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## Organic acidurias: Major gaps, new challenges, and a yet unfulfilled promise

Dimitrov, Bianca ; Molema, Femke ; Williams, Monique ; Schmiesing, Jessica ; Mühlhausen, Chris ; Baumgartner, Matthias R ; Schumann, Anke ; Kölker, Stefan

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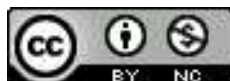
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## REVIEW ARTICLE

# Organic acidurias: Major gaps, new challenges, and a yet unfulfilled promise

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## Abstract

Organic acidurias (OADs) comprise a biochemically defined group of inherited metabolic diseases. Increasing awareness, reliable diagnostic work-up, newborn screening programs for some OADs, optimized neonatal and intensive care, and the development of evidence-based recommendations have improved neonatal survival and short-term outcome of affected individuals. However, chronic progression of organ dysfunction in an aging patient population cannot be reliably prevented with traditional therapeutic measures. Evidence is increasing that disease progression might be best explained by mitochondrial dysfunction. Previous studies have demonstrated that some toxic metabolites target mitochondrial proteins inducing synergistic bioenergetic impairment. Although these potentially reversible mechanisms help to understand the development of acute metabolic decompensations during catabolic state, they currently cannot completely explain disease progression with age. Recent studies identified unbalanced autophagy as a novel mechanism in the renal pathology of methylmalonic aciduria, resulting in impaired quality control of organelles, mitochondrial aging and, subsequently, progressive organ dysfunction. In addition, the discovery of post-translational short-chain lysine acylation of histones and mitochondrial

**Abbreviations:** CKD, chronic kidney disease; GA1, glutaric aciduria (or acidemia) type 1; GCDH, glutaryl-CoA dehydrogenase; GDH, glutamate dehydrogenase; MMA, methylmalonic aciduria (or acidemia); OAD, organic aciduria or acidemia; OGDH, 2-oxoglutarate dehydrogenase; PA, propionic aciduria (or acidemia); ROS, reactive oxygen species; TCA, tricarboxylic acid cycle.

Anke Schumann and Stefan Kölker contributed equally to this study.

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enzymes helps to understand how intracellular key metabolites modulate gene expression and enzyme function. While acylation is considered an important mechanism for metabolic adaptation, the chronic accumulation of potential substrates of short-chain lysine acylation in inherited metabolic diseases might exert the opposite effect, in the long run. Recently, changed glutarylation patterns of mitochondrial proteins have been demonstrated in glutaric aciduria type 1. These new insights might bridge the gap between natural history and pathophysiology in OADs, and their exploitation for the development of targeted therapies seems promising.

#### KEYWORDS

autophagy, mitochondria, organic aciduria, post-translational acylation, therapy, toxic metabolite

