Received: 7 December 2018 | Revised: 4 April 2019 | Accepted: 8 April 2019

DOI: 10.1002/jimd.12100

JIMD SSIEM WILEY

REVIEW ARTICLE

Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision

Johannes Häberle[1] | Alberto Burlina[2] | Anupam Chakrapani[3] | Marjorie Dixon[4] | Daniela Karall[5] | Martin Lindner[6] | Hanna Mandel[7] | Diego Martinelli[8] | Guillem Pintos-Morell[9,10,11] | René Santer[12] | Anastasia Skouma[13] | Aude Servais[14] | Galit Tal[15] | Vicente Rubio[16] | Martina Huemer[1,17] | Carlo Dionisi-Vici[8]

[1]University Children's Hospital Zurich and Children's Research Centre, Zurich, Switzerland

[2]Division of Inborn Metabolic Disease, Department of Pediatrics, University Hospital Padua, Padova, Italy

[3]Department of Metabolic Medicine, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[4]Dietetics, Great Ormond Street Hospital for Children, NHS Trust, London, UK

[5]Clinic for Pediatrics, Division of Inherited Metabolic Disorders, Medical University of Innsbruck, Innsbruck, Austria

[6]University Children's Hospital, Frankfurt am Main, Germany

[7]Institute of Human Genetics and metabolic disorders, Western Galilee Medical Center, Nahariya, Israel

[8]Division of Metabolism, Bambino Gesù Children's Hospital, Rome, Italy

[9]Centre for Rare Diseases, University Hospital Vall d'Hebron, Barcelona, Spain

[10]CIBERER_GCV08, Research Institute IGTP, Barcelona, Spain

[11]Universitat Autònoma de Barcelona, Barcelona, Spain

[12]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[13]Institute of Child Health, Agia Sofia Children's Hospital, Athens, Greece

[14]Service de Néphrologie et maladies métaboliques adulte Hôpital Necker 149, Paris, France

[15]The Ruth Rappaport Children's Hospital, Rambam Medical Center, Haifa, Israel

[16]Instituto de Biomedicina de Valencia (IBV-CSIC), Centro de Investigación Biomédica en Red para Enfermedades Raras (CIBERER), Valencia, Spain

[17]Department of Paediatrics, Landeskrankenhaus Bregenz, Bregenz, Austria

Correspondence
Johannes Häberle, University Children's Hospital Zurich, Division of Metabolism, 8032 Zurich, Switzerland.
Email: johannes.haeberle@kispi.uzh.ch
Vicente Rubio, Instituto de Biomedicina de Valencia, c/ Jaume Roig 11, 46010 Valencia, Spain.
Email: rubio@ibv.csic.es
Martina Huemer, Department of Paediatrics; Landeskrankenhaus Bregenz, Carl-Pedenz-Strasse 2, 6900 Bregenz, Austria.
Email: martina.huemer@lkhb.at
Carlo Dionisi-Vici, Division of Metabolism, Bambino Gesu Hospital Piazza, S. Onofrio

Abstract

In 2012, we published guidelines summarizing and evaluating late 2011 evidence for diagnosis and therapy of urea cycle disorders (UCDs). With 1:35 000 estimated incidence, UCDs cause hyperammonemia of neonatal (~50%) or late onset that can lead to intellectual disability or death, even while effective therapies do exist. In the 7 years that have elapsed since the first guideline was published, abundant novel information has accumulated, experience on newborn screening for some UCDs has widened, a novel hyperammonemia-causing genetic disorder has been reported, glycerol phenylbutyrate has been introduced as a treatment, and novel promising therapeutic avenues (including gene therapy) have been opened. Several factors including the impact of the first edition of these guidelines (frequently read and quoted) may have

Vicente Rubio, Martina Huemer, and Carlo Dionisi-Vici contributed equally to this study.

4, I-00165 Rome, Italy.
Email: carlo.dionisivici@opbg.net

Communicating Editor: Manuel Schiff

Funding information
Spanish Government; Fundación Inocente Inocente, Grant/Award Number: MINECO BFU2017-84264-P; Center for International Business Education and Research, University of Illinois at Urbana-Champaign-ISCIII; the Swiss National Science Foundation, Grant/Award Number: SNF 320030_176088; Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen (APS)

increased awareness among health professionals and patient families. However, under-recognition and delayed diagnosis of UCDs still appear widespread. It was therefore necessary to revise the original guidelines to ensure an up-to-date frame of reference for professionals and patients as well as for awareness campaigns. This was accomplished by keeping the original spirit of providing a trans-European consensus based on robust evidence (scored with GRADE methodology), involving professionals on UCDs from nine countries in preparing this consensus. We believe this revised guideline, which has been reviewed by several societies that are involved in the management of UCDs, will have a positive impact on the outcomes of patients by establishing common standards, and spreading and harmonizing good practices. It may also promote the identification of knowledge voids to be filled by future research.

KEYWORDS

ammonia, arginase 1, argininosuccinate lyase, argininosuccinate synthetase, carbamoylphosphate synthetase 1, GRADE, guidelines, hyperammonemia, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, N-acetylglutamate synthase, ornithine transcarbamylase, UCD, urea cycle disorders

1 | INTRODUCTION

The first version of the urea cycle disorders (UCDs) guidelines were intended for all involved care providers, including metabolic specialists, pediatricians, dietitians, neonatologists, intensive care specialists, adult physicians, neurologists, nurses, psychologists, pharmacists, and patients and their families.[1] These guidelines covered the deficiencies of the five "classical" urea cycle enzymes (Figure 1), carbamoylphosphate synthetase 1 (CPS1), ornithine transcarbamylase

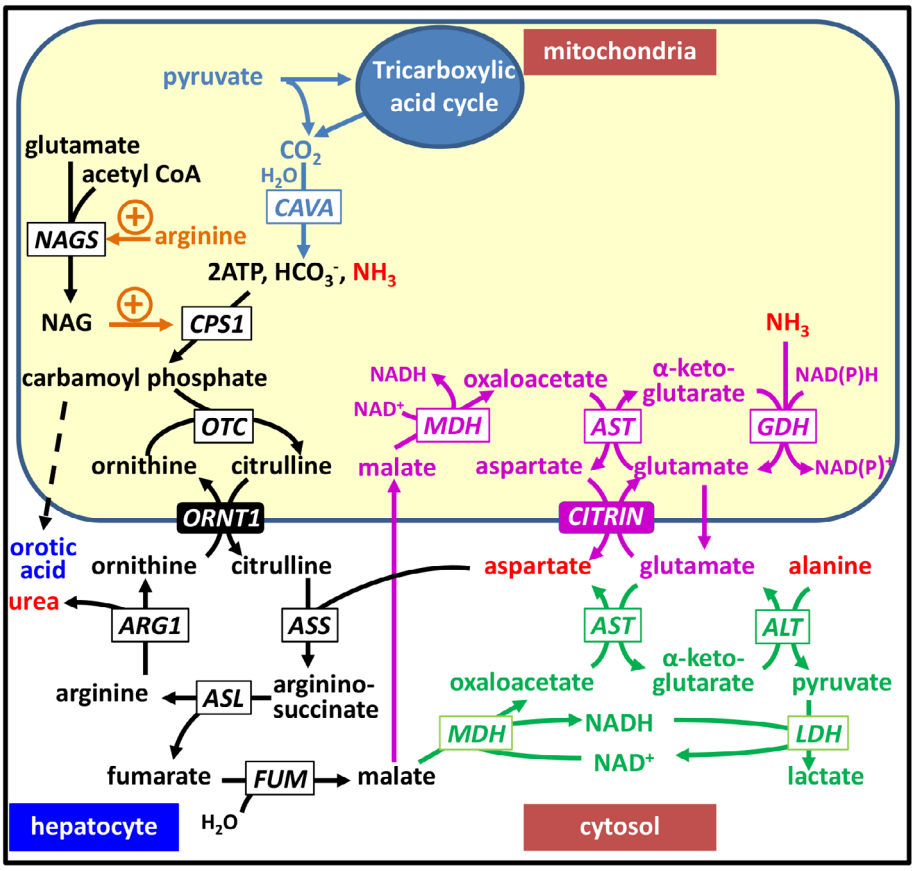

FIGURE 1 The urea cycle and associated pathways. ALT, alanine aminotransferase; ARG1, arginase 1; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; AST, aspartate aminotransferase; CAVA, carbonic anhydrase Va; citrin, mitochondrial aspartate/glutamate carrier; CPS1, carbamoyl phosphate synthetase 1; FUM, fumarase; GDH, glutamate dehydrogenase; GLS, glutaminase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; NAGS, N-acetylglutamate synthase; ORNT1, ornithine/citrulline antiporter; OTC, ornithine transcarbamylase. Modified from Reference[342]

