium homeostasis and secondary mitochondrial dysfunction (Felipo and Butterworth 2002). This theory is supported by the frequent co-finding of TCA cycle intermediates and lactate in our urea cycle disorder patients with 3-MGA-uria.

We also tentatively postulate mitochondrial dysfunction as underlying cause for 3-MGA-uria in a group of 37 patients diagnosed with other non-metabolic disorders (Table 2B). However, we can only partly prove this, as only five of the patients underwent a muscle biopsy which showed disturbed mitochondrial function before the final diagnosis was made. On the other hand often lactic acidosis or alanine elevation pointed towards mitochondrial dysfunc-tion. In 13 of these 37 patients, a muscle biopsy was only investigated histologically leading to the diagnosis of a neuromuscular disorder (see Tables 2B and 3). The consis-tent finding of 3-MGA-uria in this patient group could be a sign of mitochondrial dysfunction. For two classical neuro-muscular disorders mitochondrial dysfunction has recently been reported. In myoblasts of the *mdx mouse*, a well estab-lished mouse model of DMD, an impaired cellular energy metabolism due to abnormal calcium homeostasis, reduced amounts of OXPHOS complexes and ATP synthase as well as disorganized mitochondrial network were observed (Onopiuk et al 2009). Furthermore, energy shortage and increased mitochondrial free radical production leading to cell damage was also recently shown in a neural cell model of SMA (Acsadi et al 2009), suggesting mitochondrial dysfunction as important pathology underlying SMA.

3-MGA-uria in correlation with impaired cholesterol biosynthesis

The mevalonate or Popjak shunt links cholesterol biosynthesis with leucine catabolism (Fig. 1). In patients with Smith Lemli Opitz syndrome elevated 3-MGA-levels have been reported in seven of 35 patients (Kelley and Kratz 1995). We did not detect 3-MGA-uria in eight Smith Lemli Opitz syndrome patients on cholesterol/simvastatin treatment. However, the earlier reported patients, were untreated patients with very low cholesterol levels (< 0.129 mmol/L, reference range not given (Kelley and Kratz 1995)), suggesting that it only occurs in untreated patients on the severe end of the Smith Lemli Opitz syndrome -spectrum or with high cholesterol precursors (Kelley and Kratz 1995). We also did not detect 3-MGA-uria in three patients with Mevalonate kinase deficiency, another defect of cholesterol biosynthesis. However, the HMG salvage pathway has recently been proven to account for the elevated 3-MGA production in a zebrafish model of Costeff syndrome. The authors showed, that simvastatin inhibited mevalonate production from extramitochondrial HMG-CoA, which leads to elevated 3-MGA levels (see Fig. 1, (Pei et al 2010)). This shunt should explain the 3-MGA-uria found in 18 of our patients, later diagnosed with GSD I or IX. The finding of 3-MGA-uria was reported earlier in one patient with GSD 1b (Law et al 2003). An imbalanced homeostasis between disturbed gluconeogenesis and cholesterol synthesis is supposed to increase the shunting towards 3-MGA production. One should keep the differential diagnosis of a GSD in mind when facing a patient with elevated lactate, 3-MGA-uria and hepatomegaly clinically suspected of a mitochondrial disorder. Possibly the 3-MGA-uria in two of the patients with haematological disorders, and the Duchenne patient is also related to cholesterol metabolism as the patients were treated with glucocorticosteroids for a long time.

Differential diagnosis in patients with 3-MGA-uria

After excluding the described patient groups (see Table 2A, B, C) a group of 100 patients with 3-MGA-uria (Table 2D) remained. Primary mitochondrial dysfunction defined by either single or multiple OXPHOS deficiency or genetically proven mitochondrial disorder was found in half of the patients. No disorder could be established in spite of extensive investigations in ten patients with a single hypoglycaemia during febrile illness in early childhood and in four patients with ALTE. These children are all doing well during long years of follow up. One may postulate an underlying disorder in (energy) metabolism which is only clinically significant in a limited time window in early childhood. The remaining group of 41 patients, mostly presenting with progressive neurodegenerative disorders, in whom investigations are ongoing is surely an interesting and challenging group. However, this group is clinically very heterogeneous, and most of the patients only showed 3-MGA-uria occasionally and mildly elevated. One should therefore not overrate the diagnostic value of 3-MGA-uria in these patients, but keep an eye open for all diagnostic features of a patient (e.g. physical examination, dysmorphic features, biochemical results, radiological results).