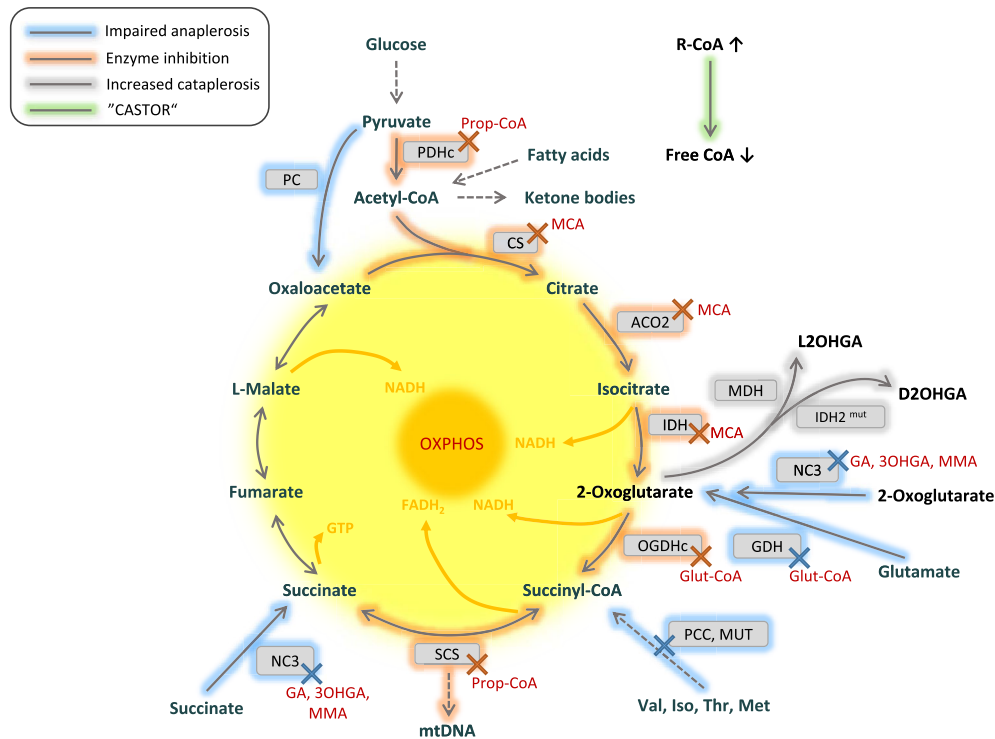
## 1 | OLD ROADS, MAJOR GAPS

Organic acidurias or acidemias (OADs) are a group of inherited metabolic diseases, which has been identified following the introduction of analytical gas chromatography techniques since the 1960s. The name-giving biochemical hallmark of OADs is the accumulation of so-called “organic acids,” that is, nonamino mono-, di- or tricarboxylic acids which can be detected in urine, plasma, and cerebrospinal fluid. In the majority of OADs, this is caused by deficient mitochondrial breakdown of CoA-activated carbonic acids such as propionyl-CoA, methylmalonyl-CoA, isovaleryl-CoA, and glutaryl-CoA. Some of these are thought to be toxic, mostly through induction of mitochondrial dysfunction, energy impairment, and oxidative stress, resulting in acute and chronic dysfunction of organs with a high energy demand<sup>1–10</sup> (Figure 1). Systematic data analysis of 181 publications on individuals with isolated methylmalonic (MMA; OMIM #25100) and propionic aciduria (PA; OMIM #606054) has recently supported the previous notion of mitochondrial impairment playing a major role in these diseases,<sup>11</sup> particularly in the manifestation of cardiomyopathy, optic atrophy, abnormalities of basal ganglia and the liver, pancreatitis, and epilepsy.<sup>11,12</sup>

Observational studies, demonstrating overlapping clinical phenotypes between OADs and phenotypic diversity in individuals with the same OAD, even in siblings,<sup>13,14</sup> have significantly changed our view on the long-term outcome of affected individuals. First, the traditional division in “classic” and “cerebral” OADs has been challenged, since the brain is most often involved in both subgroups, while cerebral OADs may also develop non-neurologic disease manifestations such as chronic kidney disease (CKD) in glutaric aciduria type 1<sup>15</sup> (GA1; OMIM #231670) and cardiomyopathy in D-2-hydroxyglutaric aciduria type 2 (OMIM #613657).<sup>16</sup> Second, CKD, originally assigned to

MMA (OMIM #251110),<sup>17</sup> is also found in PA patients, however, at a later age than in MMA patients.<sup>13,18</sup> Finally, these studies have challenged our view about the disease onset. Because of the tight metabolic coupling between a mother and her unborn child, a fetus having an OAD is commonly thought to be protected during pregnancy. This notion is supported by the fact that individuals with potentially life-threatening OADs are usually born asymptotically and start to present first symptoms after a variable postnatal time interval. However, the observation of macrocephaly, hypoplasia of the temporal lobe, and immature gyral pattern of the cortex in newborns with GA1, and of low birth weight in mut<sup>0</sup>-type MMA highlights that the maternal metabolism may not completely prevent disease manifestation in utero and that other mechanisms on a subcellular level might fuel organ-specific disease manifestations.<sup>13,19</sup>

With improved survival and longer follow-up, knowledge about OADs is increasing, unraveling major gaps and new challenges. Most strikingly, early diagnosis and adherence to recommended conservative metabolic management using combinations of dietary treatment, cofactors, carnitine supplementation, nonabsorbable antibiotics, and intensified emergency management during catabolic episodes cannot reliably prevent disease progression, which does not even spare those who have not had a single acute metabolic decompensation for years (if ever).<sup>20–25</sup> Furthermore, a growing number of individuals with OADs are found to develop cerebral neoplasms such as in L-2-hydroxyglutaric aciduria (OMIM #236792) and GA1,<sup>26,27</sup> and hepatic neoplasms like in MMA.<sup>28,29</sup> Lastly, although plasma ammonium, acid-base balance, and anion gap are useful to estimate the risk of a metabolic decompensation in a “classic” OAD patient and to guide therapeutic decision-making,<sup>30,31</sup> these basic biomarkers as well as disease-specific carbonic acids and acylcarnitines have a low predictive value for the long-term outcome.<sup>20</sup>



**FIGURE 1** Synergistic pathways to mitochondrial toxicity in organic acidurias. Accumulating CoA esters and carbonic acid such as propionyl-CoA (Prop-CoA), 2-methylcitrate (MCA), methylmalonate (MMA) in propionic and/or methylmalonic aciduria as well as glutaryl-CoA (Glut-CoA), glutarate (GA), and 3-hydroxyglutarate (3OHGA) in GA1 inhibit key enzymes of energy metabolism, with a particular focus on the tricarboxylic acid cycle (TCA). Besides metabolite-induced inhibition of key enzymes, the TCA cycle flux is further impaired by physiologic, inherited or acquired deficiency of anaplerotic pathways such as pyruvate carboxylase (in neurons), propionate oxidation (in PA and MMA), and glutamate dehydrogenase (GDH, in GA1), metabolite-induced inhibition of sodium-dependent dicarboxylic acid transporter 3 (NC3; in GA 1), and cataplerosis due to 2-oxoglutarate-dependent synthesis of L-2-(L2OHGA) and D-2-hydroxyglutarate (D2OHGA). Overall, the mitochondrial metabolism is compromised by CoA sequestration and limited availability of free CoA. ACO2, aconitase 2; CASTOR, CoA sequestration, toxicity or redistribution; CS, citrate synthase; FADH<sub>2</sub>, dihydroflavine adenine dinucleotide; GTP, guanosine triphosphate; IDH, isocitrate dehydrogenase; IDH2<sup>mut</sup>, mutated form of isocitrate dehydrogenase 2; Iso, isoleucine; MDH, malate dehydrogenase; MUT, methylmalonyl-CoA mutase; NADH, nicotinamide adenine dinucleotide; OGDHc, 2-oxoglutarate dehydrogenase complex; OXPHOS, oxidative phosphorylation; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PDHc, pyruvate dehydrogenase complex; R-CoA, acyl-CoAs; SCS, succinyl-CoA synthetase; Thr, threonine; Val, valine

The same holds true for the genotype, except in cofactor-sensitive OADs. Therefore, the term “metabolic stability” seems poorly defined for the group of OADs and even misleading.