(OTC), argininosuccinate synthetase (ASS), argininosuccinate lyase (ASL), and arginase 1 (ARG1). In addition, they dealt with the deficiencies of N-acetylglutamate synthase (NAGS), the enzyme that provides N-acetylglutamate (NAG) needed to activate CPS1, and of the mitochondrial ornithine/citrulline antiporter (ORNT1), necessary for entry of ornithine into the mitochondrion and for exit of citrulline to the cytosol for further conversion to arginine. A deficiency of the latter causes the hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (Figure 1).

Although all these deficiencies (abbreviated with "D" following the enzyme acronym) were already well-characterized before 2012, all of them having individual MIM entries (in the same order as the indicated catalysts #237300; #311250; #215700; #207900; #207800, #237310, and #238970), their relative rarity (recent estimates of their cumulative incidences, 1:35 000-1:69 000[2,3]) and dispersion among centers prompted us to make an effort to gather all the significant published evidence on them to try to draw evidence-based guidelines for diagnosis and treatment. The 2012 publication was evaluated *ex post* confirming high and sustained interest in the guidelines,[4] as reflected by the considerable number of reads and citations. Thus, they may have increased awareness and enabled streamlining and harmonization of procedures for diagnosis and effective treatment. These represent important advances since UCDs are serious disorders presenting in 50% of the cases as neonatal hyperammonemia with high mortality (25%-50%) and, frequently, with severe neurological sequelae in survivors.[5-11] The more variable clinical manifestations observed in later presentations (that may occur at any age) are also burdened with significant morbidity and mortality.[6,12-14] Furthermore, prompt diagnosis and specific treatment are imperative[15,16] to prevent subsequent cognitive impairment, known to correlate with duration and severity of hyperammonemia.[7,17-19]

Nearly 7 years after the initial publication, it was desirable to revise our guidelines, given the many changes in the UCD field and advances made. This is reflected by the addition of 151 *new* publications reporting significant advances. The advances encompass many clinically relevant aspects, including epidemiology, clinical course, and outcomes; pathogenetic mechanisms with potential to base novel therapies, exemplified with ASLD[20]; increased experience with newborn screening (NBS) for some UCDs; and a novel genetically caused hyperammonemia impacting on the urea cycle, carbonic anhydrase Va (CAVA) deficiency.[21] Furthermore, several new information has emerged, especially the availability of novel treatment options including a new drug (glycerol phenylbutyrate) and the development of novel therapeutic avenues (including gene therapy).

A postpublication criticism to our initial guidelines was the use of the Scottish Intercollegiate Guideline Network (SIGN, http://www.sign.ac.uk) methodology, which inevitably resulted in poor ratings of the strength of the guidelines' recommendations.[22] The Grading of Recommendations, Assessment, Development and Evaluation (GRADE; http://www.gradeworkinggroup.org/society/index.htm) system offered an alternative that may be more suitable for rare diseases. This first extensive revision of the UCD guidelines therefore uses the GRADE methodology for all recommendations, also those that required little update compared to the initial publication.

As in the 2012 guideline, the present revision focuses on the seven classical enzymopathies of the urea cycle and on NAGS and ORNT1 deficiencies, but also considers in the differential diagnosis citrin deficiency (citrullinemia type 2, MIM #605814 and #603471), lysinuric protein intolerance (LPI, MIM #222700), pyrroline 5-carboxylate synthetase (MIM #610652) and ornithine aminotransferase (OAT, MIM #258870) deficiencies, and CAVAD (MIM #615751), which are rare in European populations and with yet limited evidence for their management.

2 | METHODOLOGY AND OBJECTIVES

This revision is the result of a formalized consensus process from 2011 onwards, incrementing by 151 new publications the already used 263 publications of the first version of these guidelines (details on literature accretion and judgment are in Reference 1). A 17-expert panel was assembled from eight different European countries and Israel, in which R.S. was the moderator and J.H. the chairperson, and which included 10 additional pediatric metabolic specialists (A.B., A.C., C.D.-V., M.H., D.K., M.L., H.M., D.M., A.S., G.T.), a pediatric nephrologist (G.P.-M.), a metabolic specialist for adults (A.S.), and one each of the following: medical biochemist (V.R.), psychologist (M.H.), and metabolic dietitian (M.D.).

A criticism of the first version of the guideline[22] led the panel to adopt the GRADE methodology for scoring evidence levels (Grading of Recommendations Assessment, Development and Evaluation, http://www.gradeworkinggroup.org/) as High (++++), Moderate (+++), Low (++), and Very low (+).

The panel reached a consensus on the importance of the outcome parameters and key questions listed below. Outcomes were rated on a scale from 1 to 9 in increasing degree of importance for decision-making. Scores of 9 to 7 were considered to be of critical importance, 6 to 4 of importance but not critical and 3 to 1 of low importance.

Outcome/key question	Importance/ score given
1. Survival in early onset disease	Critical
How can survival be improved?	9
• How can patients be identified early and reliably?	9
• Which parameters are prognostic in the short and long-term?	8
• What interventions are appropriate in which situations?	8
2. Survival in late-onset disease	Critical
• How can survival be improved?	8
• How can patients be identified early and reliably?	8
• Which parameters are prognostic in the short and long-term?	7
• What interventions are appropriate in which situations?	8
3. Cognitive outcome	Critical
• How to preserve cognitive function?	8
4. Neurological outcome	Critical
• How to prevent neurological disease?	8
5. Liver disease	Important
• How to prevent significant liver disease?	6
6. Psychiatric outcome	Important
• How to prevent psychiatric manifestations?	6
7. Quality of life	Critical
• How to preserve quality of life?	8
• How to reduce the burden of disease?	7
• What is the burden of dietary and drug treatment?	7
8. Auxology	Important
• How to achieve normal growth and weight?	6

Medline, Web of Science, Embase, Cochrane Library, MedLink, and Orphanet were used for systematic literature searches.

The panel examined the recommendations of the first version, re-phrased and changed them as required by the updated evidence. Based on the strength of the evidence derived from the literature, recommendations are phrased according to GRADE (we strongly recommend …; we recommend …; we suggest …). Expert opinions (ie, no published evidence, result of a consensus-oriented discussion of the panelists) are labeled as such.

2.1 | Statement of intent

The goal of these guidelines remains the same, to facilitate informed decision-making in the process of UCD patient care and to be considered an evidence-based instrument to help optimize care. In dealing with individual patients, the guidelines should not substitute prudent clinical decision-taking, but they can provide an informed background for discussing diagnostic and therapeutic options. Despite the use of as sound evidence as available, they cannot guarantee by themselves satisfactory diagnosis and outcome in every patient, neither can they be considered to exhaustively cover all possibilities for diagnosis and care; they do not exclude in principle other nonmentioned but acceptable methods that have not been included.