**Table 3** Details on 13 patients with neuromuscular disorders and 3-MGA-uria

| | Diagnosis (*affected gene)* | UOA: 3-MGA value(s)* | UOA: other findings |
|---|---|---|---|
| 1 | Congenital merosin negative muscle dystrophy *(NA)* | 32 | none |
| 2 | Congenital merosin negative muscle dystrophy *(NA)* | 24, 25, 23, 17, 8, 13 | once ketotic profile, always EMA |
| 3 | Congenital Actine Filament aggregation myopathy without nemaline rods *(NA)*** | 26, 12 | none |
| 4 | Duchenne Muscular Dystrophy *(DMD)**** | 23, 13 | EMA, lactate, succinic acid |
| 5 | Lipid myopathy *(NA)* | 22 | none |
| 6 | Multi-minicore myopathy *(RYR1)* | 21 | none |
| 7 | Multi-minicore myopathy *(RYR1)* | 37, 26, 24, 18 | TCA intermediates, once ketotic profile |
| 8 | SMA *(SMN1)* | 41 | MMA, EMA |
| 9 | SMA *(SMN1)* | 23 | EMA, mild elevation of dicarbonic acids |
| 10 | SMA *(SMN1)***** | 23; 18 | EMA |
| 11 | SMA *(SMN1)***** | 27, 27, 25, 25, 19, 19, 19 | EMA, adipic and suberic acid, TCA cycle intermediates |
| 12 | SMA *(SMN1)* | 22 | EMA |
| 13 | Muscular Dystrophy *(NA)* | 50, 46 | none |

*EMA* ethylmalonic aciduria, *MMA* methylmalonic aciduria, *NA* not available, *SMA* spinal muscular atrophy, *UOA* urinary organic acid analysis, * in mmol/mol creatinine (ref. 0–20) in chronological order; ** low serum citrulline, ***prednisone treatment, **** SCADD excluded genetically

## Correlation of 3-MGA-uria with specific mitochondrial disorders

Mutations in *TAZ*, *OPA3*, *TMEM70* and *SERAC1*, respectively, are virtually always associated with 3-MGA-uria (88–100 % of cases see Table 4). These patients show repetitively and con-sistently increased urinary 3-MGA, which is also substantially higher as in other disorders (see Table 2). There are no other diagnostic urinary metabolites found beside 3-methylglutaric acid. Hence, the 3-MGA-uria is a major finding, a hallmark of the phenotype and often the key to the diagnosis. These dis-orders, as well as 3-methylglutaconyl-CoA hydratase deficien-cy (AUH defect), should be referred to as IEM with 3-MGA-uria as discriminative feature. Interestingly, they are all related to mitochondrial membrane pathology in the broadest sense. 3-MGA-uria is frequently seen in patients with mutations in *AGK* (70 %). In most cases the excretion is <40 mmol/mol creatinine. One should not label these patients IEM with 3-MGA-uria as discriminative feature as the clinical and bio-chemical phenotype is too diverse. Three patients presented with isolated cataract (Aldahmesh et al 2012), and all turned out to have 3-MGA-uria (F. Alkuraya, personal information). The other end of the spectrum of patients with *AGK* mutations is Sengers syndrome with cataracts and (cardio)myopathy with four out of seven patients being reported with 3-MGA-uria.

We found 3-MGA-uria in 11 % of all patients with a proven ("primary") mitochondrial disorder. There are three subgroups of patients in which 3-MGA-uria could be help-ful in the diagnostic work up.

The first subgroup with a high correlation of disease mechanism and 3-MGA-uria, are the patients with mito-chondrial deletion leading to the Pearson phenotype. Of

**Fig. 1** Shunting between cholesterol biosynthesis and leucine catabolism. AUH = 3-methylglutaconyl-CoA hydra-tase (enzyme deficient in 3-MGA-uria type I). HMG-CoA = 3-hydroxy-3-methylglutaryl CoA (adapted from (Wortmann et al 2010a))