These studies highlight major gaps that currently hamper the improvement of health outcomes in individuals with OADs. This review aims to critically discuss current pathogenetic concepts and to look beyond these horizons in order to identify strategies to bridge existing gaps.

## 2 | FROM DEFICIENCY TO DISEASE: ON BOTTLE NECKS, INSURMOUNTABLE BORDERS, AND SLOPPY ENZYMES

Inherited deficiencies of proteins involved in metabolic pathways are various and do not necessarily result in a

clinically apparent disease. As an example, hydroxyprolinemia (OMIM #237000) caused by deficient dehydrogenation of hydroxyproline to  $\Delta$ 1-pyrroline-3-hydroxy-5-carboxylic acid due to biallelic mutations in *PRODH2* is thought to be a benign condition.<sup>32</sup> It is a frequent cause of false positive newborn screening for maple syrup urine disease (OMIM #248600).

The group of OADs is an interesting example to study the necessary preconditions of the transfer from an inherited protein dysfunction to a disease. The formation of toxic metabolites is thought to play a pivotal role for OADs. But what makes a metabolite toxic? First, this metabolite should accumulate to exceed a certain threshold that is required to drive the adverse action. Accordingly, one or more of the following criteria should be met: (a) The metabolic block cannot be bypassed and thus compensated by an isoenzyme or another metabolic pathway acting in parallel to the deficient pathway. For

example, only glutaryl-CoA dehydrogenase (GCDH; EC 1.3.8.6) is known to catalyze the oxidative decarboxylation of glutaryl-CoA to crotonyl-CoA, while the formation of glutaryl-CoA from its precursor 2-oxoadipate can be handled by both, dehydrogenase E1 and transketolase domain-containing protein 1 (preferring 2-oxoadipate as a substrate; EC 1.2.4.2) and 2-oxoglutarate dehydrogenase (OGDH, preferring 2-oxoglutarate but also accepts 2-oxoadipate as a substrate; EC 1.2.4.2).<sup>8,33</sup> (b) Enzymatic dysfunction gives rise to (noncanonical) metabolites that cannot be further metabolized such as L-2-hydroxyglutarate<sup>34</sup> or 3-hydroxyglutarate<sup>35</sup> due to inherited loss or nonexistence of a metabolite repair enzyme. (c) The assumingly toxic metabolite cannot cross a given border, specifically a biological membrane, and hence is entrapped in an intracellular compartment. It is well known that the inner mitochondrial membrane is impermeable to free CoA and CoA esters, requiring a carnitine-dependent translocation to cross it.<sup>36</sup> Although this mechanism orchestrates mitochondrial and cytosolic metabolism, it also facilitates the mitochondrial accumulation of toxic CoA esters arising from inherited deficiencies of mitochondrial enzymes such as L-methylmalonyl-CoA mutase (EC 5.4.99.2) or propionyl-CoA carboxylase (EC 6.4.1.3). The consequences of CoA sequestration and toxicity or redistribution have been summarized previously in the so-called *CASTOR hypothesis*,<sup>36</sup> a variant form of the toxic metabolite hypothesis.<sup>37</sup> Another example of a pathomechanistically relevant biological barrier is the blood-brain barrier. In the amino acid disorders phenylketonuria and maple syrup urine disease, the expression of the LAT1 transporter in microvascular endothelial cells is the prerequisite for increased transport of the essential amino acids L-phenylalanine and L-leucine, respectively, from plasma to the brain interstitial fluid, competing with other large neutral amino acids using the same transporter and, as consequence, causing harm to the brain.<sup>38-40</sup> In OADs, the lack of an effective efflux transport for di- and tricarboxylic compounds across the blood-brain barrier relevantly contributes to the cerebral accumulation of neurotoxic glutarate and 3-hydroxyglutarate in GA1, 2-methylcitrate in MMA and PA, and methylmalonate in MMA (*entrapment hypothesis*).<sup>41,42</sup> At supraphysiologic concentrations, glutaric acid induces dysfunction of the blood-brain barrier, however, with unclear pathophysiologic relevance for GA1.<sup>10,43</sup>

Besides accumulation, a second major requirement for a toxic metabolite is the presence of one or more important intracellular targets to harm the affected organism. Enzymes are not absolutely substrate-specific at a low rate and can also accept metabolites that share similarities with their preferred substrate. An example for this “*substrate promiscuity*” is L-malate dehydrogenase

(EC 1.1.1.37), performing a side activity on 2-oxoglutarate thereby producing L-2-hydroxyglutarate. Furthermore, enzymes can catalyze an incorrect reaction on their physiological substrate (“*catalytic promiscuity*”),<sup>44</sup> resulting in side-products of canonical enzymes as well as non-canonical metabolites. Pathomechanistic studies on OADs have unraveled that enzymes can also become the target of toxic metabolites that resemble physiological inhibitors, what may be termed “*inhibitor promiscuity*”. For example, it was demonstrated that glutaryl-CoA inhibits the E2 subunit of the OGDH complex, resembling the feedback inhibition of its physiologic product succinyl-CoA and thereby causing dysfunction of the tricarboxylic acid (TCA) cycle in GA1.<sup>6</sup> In analogy, propionyl-CoA, which accumulates in MMA and PA, resembles the feedback inhibition of acetyl-CoA acting at the pyruvate dehydrogenase complex (EC 1.2.4.1) and thereby disconnects anaerobic and aerobic ATP production.<sup>45</sup>