3 | SUGGESTED GUIDELINES

3.1 | Diagnostic aspects of UCDs

3.1.1 | The clinical picture

The majority of the UCDs covered in this guideline were already well known in 2011 and thus their clinical presentations were fully represented in the first version of these guidelines, with little substantial change since then. Therefore, we refer to the earlier version for more detail and we provide a slightly modified table (Table 1) to summarize the clinical features and triggering factors in these disorders.

In short, UCDs can have acute, chronic, and intermittent clinical manifestations occurring at any age,[23-29] having as clinical hallmark the hyperammonemic crisis, which is mostly triggered by the change from intrauterine to neonatal life and by catabolic events, protein overload or intake of certain drugs (Table 1). Concerning drugs, a novel realization is that attention should be payed to inhibitors of carbonic anhydrases (like topiramate[30]), since bicarbonate is a substrate of CPS1 and its local production in liver mitochondria by CAVA can limit the rate of CPS1. This has been revealed by the report of hyperammonemia due to CAVAD.[21]

The fragility and structural alterations of hair (trichorrhexis nodosa) of ASLD[23,31-33] and the progressive spastic diplegia of ARG1D and HHH syndrome (frequently without a history of hyperammonemia)[29,34-36] are relatively specific manifestations of UCDs that are apparent on clinical examination.

As symptoms of UCDs are nonspecific and they are manifestations of ammonia-triggered encephalopathy, UCDs should be immediately suspected when unexplained encephalopathy occurs at any age, but particularly in neonates.[37,38] Table 1 reflects the occurrence of less frequent and largely nonspecific hepatic-digestive and other neurological and/or psychiatric manifestations in UCDs, including acute or chronic liver failure as presenting manifestation in OTCD, ASSD, ARGD, and HHH syndrome[39-46] and, more rarely, stroke-like episodes (metabolic strokes; detectable by diffusion magnetic resonance imaging [MRI]) that can be reversed with prompt treatment of the UCD[17,47-51]; chorea[52];

TABLE 1 Clinical signs and symptoms of acute and chronic presentations of UCDs, and triggering factors for hyperammonemia in UCD patients

Acute presentation	Chronic presentation
• **Altered level of consciousness (from lethargy and somnolence to coma) mimicking encephalitis or drug intoxication**	• **Confusion, lethargy, dizziness**
• **Acute encephalopathy (see below)**	• **Headaches, migraine-like, tremor, ataxia, dysarthria flapping tremor (in adults)**
• **Seizures (mostly under situation of altered level of consciousness)**	• **Learning disabilities, cognitive impairment**
• Ataxia: mostly under situation of altered level of consciousness	• Epilepsy
• Stroke-like episodes	• Chorea, cerebral palsy
• Transient visual loss	• Protracted cortical visual loss
• **Vomiting and progressive poor appetite**	• Progressive spastic diplegia or quadriplegia starting in childhood (described in ARG1D and HHH syndrome)
• Liver failure, coagulopathy (esp. in OTCD and HHH)	• **Protein aversion, self-selected low-protein diet**
• **Multiorgan failure**	• **(Recurrent) abdominal pain, vomiting**
• **Peripheral circulatory failure**	• **Failure to thrive**
• **Psychiatric symptoms (hallucinations, paranoia, mania, emotional or personality changes)**	• **Hepatomegaly, elevated liver enzymes**
• "Post-partum psychosis"	• **Psychiatric symptoms: hyperactivity, mood alteration, behavioral changes, aggressiveness**
• **In neonates: sepsis-like picture, temperature instability, respiratory distress, hyperventilation**	• Self-injurious behaviour
	• *Autism-like symptoms*
	• **Fragile hair (mainly in ASLD)**
	• *Dermatitis*
	• **Episodic character of signs and symptoms**
	• Specific neuropsychological phenotype in heterozygous OTC females

Potential triggers of hyperammonemic crises in UCD patients
• Birth of the patient: passage from intrauterine to extrauterine life
• Infections
• Fever
• Vomiting
• Gastrointestinal or internal bleeding
• Decreased energy or protein intake (eg, fasting pre surgery, major weight loss in neonates)
• Catabolism and involution of the uterus during the postpartum period (mostly OTC females)
• Chemotherapy, high-dose glucocorticoids
• Prolonged or intense physical exercise
• Surgery under general anesthesia
• Unusual protein load (eg, a barbecue, parenteral nutrition)
• Drugs: Mainly **valproate and L-asparaginase/pegaspargase.** Topiramate, carbamazepine, phenobarbitone, phenytoine, primidone, furosemide, hydrochlorothiazide and salicylates have also been associated with hyperammonemic decompensation.

Bold: typical signs and symptoms; standard: uncommon signs and symptoms; *italics*: signs and symptoms only reported in single patients.

cerebral palsy without patent hyperammonemia or cerebral edema[53,54]; episodic cortical visual losses[55,56]; autism-like symptoms[13,57]; behavioral problems during childhood[13] and in postpuberal patients; and other episodic psychiatric symptoms that may be the only manifestation of a UCD.[58] The dermatitis observed in some UCD patients likely reflects protein malnutrition.[59,60]

Concerning severity, symptoms can be subtle in partial deficiencies, even with spontaneous resolution of acute episodes without targeted intervention. This is quite characteristic for female carriers for OTCD, in whom there is ample variability of disease expression largely due to variable lyonisation, since this is an X-linked defect.[15,61] Postpartum coma has been reported as a first manifestation in females with partial OTCD, CPS1D, and ASSD.[62-64] Manifestations of partial UCDs can vary among individuals having the same mutant genotype, exemplified best with partial OTCD and CPS1D in different members of the same family.[65-67]

The family history may indicate X-linked inheritance for OTCD and show consanguinity in other UCDs, all of which are autosomal recessive conditions. It is mandatory with suspected UCD patients to take a careful medical history, investigating the occurrence in the family of unexplained neonatal deaths, of neurological disorders, or of protein avoidance, and asking about drug intake by the patient.

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #1: We strongly recommend considering a UCD at any age in any acute or intermittent neurological deterioration or psychiatric illness, acute liver failure, suspected intoxication, or in the differential diagnosis of neonatal sepsis. Catabolism or protein load may represent triggering factors.
Quality of evidence: moderate (8/12 panelists voted "moderate," 4/12 panelists voted "high")

3.1.2 | Laboratory findings

Hyperammonemia is the hallmark of UCDs with peak ammonia concentrations >500 μmol/L in most neonatal patients at presentation.[38] Normal ammonia virtually excludes a UCD in a symptomatic newborn (but not in an older patient). Immediate ammonia measurement in an emergency setting is crucial since patient outcome correlates with the length of hyperammonemia.[5,7,19] To shorten the time to diagnosis, an electronic medical record-based warning system to consider ammonia measurement has been suggested for neonates aged 2 to 7 days, on whom blood gas analyses

are undertaken.[68] Respiratory alkalosis in a newborn is present in up to 50% of acute UCDs and should prompt immediate ammonia measurement.[69] There are clinically relevant pre-analytical pitfalls with respect to collection, handling, storage, and analysis of blood samples for ammonia analysis.[70,71]

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #2: We strongly recommend determining ammonia in all conditions defined by recommendation #1 as an emergency analysis. Be aware of pre-analytical pitfalls.
Quality of evidence: high (9/12 high, 3/12 moderate)