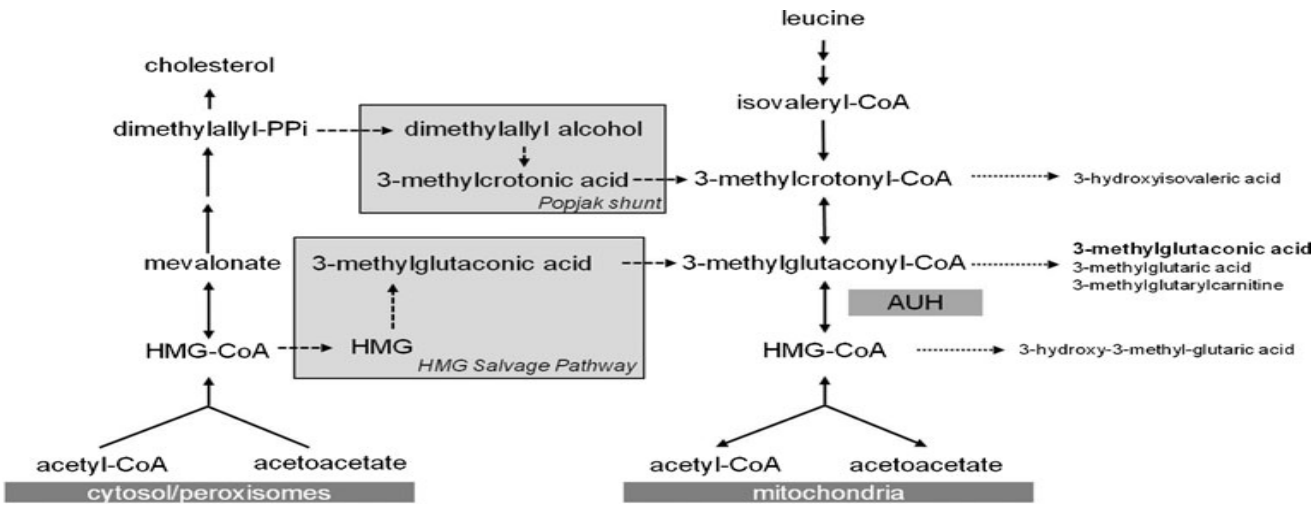

**Table 4** Presence of 3-MGA-uria in 591 patients with known mutations

| Gene function | MtDNA | Nuclear DNA | Own | Literature*** | All |
|---|---|---|---|---|---|
| Structure and assembly of C I | *MTND2 (1/3); MTND3; MTND4; MTND5; MTND6* | *NDUFS2; NDUFS6; NDUFS7 (1/3); NDUFS8; NDUFV1; NDUFAF2; C20orf7; ACAD9* | 2/20 | 0/15 | 2/35=5.7 % |
| Structure and assembly of C II | – | *SDHA* | 0/1 | NR | 0/1 |
| Structure and assembly of C III | | *BCS1L* | – | 0/6 | 0/6 |
| Structure and assembly of C IV | *MTCO1;MTCO2* | *COX10; COX15; COX6B1; FASTKD2; SCO1; SCO2; SURF1* | 0/3 | 0/11 | 0/14 |
| Structure and assembly of C V | *MTATP6 (1/8); MTATP8* | *ATP5E (1/1); ATP12(1/1)* | 1/11 | 2/18 | 3/29=10.3 % |
| | | ***TMEM70*** | **7/7** | **55/58** | **62/65=95 %** |
| MtDNA replication, nucleotide synthesis and transport | | *POLG (6/41); DGUOK; TK2; TYMP; SLC25A3; SUCLG1(2/12)*; SUCLA2(5/6)*; TWINKLE (1/21); RRM2B; MPV17* | 13/80 | 1/36 | 14/116=12 % |
| MtDNA translation | *MTTL (m.3243A>G (4/26)),MTTS* | *EFG1; MRPS22; PUS1; TRMU; DARS2; RARS2* | 3/26 | 1/35 | 4/61=6.5 % |
| Mt protein import | | *TIMM8A* | 0/5 | NR | 0/5 |
| | | ***DNAJC19*** | **–** | **19/19** | **19/19=100 %** |
| Mt membrane phospholipid remodelling | | ***TAZ*** | **3/3** | **46/53** | **49/56=88 %** |
| | | ***SERAC1*** | **18/18** | | **18/18=100 %** |
| Mt membrane biogenesis and maintenance | | *OPA1* | 0/2 | 0/31 | 0/33 |
| | | ***OPA3*** | **1/1** | **44/44** | **45/45=100 %** |
| | | *AGK* | *6/9* | *1/1* | *7/10= 70 %* |
| Mt other | *Deletion (8/24)* | | 0/5 | 8/19 | 8/24=30 % |
| CoQ10 related | | *COQ2; PDSS1; ETFDH (3/35)* | 1/7 | 2/34 | 3/41=7.3 % |
| Pyruvate metabolism | | *PDHA1; PDP1* | 0/2 | 0/2 | 0/4 |
| TCA cycle related | | *FH;* alpha-ketoglutarate DH** | – | 0/7 | 0/7 |
| **IEM with 3-MGA-uria as discriminative feature** | | | **31/31** | **164/174** | **195/205= 95 %** |
| | | | 26/171 | 15/215 | 41/386=11 % |
| Other | | | | | |
| All | | | 57/202 | 179/389 | 236/591 |