### 3 | NEW CONNECTIONS: FROM MITOCHONDRIAL DYSFUNCTION TO IMPAIRED MITOPHAGY

Previous studies have shown that accumulating toxic acyl-CoAs and carbonic acids affect mitochondrial energy metabolism in a synergistic way (Figure 1). Underlying mechanisms have been reviewed previously<sup>4,9-12</sup> and are therefore only briefly summarized here. In GA1, it is postulated that glutaryl-CoA inhibits the TCA cycle via the OGDH complex,<sup>6</sup> while glutarate and 3-hydroxyglutarate impair the exchange of TCA cycle intermediates between astrocytes and neurons via inhibition of sodium-dependent dicarboxylate transporters, disrupting their bioenergetic coupling.<sup>46,47</sup> Furthermore, glutarate impairs astroglial glutaminolysis, an important anaplerotic mechanism, via inhibition of glutamate dehydrogenase (GDH, 1.4.1.3).<sup>48</sup> Also, energy supply of the brain could be further impaired by 3-hydroxyglutaric acid-induced vascular dysfunction and glutarate-mediated alteration of capillary pericyte contractility,<sup>49,50</sup> which may explain disturbed autoregulation of cerebral blood flow.<sup>51</sup> As a consequence of mitochondrial dysfunction and excitotoxic mechanisms the production of reactive oxygen species (ROS) is increased.<sup>10</sup> In MMA and PA, the primary enzymatic defects are located in the propionate pathway that fuels succinyl-CoA into the TCA cycle, another important anaplerotic mechanism. Intramitochondrially accumulating propionyl-CoA impairs energy metabolism via inhibition of the pyruvate dehydrogenase complex<sup>45</sup> and succinate-CoA synthetase (EC 6.2.1.4),<sup>52</sup> and ureagenesis via inhibition of N-acetylglutamate synthase (EC 2.3.1.1).<sup>53</sup> The TCA cycle flux is further disturbed by

2-methylcitrate-mediated inhibition of the TCA enzymes citrate synthase (EC 2.3.3.1), aconitase 2 (EC 4.2.1.3), and isocitrate dehydrogenase (EC 1.1.1.42),<sup>54</sup> and by methylmalonate-induced inhibition of mitochondrial succinate uptake.<sup>55</sup> Mitochondrial dysfunction, stress signaling, and apoptosis were demonstrated via altered miRNA expression in a PA mouse model,<sup>56</sup> and oxidative stress was shown in MMA and PA patient fibroblasts.<sup>57,58</sup>

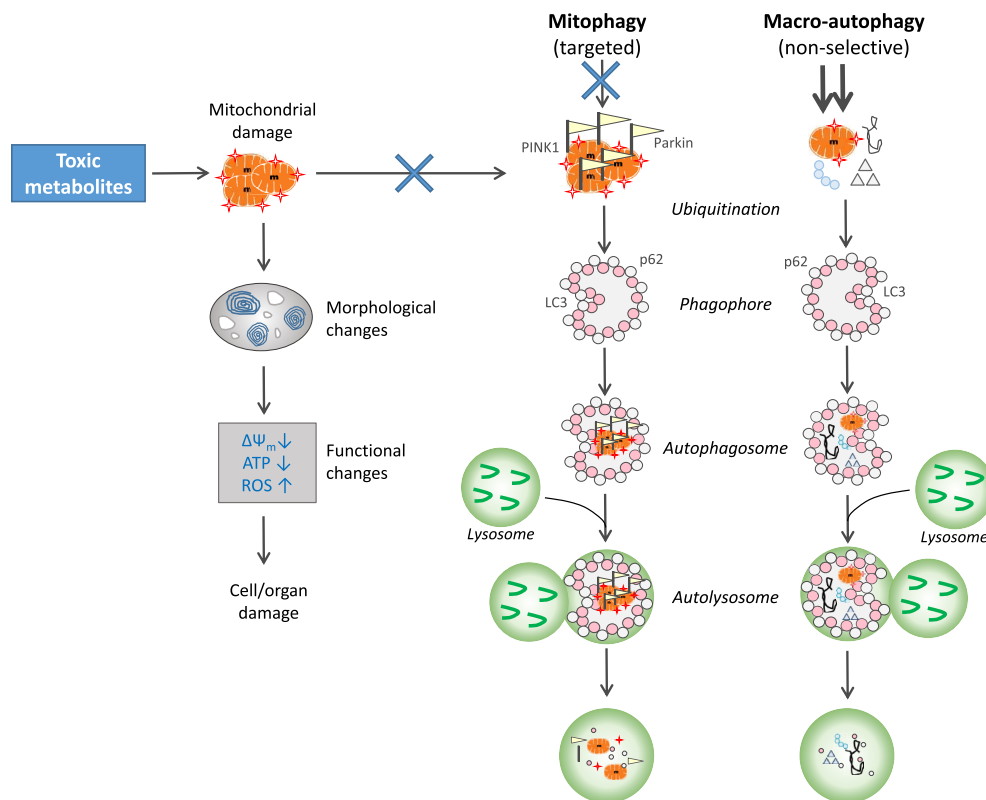
The major strength of this concept is that it can explain acute metabolic decompensations via synergistically acting pathways leading to mitochondrial dysfunction. These inhibitory mechanisms, however, are potentially reversible with decreasing toxic metabolite concentrations. Therefore, the weak point of this concept is the progressive organ dysfunction in supposedly “metabolically stable” individuals with an OAD, which might be explained by sustained mechanisms that aggravate organ dysfunction with age. Evidence for sustained mitochondrial pathology was found in *Gcdh*<sup>-/-</sup> mice, showing enlarged mitochondria with reduced electron density during induced metabolic crises,<sup>59</sup> and in HeLa cells transfected with mutant GCDH, revealing elongation of mitochondria and disturbed inner mitochondrial membrane organization.<sup>60</sup> Progression of mitochondrial dysmorphology and dysfunction was also demonstrated in liver, kidney, skeletal and heart muscle of MMA and PA patients, showing multiple deficiencies of respiratory chain complexes and thus partially resembling mitochondrial DNA depletion syndromes. In analogy, reduced cytochrome *c* oxidase was shown in *Mut*<sup>-/-</sup> mice, being accompanied by progressive organ dysfunction and the formation of mega-mitochondria.<sup>1,45,61,62</sup> Chronic depletion of mitochondrial DNA, possibly caused by propionyl-CoA induced inhibition of succinyl-CoA synthetase, was initially suggested as putative mechanism but was not unequivocally supported by experimental data.<sup>1,45,61</sup>