If hyperammonemia is confirmed, plasma amino acids, blood or plasma acylcarnitines, and urine organic acids and orotic acid should be analyzed urgently. The results should be available within 24 hours, but treatment must not be delayed due to pending results. In patients with fatal outcome, it is recommended to obtain anticoagulated blood for DNA isolation and to keep frozen aliquots of plasma, serum, urine, and cerebrospinal fluid (CSF) samples.[26,72]

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #3: If ammonia is elevated, we strongly recommend taking blood samples for urgent analysis of amino acids and acylcarnitines. Then start treatment while awaiting the results. Urine for analysis of organic acids and orotic acid should also be requested.
Quality of evidence: moderate (6/12 moderate, 5/12 high, 1/12 low)

3.1.3 | Differential diagnosis

Neonatal sepsis is the most common misdiagnosis in UCD patients with early manifestation. Several other conditions, by increasing ammonia production and/or impairing ammonia detoxification, can mimic a UCD.[26,73-77] *Neonatal hyperammonemia,* can also be secondary in other inborn errors,[78] liver failure, or congenital infection. Premature infants can have a transient hyperammonemia of the newborn.[79-82] *Late-onset hyperammonemia* can be triggered by most conditions causing neonatal hyperammonemia and by increased protein catabolism (eg, post-partum, chemotherapy, steroids, trauma, gastrointestinal hemorrhage), acute or chronic liver failure, exogenous intoxications, drugs (eg, valproic acid), porto-systemic shunting, "Reye syndrome," and by conditions that vastly increase direct ammonia production (eg, asparaginase treatment, urease-positive bacteria overgrowth, or genito-urinary infection), or excessive nitrogen supply (reported in total parenteral nutrition or after glycine-solution irrigations in transurethral prostate resection).[6,31,83-89] Table 2 lists inborn errors of metabolism causing hyperammonemia and guides bedside differentiation.

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #4: As the most common misdiagnosis of early onset UCD patients is neonatal sepsis, we strongly recommend considering the possibility of a UCD in the differential diagnosis.
Quality of evidence: moderate (8/12 moderate, 4/12 high)

3.1.4 | Biochemical and enzymatic analysis

Standard clinical and analytical procedures can generally differentiate between hyperammonemia due to inborn errors or due to other conditions.[26,90-93] Figure 2 provides an algorithm for differential diagnosis of hyperammonemia. Identification is mainly based on plasma/urine analytical parameters. Importantly, the metabolite pattern generally is more informative than absolute levels of single metabolites. Only some UCDs have a specific biochemical pattern:

ARG1D: plasma arginine >300 μmol/L
ASLD: elevated plasma/urinary argininosuccinate (ASA)
ASSD: high plasma citrulline in the absence of ASA
HHH syndrome: high urinary homocitrulline
OATD: high plasma ornithine

If the metabolite pattern is not diagnostic, the final diagnosis can be achieved by activity assays of urea cycle enzymes mainly in liver (all urea cycle enzymes), red blood cells (ASL and ARG1[94]), intestinal mucosa (CPS1, OTC), or fibroblasts (ASS, ASL, HHH). However, enzyme analysis is not considered the method of choice if genetic testing is

TABLE 2 Bedside differential diagnosis of an inherited error of metabolism presenting with hyperammonemia

	Condition								
Parameter	**UCDs**	**Organic acidurias**	**β-oxidation defects**	**Carbonic anhydrase Va def.**	**HMG-CoA lyase def.**	**HI HA syndrome**	**Pyruvate carboxylase def.[g]**	**PEPCK def.**	**TMEM70, SERAC1 def.**
Acidosis	+/−	+[e]	+/−	+	+	−	+	+	+
Ketonuria[a]	−	+	absent	+	absent	−	++	+	+
Hypoglycemia[b]	−	+/−	+	+/−	+	+	+	+/−	+/−
↑ Lactic acid[c]	−	+	+/−	+	+/−	−	+	+/−	++
↑ AST and ALT	(+)[d]	−	+	−	+/−	−	+/−	++	−
↑ CPK	−	−	+	−	+/−	−	−	−	−
↑ Uric acid	−	+	+/−	−	+	−	−		++
↓ WBC/RBC/Plt	−	+	−	−	+/−	−	−	−	−
Weight loss	−	+[f]	−	−	+/−	−	+		−

Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPK, creatinephosphokinase; def, deficiency; HI-HA, hyperinsulinism-hyperammonemia; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; PEPCK, Phosphoenolpyruvate carboxykinase; SERAC1, serine active site-containing protein 1; TMEM70, transmembrane protein 70.

In addition to the conditions indicated in the table, mitochondrial oxidative phosphorylation defects, citrin deficiency, lysinuric protein intolerance or ornithine aminotransferase deficiency can also cause hyperammonemia.

[a]In neonates ketonuria (++, −, +++) suggests organic aciduria.

[b]Hypoglycemia and hyperammonemia ("pseudo-Reye") can be predominant manifestations of the organic aciduria 3-HMG-CoA-lyase deficiency.

[c]Blood lactate >6 mmol/L, since lower high lactate levels (2-6 mmol/L) may be due to violent crying or to extensive muscle activity.

[d]AST/ALT elevations can be found but are not constant in UCDs.

[e]Can be absent in neonates.

[f]Occurrence only in neonates.

[g]Only type B associated with hyperammonemia but not types A and C.