3-MGA= 3-methylglutaconic acid, 3-MGA-uria= 3-methylglutaconic aciduria, DH= dehydrogenase, IEM = inborn error of metabolism, C = complex of the respiratory chain, CoQ= Coenzyme Q10, Mt =mitochondrial, NR = not reported in literature, TCA= Tricarboxylic acid cycle* part of TCA cycle, but clinical and biochemical phenotype of mtDNA depletion syndrome, ** gene unknown, maps to 7q14-1. References are presented in the supplementary data. Gene names in italics. 3-MGA= 3-methylglutaconic acid, 3-MGA-uria= 3-methylglutaconic aciduria, DH= dehydrogenase, IEM = inborn error of metabolism, C = complex of the respiratory chain, CoQ= Coenzyme Q10, Mt =mitochondrial, NR = not reported in literature, TCA= Tricarboxylic acid cycle* part of TCA cycle, but clinical and biochemical phenotype of mtDNA depletion syndrome, ** gene unknown, maps to 7q14-1. *** References are presented in the supplementary data. Gene names in italics. IEM with 3-MGA-uria as discriminative feature in bold

these patients 30 % are reported with 3-MGA-uria (Gibson et al 1992; Jakobs et al 1991; Knerr et al 2003; Krauch et al 2002; Lichter-Konecki et al 1993). Curiously, mitochondrial deletions presenting with the Kearns-Sayre phenotype do not lead to 3-MGA-uria. The finding of 3-MGA-uria in a patient presenting with refractory anemia should lead the physician to mitochondrial DNA deletion screening in several tissues, hence sparing the patient a bone marrow aspiration.

Two other patient subgroups show 3-MGA-uria in 10.3 and 12 % of patients, respectively. These are patients with ATPase deficiency related pathology and patients with mitochondrial depletion syndromes.

3-MGA-uria is seen less frequently in patients with complex I-related mutations (5.7 %) and until now not found in relation with complex II, III or IV mutations. In contrast, both patients with ATPase related mutations (*ATP5E, ATP12*) did have 3-MGA-uria, as had one of the *MTATP6* patients.

Combining this with the consistent 3-MGA-uria found in 95 % of patients with *TMEM70* defect, this makes a link of 3-MGA-uria and ATPase-dysfunction or -related processes affecting the mitochondrial membrane more likely. One should still keep in mind, that the investigated mutations are very rare and often only one patient in each category is reported.

Furthermore, patients with mitochondrial depletion syndromes (*POLG, SUCLG1, SUCLA2* and *TWINKLE* mutations) more often show 3-MGA-uria. Strictly *SUCLG1* and *SUCLA2* are genes involved in the TCA cycle, given the fact that they lead to a typicial mitochondrial depletion syndrome, we chose to group the patients here (Morava et al 2009). In patients with a phenotype suggestive for a mitochondrial depletion syndrome 3-MGA-uria makes this suspicion stronger. One limitation of this group is, that 3-MGA-uria was mainly found in the patient population of the contributing authors. This could be due to the fact, that these laboratories are able to quantify 3-MGA in urine and therefore are able to detect slight elevations (20–40 mmol/mol creatinine). Other laboratories may often report 3-MGA-uria only if more substantial (>40 mmol/mol creatinine ) as is the case in the IEM with 3-MGA-uria as discriminative feature. Furthermore, there is again a limited number of patients reported, often in genetic or neurological journals without describing the metabolic findings beside lactic acidosis. In addition there are depletion syndromes with pure myopathic presentation in which a muscle biopsy rather than metabolic work-up is chosen as diagnostic approach. However, the correlation could help the physician in search for the diagnosis.

3-MGA-uria has not been found in association with defects in mitochondrial translation, again with the limitation of small patient numbers of these newly found group of diseases.

Mutations in *DNAJC19* underlie *DCMA* syndrome. All described 19 patients had 3-MGA-uria. DNAJC 19 is suspected to be involved in mitochondrial protein import. In contrast, we did not find 3-MGA-uria in five patients with Mohr-Tranebjaerg syndrome *(TIMM8A)*, a dystonia-deafness syndrome in which a similar pathomechanism is suspected.

TAZ and OPA3 defect are both suspected to alter mitochondrial membrane biogenesis and maintenance ("fusion/fission"). Contradictory no patients with 3-MGA-uria and *OPA1* mutations, also leading to a defective fusion/fission, have been found.

3-MGA-uria is seen in multiple acyl-CoA dehydrogenase deficiency (MADD, Glutaric aciduria IIc) patients with mutations in *ETFDH*. The defect affects fatty acid, amino acid and choline metabolism, but certainly mitochondrial dysfunction is present in this complex disorder.

## Conclusions

3-MGA-uria is a rather common finding in patients suspected of a metabolic disorder. In most patients it is seen in association with mitochondrial dysfunction. The majority of patients can be diagnosed upon routine metabolic screening including urine oligosaccharide screening for the differential diagnosis of GSD. In the latter the 3-MGA probably stems from the cholesterol biosynthesis. The minority of patients suffers an IEM with 3-MGA-uria as discriminative feature, a diagnostic flowchart and more details on a pathomechanism based classification and nomenclature of these disorders is presented in an accompanying article in this issue of JIMD (Wortman et al 2013).

**Conflict of interest** None.

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