A recent publication demonstrated increased expression and activities of mitochondrial enzymes including cytochrome *c* oxidase in cultivated kidney cells of *mut*<sup>0</sup>-type MMA patients, which unlike control cells were unresponsive to mitochondrial inhibitors such as rotenone.<sup>63</sup> In the same model, the observation of unbalanced autophagy opened a new perspective for pathophysiological research in OADs, linking mitochondrial dysfunction to subsequent organelle aging. Electron microscopy revealed an increased number of lamellar bodies in mutase-deficient kidney cells as well as in *Mut*<sup>-/-</sup>;Tg<sup>INS-Alb-Mut</sup> mice, indicating increased production of autophagosomes and autolysosomes and linking ultrastructural changes in the proximal tubule mitochondria to the manifestation of tubulointerstitial nephritis and, subsequently, chronic kidney failure, which aggravated the accumulation of methylmalonic acid.<sup>64</sup>

Enhanced macro-autophagy (or nonselective autophagy), supposedly induced by mitochondria-derived ROS, was also supported by increased levels of LC3-II and p62 under metabolic stress and was explained by reduced expression of mTORC1.<sup>65</sup> Similar morphologic alterations were obtained in the livers of transplanted MMA patients.<sup>5</sup> In MMA kidney cells, altered homeostasis of the mitochondrial network and reduced membrane potential was found to be accompanied by oxidative stress and increased macro-autophagy.<sup>65</sup> Macro-autophagy is an evolutionary conserved mechanism that enables the degradation of cells containing dysfunctional mitochondria and subsequent renewal through biogenesis.<sup>66</sup> While macro-autophagy and autolysosomal biogenesis are promoted via regulation of upstream signaling cascades in MMA, patient-derived kidney cells and primary proximal tubular cells of *mmut*-deleted mice fail to clear dysfunctional mitochondria through PINK1/Parkin-mediated mitophagy (or organelle-specific, targeted autophagy), despite unchanged lysosomal dynamics. Concomitantly increased macro-autophagy and disturbed mitophagy highlight unbalanced autophagy. Mitophagy is an important mechanism by which exhausted and selectively targeted mitochondria are cleared. Deficiency of the mitophagy pathway in MMA patients hence explains CKD by accumulation of dysfunctional ROS-producing organelles and enhanced renal epithelial stress (Figure 2). Noteworthy, treatment with the mitochondria-targeted antioxidant mito-TEMPO partially restored mitochondrial function and mitophagy. These findings underline the importance of the mitochondrial quality control system and offer potentially targetable aims which might be exploited for innovative therapies. In conclusion, theoretically reversible mitochondrial dysfunction induced by increased toxic metabolite concentrations during catabolism or protein intake beyond individual tolerance might be sustained by impairment of an important quality control system that is required to eliminate damaged organelles, to maintain functional mitochondria, and to meet energy demands. It needs to be evaluated carefully whether this mechanism also underlies progressive dysfunction in other organs of MMA patients and in other OADs.

#### 4 | FROM THE MITOCHONDRIAL CONFLUENCE OF COA ESTERS TO POST-TRANSLATIONAL PROTEIN ACYLATION

An increasing number of intracellular short-chain mono- and dicarboxylic acyl-CoAs have been shown to be involved in post-translational short-chain acylation



**FIGURE 2** Unbalanced autophagy: impairment of mitochondrial quality control system. Mitochondria of MMA patients experience functional and morphological changes due to the accumulation of toxic metabolites (left). Because of a defective mitochondrial quality control system (controlled by its key players PINK1/parkin) dysfunctional mitochondria cannot be correctly delivered to the autophagy-lysosome system inducing epithelial stress and concomitant tissue damage (middle). Macro-autophagy is activated as a compensatory mechanism (right)

Source: With modifications from: Ruppert, Schumann<sup>63</sup>; Manoli<sup>5</sup>; Luciani<sup>65</sup>

of the  $\epsilon$ -group of lysine residues in histones and enzymes,<sup>67</sup> regulating gene expression and enzymatic activity and hence adapting metabolic activities to the current demands.