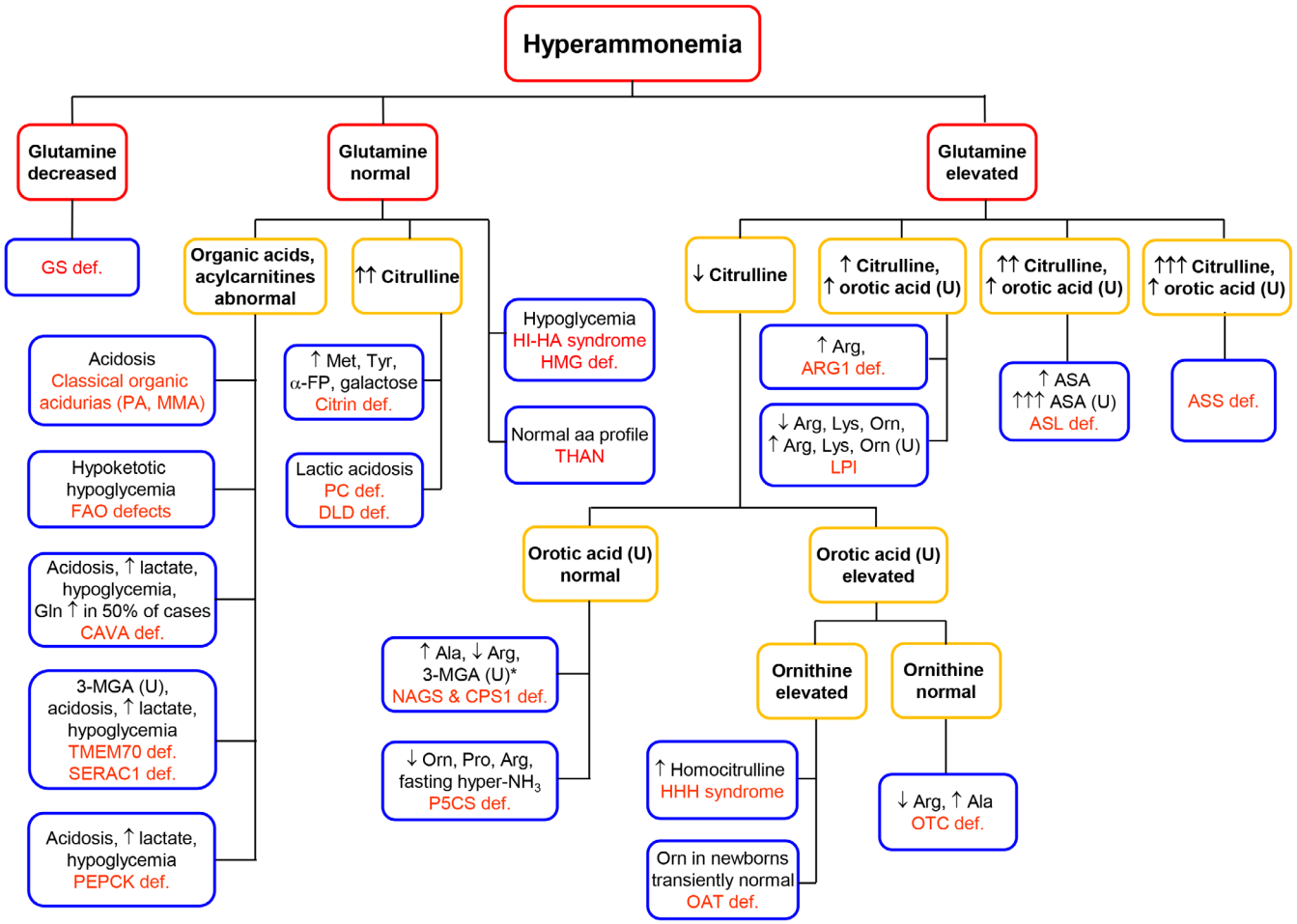

FIGURE 2 Diagnostic algorithm for neonatal hyperammonemia. Unless indicated, with (U) to indicate urine, plasma is used for the analytical determinations. α-FP, α- fetoprotein; Ala, alanine; ASA, argininosuccinic acid; ASL, argininosuccinate lyase; Arg, arginine; ARG1, arginase 1; ASS, argininosuccinate synthetase; CAVA, carbonic anhydrase Va; CPS, carbamoylphosphate synthetase; Def., deficiency; DLD, dihydrolipoamide dehydrogenase; FAO, fatty acid oxidation; Gln, glutamine; GS, glutamine synthetase; HHH, hyperornithinemia-hyperammonemia-homocitrullinuria; HI-HA, hyperinsulinism-hyperammonemia; HMG, 3-hydroxy-3-methylglutaryl-CoA lyase; LPI, lysinuric protein intolerance; Met, methionine; 3-MGA, 3-methylglutaconic acid; MMA, methylmalonic aciduria; NAGS, N-acetylglutamate synthase; OAT, ornithine aminotransferase; Orn, ornithine; OTC, ornithine transcarbamylase; PA, propionic acidemia; PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; P5CS, Δ^1−pyrroline-5-carboxylate synthetase; Pro, proline; SERAC1, serine active site-containing protein 1; THAN, transient hyperammonemia of the newborn; TMEM70, transmembrane protein 70; Tyr, tyrosine; U, urine. *, this asterisk indicates that 3-MGA was only found in few CPS1D patients but not yet in NAGSD

available, but still has a role in situations where molecular analysis is negative and/or for research for validating unclear genetic results.

3.1.5 | Molecular genetic analysis

For all urea cycle enzyme genes (homonymous with the enzymes) and the genes for citrin (*SLC25A13*) and the ornithine transporter (*SLC25A15*) involved in citrullinemia type II and the HHH syndrome, respectively, mutations have been reported in many patients (see https://www.ncbi.nlm.nih.gov/omim). Although sensitivity is less than 100% (~80% in OTCD studies[95] but probably higher for other UCDs), mutation detection is the preferred method for diagnosis when metabolite profiles are not informative. To improve genetic diagnosis, additional methods such as array-comparative genomic hybridization (CGH), RNA-based sequencing, or MLPA are necessary.[96-99] Genotyping allows for pedigree analysis, carrier identification, prenatal diagnosis, and genetic counseling. It is the basis for establishing genotype-phenotype correlations[25,100-103] and for, conceivably, future therapies such as nonsense read-through approaches or use of chaperones.

DNA (from peripheral blood cells, tissues, cultured cells, or dried blood spots) is the preferred sample. RNA is frequently used for *CPS1* mutation analysis given the numbers of exons of this gene.[104] RNA from liver tissue can be useful in OTCD and other UCDs when routine DNA analysis is

negative.[97,105,106] Mutation analysis should include investigations of known regulatory domains.[107,108]

Outcome: Survival
Key questions: How can UCD patients be identified reliably and early? Which measures are prognostic?
Recommendation #5: We strongly recommend genetic testing in UCDs to confirm the diagnosis, allow for genetic counseling and in some instances provide information on the disease course. We strongly recommend preserving DNA, fibroblasts, and/or frozen liver tissue from deceased patients with a suspicion of UCD.
Quality of evidence: moderate (9/12 moderate, 3/12 high)

3.1.6 | Prenatal testing

Prenatal testing requires the confirmed diagnosis in an index patient. In following pregnancies, early, fast, and safe prenatal testing is of interest since most UCDs are considered severe conditions. Even in milder disease variants expected to require less intensive treatment and to achieve a good outcome or for NAGSD (for which curative life-long substitutive therapy exists), prenatal testing may be considered by parents and physicians to prepare for peri- and postnatal management.[109-111] The method of choice is mutation/disease allele-tracking in chorionic villus samples or in amniotic fluid cells (or in cultures thereof).[112,113] Citrulline and ASA determinations in amniotic fluid are also highly reliable for prenatal diagnosis of ASSD and ASLD, respectively, but require the prior definition of ranges in amniotic fluid from carrier mothers.[113-116] Table 3 lists recommended analyses and sample requirements.

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #6: We strongly recommend molecular genetic analysis as the preferred prenatal testing method for all UCDs.
Quality of evidence: high (8/12 high, 4/12 moderate)

3.1.7 | Newborn screening

The current knowledge on possible benefits of NBS for UCDs relies on a small number of publications.[117,118] It is a general concern that UCD patients with severe hyperammonemia very early in life would not benefit from NBS given their limited prognosis and the very early onset often prior to the result of NBS.[117,119,120] Although efforts are made to establish analytical algorithms for NBS of NAGSD, CPS1D, and OTCD,[121] most programs do not currently screen for these conditions, given the general instability of glutamine and the low specificity and sensitivity of low citrulline levels.[122] The benefits of screening for ASSD, ASLD, and ARG1D remain to be evaluated. For severe ASSD and ASLD there appear to be few false positive and no false negative cases.[123-125] Nevertheless, screening for ASLD was abandoned in Austria in 2000 because of the high rate of identified newborns with partial deficiency who remained asymptomatic.[126] It is not known how many and which of these mild cases are at risk of decompensation later in life.[28,127] The sensitivity of NBS for ARG1D is unknown, since in this disease arginine levels may be within the normal range in the first days of life. The same may be true for ornithine in HHH patients.[128] Concerning HHH, another obstacle is the potential production of ornithine by red cell arginase during blood spot drying. If NBS for UCDs is performed, follow-up should be done in specialized metabolic units.

Outcome: Survival
Key question: How can UCD patients be identified reliably and early?
Recommendation #7: As patients with UCDs may benefit from early diagnosis, reliable NBS is desirable. We recommend considering NBS for ASSD and ASLD. At present, there are insufficient data for a recommendation on NBS programs for NAGSD, CPS1D, OTCD, ARG1D, and HHH syndrome.
Quality of evidence: moderate (6/12 moderate, 4/12 low, 2/12 high)

4 | MANAGEMENT OF ACUTE HYPERAMMONEMIA

4.1 | Initial management

Since the prognosis is strongly influenced by the duration of coma at presentation[7,19] and peak ammonia levels,[18,118,129-131]

TABLE 3 Prenatal testing of UCDs: Recommended analyses and sample requirements

Disorder	Tests recommended
NAGSD	Mutation analysis using DNA from CVS or AFC[a]
CPS1D	**Mutation analysis using DNA from CVS or** AFC Enzyme analysis, late fetal liver biopsy[b]
OTCD	**Mutation analysis using DNA from CVS or** AFC[c] Enzyme analysis, late fetal liver biopsy[b,d]
ASSD	**Mutation analysis using DNA from CVS or AFC** **Citrulline in amniotic fluid** (calculation of ratios) Enzyme analysis, intact or cultured CVS or cultured AFC
ASLD	**Mutation analysis using DNA from CVS or AFC** **Argininosuccinate and its anhydrides in amniotic fluid** Enzyme analysis, intact or cultured CVS or cultured AFC
ARG1D	**Mutation analysis using DNA from CVS or AFC** **Enzyme assay in fetal blood erythrocytes (mid-gestation sampling)**
HHH syndrome	**Mutation analysis using DNA from CVS or AFC** Functional assay in CVS or cultured AFC

Abbreviations: CVS, chorionic villous sample; AFC, amniotic fluid cells.
Bold: first choice.
[a]In case of a request for prenatal testing one should keep in mind that NAGSD is a treatable disorder.
[b]Described in single patients but not widely available and very limited experience.
[c]In the female fetus the genotype is only able to exclude OTC mutations. Because of the lyonisation it has no predictive value for the resulting phenotype if affected.
[d]Feasible in male, but interpretation not clear in females due to X-mosaicism.

therapy must not be delayed. Hospitals should always have available the first-line medications and consensus-based written protocols on how to proceed.

First actions should be

1. Stop protein intake
2. Start intravenous (IV) glucose with appropriate electrolytes (Na^+, K^+)
3. Initiation of first-line medications as outlined in Table 4
4. Collection of plasma and urine for diagnostic purposes without postponing initiation of treatment
5. Transfer the patient with a hyperammonemic crisis to a specialist center without delay

Outcomes: Survival, cognitive outcome
Key questions: How can survival of an acute hyperammonemic episode be improved? How can we preserve cognitive function?
Recommendation #8: We strongly recommend immediately commencing measures to reverse endogenous protein catabolism and to promote ammonia detoxification (as detailed in Table 4).
Quality of evidence: moderate (8/12 moderate, 4/12 high).

Before treatment of acute hyperammonemia, the prognosis regarding neurodevelopmental outcome needs to be considered and may influence the decision whether to continue specific treatment or to start palliative care.

The prognosis is considered very poor in patients with any of the following characteristics:

1. Coma >3 days
2. Significantly elevated intracranial pressure
3. Ammonia concentration in plasma >1000 μmol/L generally correlates with a less favorable prognosis.[5,19] However, very high ammonia concentrations are not an absolute criterion, they always have to be evaluated in association with the clinical situation and the duration of hyperammonemia. Single patients with a normal outcome despite initial ammonia >1000 μmol/L have been reported.[16,132]

4.2 | Drugs and dosages in acute UCDs decompensations

The nitrogen scavengers benzoate and phenylacetate are the mainstay drugs for bypassing the urea cycle. Benzoate conjugates with glycine to form hippurate and glutamine is converted to phenylacetylglutamine (PAGN) by phenylacetate or its prodrug phenylbutyrate (PBA). Both conjugated metabolites are excreted into urine.[26,90,91,93,129,133,134] The administration of arginine and/or citrulline aims at maximizing ammonia excretion through the urea cycle.[23-26,135] N-carbamylglutamate replaces the CPS1 activator N-acetylglutamate.[23-26,135] The doses given in Table 5 reflect a consensus based on available literature.[26,90,91,93,129,133,134] If a nitrogen scavenger bolus is given, ondansetron (0.15 mg/kg) may be administered to prevent vomiting.[134,136] Repeated boluses or very high doses of benzoate or phenylacetate can saturate the scavenger-converting systems, increasing the risk of drug accumulation and toxicity.[136,137]

4.3 | Management of a neonate at risk of a UCD at birth

These recommendations adapt the "BIMDG Management Protocol of a baby at risk of a urea cycle disorder" (http://www.bimdg.org.uk/).

When the previous sibling had early onset presentation:

- Take the "metabolic history" of the index case including specific diagnosis
- Perform pedigree analysis
- Consider measures for minimizing stress of delivery
- Plan delivery at a hospital with a specialized metabolic unit
- Transfer newborn to the neonatal unit immediately
- Start IV glucose 10% (4 mL/kg/h; 6-8 mg/kg/min) with appropriate electrolytes (Na^+, K^+) within 30 minutes and initiate protein-free feed/infant formula. Express breast milk initially.
- Start 6-hourly 50 mg/kg of both sodium benzoate and L-arginine
- Measure plasma ammonia at 6 hours and if <80 μmol/L again every 6 hours; if ammonia reaches 80 to 150 μmol/L, re-assay in 4 hours; if ammonia >150 μmol/L or if the baby becomes unwell repeat ammonia assay immediately, stop feeds and see Table 4 for actions to be taken

TABLE 4 Levels of hyperammonemia and suggested actions in case of symptomatic patients

Ammonia level (μmol/L)	Action in undiagnosed patient	Action in known UCD patient	Comments
Increased above upper limit of normal	• Stop protein intake • Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate, 8 mg/kg/min in infants, and 6 mg/kg/min in all others) ± insulin[b] • Monitor ammonia blood levels every 3 h	• Stop protein intake • Give IV glucose at an appropriate dosage to prevent catabolism (10 mg/kg/min in a neonate, 8 mg/kg/min in infants, and 6 mg/kg/min in all others) ± insulin[b] • Monitor ammonia blood levels every 3 h	• Stop protein for maximum 24 h • Avoid exchange transfusions as cause of catabolism • Hyperglycemia can be extremely dangerous (hyperosmolarity) • If major hyperglycemia occurs with high lactate (>3 mmol/L) reduce glucose infusion rate rather than increase insulin • Avoid hypotonic solutions • Add sodium and potassium according to the electrolyte results • Consider the sodium intake if sodium benzoate or sodium PBA are used[d] • L-arginine not to be given in ARG1D • Some concerns of sodium benzoate use in OAs • Avoid repetitive drug boluses • Monitor phosphate levels and supplement early especially during hemodialysis
In addition when >100 and <250[a]	• Start drug treatment with IV L-arginine and sodium benzoate (see Table 5) • Start carbamylglutamate, carnitine, vitamin B_{12}, biotin (see Table 5 and its legend)	• Continue drug treatment with L-arginine (plus continue or add L-citrulline for mitochondrial UCDs) and sodium benzoate ± sodium PBA/phenylacetate[c] (see Table 5), increase dose or give IV • Consider protein free energy (glucose polymer and lipid emulsions) by NG tube unless the child is vomiting (10 g CHO from glucose polymer and 4 g lipids = ~76 kcal/100 mL)	
In addition when 250 to 500	• As above • Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) • Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 h	• As above, but all drugs per IV • Prepare hemo(dia)filtration if significant encephalopathy and/or early high blood ammonia level or very early onset of disease (day 1 or 2) • Begin hemo(dia)filtration if no rapid drop of ammonia within 3-6 h	
In addition when 500 to 1000	• As above • Start hemo(dia)filtration immediately	• As above • Start hemo(dia)filtration as fast as possible	
In addition when >1000	• Evaluate whether to continue specific treatment or to start palliative care	• Evaluate whether to aim at curative treatment or palliative care	

[a]This limit of action applies for patients outside the neonatal period; for neonates use >150 and <250.

[b]Monitor blood glucose after 30 minutes and subsequently every hour, because some neonates are very sensitive to insulin.