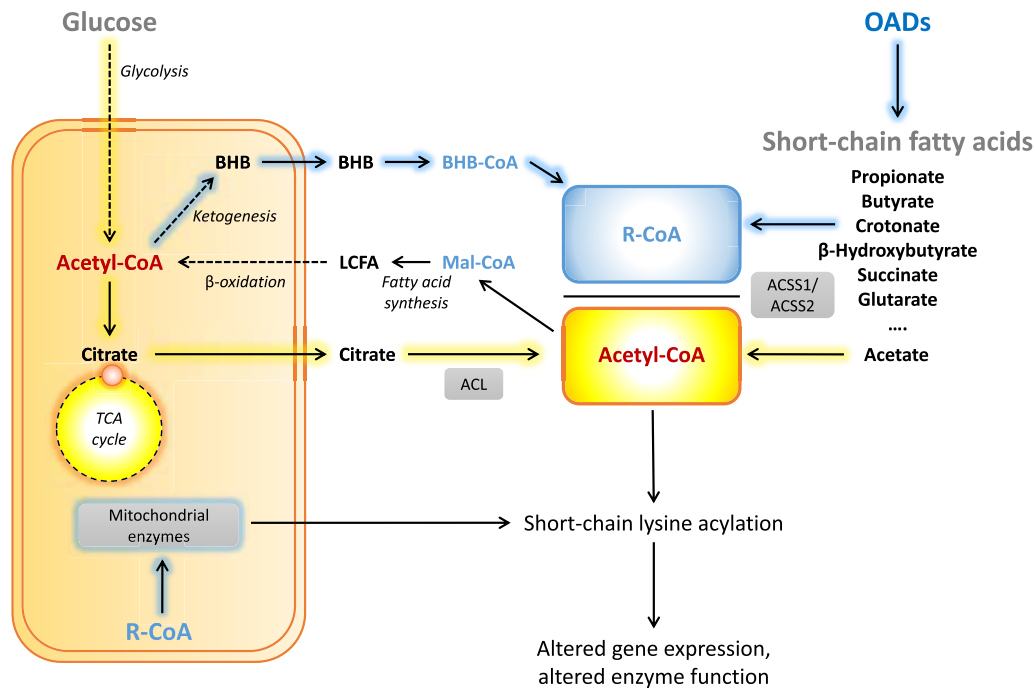
In 1963, enzymatically driven histone acetylation was the first discovered protein acylation,<sup>68,69</sup> and further post-translational modifications such as butyrylation, propionylation, 2-hydroxyisobutyrylation, succinylation, malonylation, crotonylation,  $\beta$ -hydroxybutyrylation, and glutarylation were subsequently described (Figure 3). Short-chain acylation changes the chemical properties of the modified amino acid residues by adding a hydrophobic, polar, or acidic group, and hence modulates the ability to form hydrogen bonds, electrostatic interactions with negatively charged residues, and van der Waals interactions with other proteins.<sup>67</sup> The general amount of acyl-CoAs can influence histone acylation and therefore affect gene expression.<sup>70</sup> For example, under low glucose conditions more nonacetyl histone acylations are present, while under high glucose conditions and concomitantly increasing acetyl-CoA concentration histone acylation becomes predominant.<sup>71</sup> Changing the abundance of post-translational modifications of histones enables an organism to adapt its biological processes and cellular metabolism to nutrient supply by fundamental metabolite sensing.<sup>72,73</sup>

In contrast to histone acylation, short-chain acylation of mitochondrial proteins is enzyme-independent. In individuals with OADs, disease-specific acyl-CoAs are formed

in the mitochondria. Since the inner mitochondrial membrane is impermeable to acyl-CoAs, their accumulation in the mitochondrial compartment is facilitated, whereas a direct impact of fluctuating acyl-CoAs on regulatory proteins in the nucleus is limited.<sup>74,75</sup> In other words, different branches of metabolism generate specific classes of acyl-CoAs whose regulatory impact is determined by intracellular compartments. In OADs, the variety of disease-specific acyl-CoAs drives the diversity of acylations of metabolic proteins, particularly in mitochondria.<sup>76-78</sup> Under physiologic conditions, most adaptations are short-term and constantly changing with the metabolic state. In case of OADs like GA1, PA, and MMA, however, the permanent and massive increase in certain mitochondrial acyl-CoAs may result in chronically enhanced nonenzymatic, metabolite-sensitive mitochondrial protein acylation, even during intervals of supposedly “metabolic stability”.<sup>79,80</sup>

In line with these assumptions, *Gcdh*<sup>-/-</sup> mice revealed markedly increased global protein glutarylation already under standard conditions.<sup>81</sup> Among them, hyperglutarylated carbamylphosphate synthetase 1 (EC 6.3.4.16), a mitochondrial enzyme involved in ureagenesis, was less active than in wild-type mice but showed increased enzyme expression, presumably to compensate for reduced *GCDH* enzymatic activity. Further investigation of the glutarylomes in liver and brain homogenates of *Gcdh*<sup>-/-</sup> mice identified 148 and 35 glutarylated proteins, respectively, varying in their number of glutarylation sites