[c]If available, an IV equimolar solution of sodium benzoate and sodium phenylacetate can be used: 250 mg/kg as bolus IV/90-120 minutes, then 250 mg/kg as continuous IV infusion over 24 hours. The combination of sodium benzoate and sodium phenylacetate is available as a drug, registered by the FDA (available in the EU on Named Patient Basis) and indicated as adjunctive therapy for the treatment of acute hyperammonemia and associated encephalopathy in patients with deficiencies in enzymes of the urea cycle.

[d]1g sodium benzoate and sodium PBA contain 7 and 5.4 mmol Na, respectively.

- Measure plasma amino acids (quantitatively) urgently at 12 hours

When the previous sibling had late-onset presentation:

- Start glucose infusion only if birth was complicated (birth asphyxia, etc.)
- Give first stage infant formula ≤6 g protein/d (corresponding to 130 mL infant formula/kg/d for a 3.5 kg baby). Newborn babies usually take around 50 to 60 mL/kg on day 1, then increase over a few days to 150 mL/kg/d.
- Breast feeding on demand is possible, but top-up feeds are required to compensate for low volume and low energy on days 1and 2
- Determine plasma ammonia and amino acids (quantitatively) at 24 hours of age
- If ammonia <60 μmol/L, reanalyze in 24 hours
- If ammonia 60 to 150 μmol/L, reanalyze in 12 hours
- If ammonia >150 μmol/L or if the baby becomes unwell repeat ammonia assay immediately and see Tables 4 and 5 for actions to be taken
- If at 48 hours ammonia is <80 μmol/L, continue infant formula at 3-hourly intervals up to 150 mL/kg/d and observe
- If ammonia is 80 to 150 μmol/L at 48 hours or at any other time after 24 hours and the baby is well, reanalyze at 12-hourly intervals, request results of plasma amino acids, and change to protein-free formula

TABLE 5 Dosages of drugs to be used in acute hyperammonemia and acute decompensations of UCDs

Disorder	Sodium benzoate (to be given IV in glucose 10%)	Sodium PBA/Sodium phenylacetate (to be given IV in glucose 10%)	L-arginine hydrochloride (to be given IV in glucose 10%)	N-carbamylglutamate (only available as oral/enteral drug)
Undiagnosed patient[a]	250 mg/kg as bolus in 90-120 minutes, then maintenance 250-500 mg/kg/d[c]>20 kg bw: 5.5 g/m^2/d	250 mg/kg as bolus in 90-120 minutes, then maintenance: 250-500 mg/kg/d[c]	250(-400) mg/kg (1–2 mmol/kg) as bolus in 90-120 minutes, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	100 mg/kg bolus per NG tube then 25-62.5 mg/kg every 6 h
NAGSD	Same[c]	Same[c]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 minutes, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	Same
CPS1D and OTCD	Same[c]	Same[c]	Same	-
ASSD	Same[c]	Same[c]	Same	-
ASLD[d]	Same[c]	Same[c]	200-400 mg/kg (1–2 mmol/kg) as bolus in 90-120 minutes, then maintenance 200-400 mg/kg/d (1–2 mmol/kg/d)	-
ARG1D[b]	Same[c]	-	**AVOID**	-
HHH syndrome	Same[c]	Same[c]	250 mg/kg (1.2 mmol/kg) as bolus in 90-120 minutes, then maintenance 250 mg/kg/d (1.2 mmol/kg/d)	-

Cave: The doses indicated in this table can be used at the start of treatment but must be adapted depending on plasma ammonia and amino acids. Sodium benzoate and sodium PBA/phenylacetate should be given in parallel in severe acute decompensation. In less severe cases, a step-wise approach with initial sodium benzoate and, if hyperammonemia persists or worsens, the addition of sodium PBA/phenylacetate can be chosen. Since hyperammonemia causes brain edema, sufficient NaCl should be added so that solutions are not hypotonic. The sodium load in the other intravenous medications should be taken into account.

Maximal daily drug dosages: sodium benzoate 12 g/d, sodium PBA 12 g/d, L-arginine 12 g/d.

[a]In undiagnosed patients, use of a combination of the drugs in this table seems justified, consider additional use of carnitine 100 mg/kg IV/d, hydroxycobalamin 1 mg IM/IV/d, and biotin 10 mg IV/PO/d.

[b]The risk for acute hyperammonemic decompensation is low in ARG1D.

[c]If on hemodialysis/hemodiafiltration, doses should be increased to 350 mg/kg/d (maintenance dose).

[d]In ASLD, L-arginine therapy for acute decompensations might be sufficient for some patients.

4.4 | Extracorporeal detoxification

In neonates and children, the response to emergency medical management should be evaluated after 4 hours (the estimated time window for alerting the dialysis team and to prepare vascular access[129,138]). If considered inadequate, continuous renal replacement therapy should be started. Peritoneal dialysis is much less effective[19] and should therefore only be used if no other form of dialysis is available and the patient cannot be rapidly transferred. Patients receiving efficient extracorporeal detoxification may still have a poor cognitive prognosis.[16,18] A recent systematic review of clinical and biochemical data from published neonatal onset UCD patients found that following current practice, dialysis did not improve outcome. Authors concluded that "it may be essential for improving outcome to initiate all available treatment options, including dialysis, as early as possible."[16] Kido et al even advocate that patients with a "peak ammonia level >180 μmol/L at the onset should receive hemodialysis."[18]

Outcomes: Survival, cognitive outcome, neurological situation
Key questions: How can survival be improved by the use of extracorporeal detoxification? How can we preserve cognitive function? How can we prevent neurological disease?
Recommendation #9. In parallel with medical treatment, we strongly recommend preparing extracorporeal detoxification in patients with severe neurological symptoms induced by hyperammonemia. Extracorporeal detoxification should be started as soon as possible, unless initial medical treatment has already led to sufficient improvement of ammonia levels and the clinical situation.
Quality of evidence: moderate (10/12 moderate, 2/12 high)

In adults, hemodialysis (HD) or continuous veno-venous hemofiltration (CVVH) is the first-line treatment in acute decompensations in many institutions since it is readily available in most intensive care units. It can be started quickly even before transfer to a specialized center and it is a low-risk procedure. It may be recommended even if the diagnosis is not yet certain. Since intracranial hypertension and cerebral edema appear earlier in adults than in children, extracorporeal detoxification should be considered if ammonia exceeds 200 μmol/L under consideration of comorbidities, availability of and tolerance to medication.

Outcomes: Survival, cognitive outcome, neurological situation
Key questions: Can survival be improved in adults by the use of extracorporeal detoxification? How can we preserve cognitive function? How can we prevent neurological disease?
Recommendation #10: We recommend extracorporeal detoxification be considered as a first-line treatment in acute hyperammonemic decompensations in adults.
Quality of evidence: low (no voting as recommendation follows BIMDG advice)

HD provides the highest ammonia extraction, as solute clearance is related to dialysate flow rate and blood flow rate, in addition to the surface area of the dialytic membrane. After discontinuation of HD, some patients may have an acute relapse of hyperammonemia.