**FIGURE 3** Metabolic regulation of short-chain lysine acylation. The concentrations of intracellular acyl-CoA esters such as acetyl-CoA and short chain acyl-CoAs (R-CoA) reflect metabolic activity and the availability of energy substrates. Besides many other metabolic functions, acyl-CoA esters can also serve as a substrate for post-translational acylation of lysine residues of histones and enzymes, changing gene expression and enzymatic activity to adapt metabolic activity in a coordinated way by metabolite sensing. Chronic increase in R-CoA esters, however, might result in metabolic maladaptation in the long run. ACL, ATP-citrate lyase; ACSS1, acyl-CoA synthetase short-chain family, member 1; ACSS2, acetyl-CoA synthetase short-chain family, member 1; BHB, β-hydroxybutyrate; BHB-CoA, β-hydroxybutyryl-CoA; LCFA, long-chain fatty acid; Mal-CoA, malonyl-CoA; OAD, organic aciduria or acidemia; TCA, tricarboxylic acid cycle  
Source: With modifications from: Sabari<sup>67</sup>

and their role in various metabolic pathways. Ultrastructural analysis unraveled glutarylated proteins in the brain to be exclusively localized in glial cells, with specifically reduced catalytic activities of glutarylated GDH and brain-specific carbonic anhydrase 5b (EC 4.2.1.1).<sup>82</sup> The exclusive astroglial localization of glutarylated proteins was unexpected since in the brain GCDH is predominantly expressed in neurons<sup>83</sup> and—less pronounced—in cortical astrocytes. Although murine *Gcdh*<sup>-/-</sup> astrocytes seem to be able to produce low amounts of glutaric and 3-hydroxyglutaric acid, and although astrocytic production can be stimulated by supraphysiological concentrations of lysine, it remains to be elucidated why mitochondrial proteins in neurons escape enhanced glutarylation as mitochondria of neurons are assumed to be the major source of glutaryl-CoA in the brain.<sup>83</sup>

The net effect of glutarylated astroglial proteins is also far from being understood. While protein glutarylation was originally supposed to limit the astroglial synthesis of glutamate and thus to adapt glial cells to the metabolic demands of neighboring *Gcdh*<sup>-/-</sup> murine neurons, the opposite effect seems even more likely, since GDH is an important anaplerotic enzyme that feeds 2-oxoglutarate in the TCA cycle. Three consequences of increased GDH

glutarylation seem possible: First, it may lead to decreased astroglial GDH activity and, subsequently, increased glutamate levels in the synaptic cleft. Second, it may deplete the mitochondrial 2-oxoglutarate pool, impairing anaplerosis and energy production, and third, it may affect the formation of GDH- and GCDH-containing enzymatic supercomplexes. These mechanisms may further impair the astroglial provision of TCA cycle intermediates for neurons and hence the metabolic coupling of astrocytes and neurons,<sup>41,47</sup> thereby promoting excitotoxic mechanisms.<sup>84</sup>

It remains to be unraveled whether selective astroglial protein glutarylation underlies white matter changes predominantly found in GA1 patients with a high excretor phenotype, and progress with age despite adherence to metabolic therapy.<sup>85,86</sup>

## 5 | THE YET UNFULFILLED PROMISE OF SAFE AND DISEASE-CHANGING THERAPIES

Evidence-based recommendations for the diagnosis, therapy, and long-term management of individuals with GA1,

MMA, and PA have been proposed.<sup>20,87</sup> Standard long-term management for these OADs is based on conservative management including low lysine diet with precursor-free, arginine-fortified amino acid supplements, and carnitine supplementation in GA1 patients, and low protein diet with or without precursor-free amino acid supplements, hydroxocobalamin (in responsive MMA patients), carnitine supplementation, and antibiotic-mediated reduction of intestinal propionate-producing microbiota in MMA/PA patients. For all OADs, emergency treatment is recommended to maintain anabolism during potentially harmful situations and to reduce the production and enhance the detoxification of toxic metabolites.

Recommended metabolic therapy for MMA and PA patients does not reliably protect against recurrent metabolic decompensations and the manifestation of long-term complications.<sup>11,12</sup> Furthermore, iatrogenic prescription of dietary treatment may result in chronic changes of plasma amino acids and other nutrients, possibly affecting growth and interfering with important cellular mechanisms such as autophagy.<sup>88</sup> In contrast, adherence to recommended therapy markedly reduces the frequency of striatal damage in screened individuals with GA1,<sup>89-93</sup> while therapeutic deviation increases the risk of dystonia and untimely death.<sup>15,94</sup> But careful evaluation of the long-term follow-up of GA1 patients, however, identified (a) an age-dependent CKD which did not seem to be impacted by recommended therapy,<sup>15</sup> (b) an increased risk of cerebral accumulation of glutarate and progressive neuroaxonal compromise in high excretor-type GA1 patients,<sup>85</sup> and (c) the manifestation of malignant brain tumors in three patients with poor adherence to or late start of therapy.<sup>27</sup> Although recommended therapy might be beneficial for the majority of pediatric GA1 patients, as it is for many MMA and PA patients, recent observations raise the question about whether it reliably protects against long-term complications in adolescents and adults. Novel therapeutic strategies aiming to reduce the cerebral lysine oxidation are currently under investigation. One of these strategies, that is, pharmacologic inhibition of dehydrogenase E1 and transketolase domain containing 1 aiming to reduce the production of glutaryl-CoA, failed to rescue the biochemical and clinical phenotype of *Gcdh*<sup>-/-</sup> mice since OGDH provides an alternative pathway for the generation of glutaryl-CoA.<sup>33</sup>

While liver transplantation is not thought to be a therapeutic option for individuals with GA1,<sup>87</sup> liver transplantation (PA, MMA), and kidney or combined liver/kidney transplantation (MMA) can be considered in MMA and PA patients with frequent metabolic decompensations.<sup>20</sup> Nevertheless, experience is still limited and decisions are highly individualized. While liver or combined liver/kidney transplantation in MMA patients has

become an effective alternative treatment option with favorable short-term outcome, graft survival, and survival,<sup>95-99</sup> and has led to partial correction of the metabolic derangement, and markedly reduced levels of the hepatokine and metabolic key regulator FGF21,<sup>5,100</sup> this intervention does not cure the disease but attenuates the biochemical and clinical phenotype. As a consequence, conservative metabolic management, although less strict than before transplantation, is continued in order to lower the remaining risk of neurologic and renal deterioration.<sup>20</sup> Furthermore, the choice of a renal-sparing immunosuppressive regime is recommended, and potential neurological complications of certain immunosuppressive medications need to be taken into account.<sup>101</sup>

Compared to MMA, the benefits and risks of liver transplantation in PA patients are less clear. Some studies provided evidence that liver transplantation might be more cost-effective and beneficial than conservative management<sup>102,103</sup> and might halt or even reverse dilated cardiomyopathy.<sup>23,104</sup> This view has been challenged by the demonstration of high post-transplant mortality, the development of severe, unusual and unexpected perioperative complications, and worsening of pre-existing kidney dysfunction.<sup>105</sup> Furthermore, the post-transplant lack of well-known metabolic “red flags” may conceal metabolic derangements of the “hidden” brain compartment. This highlights the need to carefully evaluate the individual risk of perioperative and post-transplant complications and to take all necessary steps to minimize these risks in advance.

Innovative therapeutic approaches with promising results in mouse models include liver-directed recombinant AAV gene delivery (MMA, PA) and systemic mRNA therapy (MMA).<sup>106-109</sup> In analogy to liver transplantation and animal studies, this novel approach will not cure the OAD patient but will likely produce an attenuated phenotype. Although this approach spares patients from the risks of organ transplantation and immunosuppressive therapy, long-term safety and efficacy of these therapies need further attention. For example, it was demonstrated that the AAV vector dose, enhancer/promotor selection, and the timing of gene delivery are critical factors that determine the risk of developing hepatocellular carcinoma after AAV gene delivery to the liver.<sup>110</sup> Furthermore, both approaches will or are likely to result in a transient improvement of hepatic enzyme activity. For mRNA therapy, repetitive applications similar to the frequency of enzyme replacement therapies would be required to prevent metabolic deterioration.<sup>108</sup> In analogy, liver-directed gene delivery, although being much more sustained than mRNA therapy, will unlikely result in life-long rescue of hepatic enzyme deficiency if transgene DNA copies mostly persist as nonintegrated

episomes and hence are not be transferred to sister cells. Finally, repetitive applications might be challenging (if possible at all) with regard to immunologically driven adverse events and health expenditures.

## 6 | OUTLOOK: BUILDING BRIDGES

Recent studies have started to bridge the knowledge gap between the primary defect of OADs and late onset disease complications, which develop in the absence of acute metabolic decompensations. Two new pathophysiological mechanisms thought to sustain and aggravate metabolite-driven mitochondrial dysfunction and metabolic maladaptation in OADs described are: (a) unbalanced autophagy, resulting in impaired quality control of organelles and thus facilitating organelle aging, and (b) metabolite-driven short-chain lysine acylation, modulating enzyme function and gene expression. These mechanisms are complementary to previously described mechanisms induced by accumulating acyl-CoAs and carbonic acids, synergizing in impaired mitochondrial energy metabolism. Exploitation of these mechanisms might identify selective drug targets in the future that are suitable to prevent disease progression. At present, organ (liver, kidney) transplantation in MMA and PA patients experiences a revival but requires careful pretransplant evaluation and optimal post-transplant follow-up by a multiprofessional team including a metabolic specialist to ensure a favorable long-term outcome. Organ transplantation, similar to a systemic and liver-directed AAV-mediated mRNA therapy, does not cure the disease, whereas liver-directed therapies may result in a better long-term outcome than conservative metabolic management. The next years will be substantially determined by the translation of novel mechanistic findings to targeted therapies, clinical trials on innovative therapies, the identification of predictive biomarkers such as FGF21 to identify high risk patients and to monitor therapies, and the development of evidence-based and severity-adjusted algorithms for therapy stratification. These coordinated efforts are inevitable to fulfill the promise of safe and effective therapies for individuals with OADs.

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### AUTHOR CONTRIBUTIONS

All authors designed the concept of this review. Bianca Dimitrov, Anke Schumann, and Stefan Kölker produced the first draft of the manuscript, while all authors revised it thoroughly.

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Not applicable.

### PATIENT CONSENT STATEMENT

Not applicable.

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