In neonates, frequent technical and hemodynamic complications related to HD may hamper ammonia removal,[139] but, if feasible, intermittent HD is safe and efficient.[140] Continuous veno-venous hemodialysis (CVVHD) or continuous hemodiafiltration (CVVHDF) may be better tolerated, providing a continuous extraction with excellent ammonia clearance and can be considered as the first-line therapy in young infants.[19,130,141,142]

Outcomes: Survival, cognitive function, neurological situation
Key questions: Can survival be improved by the use of extracorporeal detoxification? How can we preserve cognitive function? How can we prevent neurological disease?
Recommendation #11: We recommend continuous veno-venous hemodialysis or hemodiafiltration as the method of choice for ammonia detoxification in newborns. We recommend considering peritoneal dialysis, which is less effective for ammonia removal, as a bridging technique when no hemodialysis is available and for transfer of patients to a metabolic center. We strongly recommend against performing exchange transfusion to treat hyperammonemia.
Quality of evidence: high (7/12 high, 4/12 moderate, 1/12 low)

4.5 | Dietary management of acute decompensation

It is crucial to stop/prevent catabolism by promoting and maintaining anabolism in any patient with an acute hyperammonemia. In most, oral feeding will not be feasible during the acute phase because of impaired consciousness and vomiting. While a 10% glucose infusion (with appropriate electrolytes, ie, Na^+, K^+) via a peripheral IV line is started, a central venous line should be inserted in the severely ill patient, to help maximize energy intake. A glucose infusion should be started as quickly as possible; if hyperglycemia occurs, continuous IV insulin should be given (see Table 5). Administration of lipids (1-2 g/kg/d) will provide additional energy and help promote anabolism. The reintroduction of protein/amino acids/essential amino acids (EAA) must not be delayed for more than 24 (−48) hours. Some authors advocate to consider supplementation with EAA or branched chain amino acids (BCAA) at the start of acute treatment; supplementation should preferably be given via the enteral route because of the contribution of the splanchnic system to protein retention and metabolism.[143,144] If the patient cannot be fed enterally, IV amino acids (using a standard parenteral amino acid solution) should be commenced at 0.5 g/kg/d, and, based on ammonia results, increased daily by 0.5 g/kg/d to at least the final safe intake of protein for age.[145]

Enteral feeding should be restarted as soon as possible. It may initially have to be protein-free (Table 6), and nasogastric (NG) feeding may temporarily be needed. Enteral fluids/feeds (with the same energy and protein content) should be increased as IV/PN fluids are decreased, so an adequate intake be constantly provided and tolerance assessed. Consider the sodium content in nitrogen scavengers (see Table 4) when calculating total electrolyte intake. Over-concentrated feeds can cause osmotic diarrhea. Dietary protein is usually reintroduced over 2 to 4 days based on ammonia results. If ammonia increases with protein reintroduction, EAA supplements for UCDs can be used in combination with natural protein. Energy intake should be approximately 120% of age-adjusted requirements, initially to promote anabolism.

Outcomes: Survival, metabolic stability
Key questions: What dietary interventions are appropriate in acute hyperammonemia? How can we improve metabolic stability?
Recommendation #12: For the treatment of acute hyperammonemia we strongly recommend establishing and maintaining anabolism by providing high-dose glucose ± insulin with appropriate electrolytes (Na^+, K^+). Lipids should be added as soon as fatty acid oxidation disorders have been excluded. We recommend protein-free nutrition should not exceed 24 (−48) hours.
Quality of evidence: moderate (7/12 moderate, 3/12 high, 2/12 low)

5 | LONG-TERM MANAGEMENT OF UCDS

5.1 | General aspects

Long-term treatment of UCDs is challenging for patients and families because of the poor palatability (particularly of EAA supplements), the volume and frequency of diet and drug administrations; all these are serious barriers to adherence.[146] The aims are to maintain stable metabolic control, to eliminate chronic complications,[23,26,147] and to achieve normal development and growth. Patients will need a combination of:

TABLE 6 Emergency regimen for protein-free feeding in infants and children (adapted from Dixon et al., 2015[343])

Age	Glucose polymer concentration (%)	Energy/100 mL		Suggested daily intake (ml/kg)	Feeding frequency
		kcal	kJ		
Up to 6 mo	10	40	167	150 mL/kg up to a maximum of 1200 mL	2 to 3 hourly oral/bolus day and night or continuous tube feeds using enteral feeding pump
7-12 mm	10-15	40-60	167-250	120 mL/kg up to a maximum of 1200 mL	
1 y	15	60	250	Estimate as indicated[a]	
2-9 y	20	80	334	Estimate as indicated[a]	
>10 y	25	100	418	Estimate as indicated[a]	

[a]For children >10 kg emergency regimen fluid requirements can be calculated as.11-20 kg: 100 mL/kg for the first 10 kg, plus 50 mL/kg for the next 10 kg. >20 kg: 100 mL/kg for the first 10 kg, plus 50 mL/kg for the next 10 kg, plus 25 mL/kg thereafter up to a maximum of 2500 mL/d.

- medications to increase waste nitrogen excretion
- low-protein diet
- supplementation of arginine and/or citrulline
- supplementation of essential nutrients such as vitamins and minerals
- supplementation of EAA (some patients)
- emergency regimen for treatment of intercurrent illnesses

A detailed management plan in writing should be made available to parents/caregivers and to the nursery/school. The management plan should include instructions on when and how to contact the metabolic team or the local hospital, where a written prescription for emergency treatment should be available. Instruction visits in nurseries and schools by a clinical nurse specialist or metabolic dietitian are recommended.

5.2 | Low protein diet

Although not supported by controlled studies, this cornerstone of long-term management is firmly based on physiological rationale, the patients' self-selection of low-protein foods, and the existing clinical experience. The low protein diet should minimize protein supply without compromising growth and prevent endogenous catabolism. Adequate protein and energy supply can be based on the FAO/WHO/UNU 2007 "safe levels of protein intake" (summarized in Table 7 for patients with normal physical activity),[145] although lower protein and/or energy intakes may be adequate for example, for less active patients.[148] Over-restriction of protein may compromise growth and well-being and can cause metabolic instability.[54,149] Optimal protein intake must be determined by individual titration in every patient. If protein tolerance is very low, EAA have to be supplemented (see below). NG tube or gastrostomy feeding (see below) may be necessary to ensure sufficient energy and/or protein intake. Ideally the diet should be provided by a combination of low and some high biological value protein to ensure adequate intake of all EAA as some low biological value protein foods have limited amounts of certain amino acids, such as lysine in cereals. Protein from food rather than EAA supplements will help maximize palatability, improve quality of life, and minimize cost, although EAA may be needed by some to achieve metabolic stability.

The daily protein is divided equally between three to four meals, and a late-night snack shortens the overnight fast. Regular review and monitoring of diet, growth, and clinical status are essential, as protein requirements and tolerance vary with age, growth velocity, nature and severity of the disorder, and the frequency of intercurrent illnesses.[150] Metabolic control may be easier in early infancy, when rapid growth results in increased protein tolerance, than in older children.[26,145]

TABLE 7 Safe levels of protein intake for different age groups, as well as during pregnancy and lactation, according to FAO/WHO/UNU[145]

PROTEIN INTAKE				
Age (months)	**Intake (g/kg bw/d)**			
1	1.77			
2	1.50			
3	1.36			
6	1.14 (if weaned: 1.31)			
12	1.14			
years	g/kg bw/d			
1.5	1.03			
2	0.97			
3	0.90			
4–6	0.87			
7–10	0.92			
Years	**Females (g/kg bw/d)**	**Males (g/kg bw/d)**		
11	0.90	0.91		
12	0.89	0.90		
13	0.88	0.90		
14	0.87	0.89		
15	0.85	0.88		
16	0.84	0.87		
17	0.83	0.86		
18	0.82	0.85		
>18	0.83	0.83		
Pregnancy:				
Total extra protein intake per trimester in g/d				
First	1			
Second	10			
Third	31			
Lactation:				
Total extra protein intake per months in g/d				
1-6	19			
>6	13			
Energy requirements				
Age	**Females**	**Males**	**Females**	**Males**
Years	kJ/kg bw/d		kcal/kg bw/d	
0.5	340	335	81.3	80.0
2.5	334	348	79.8	83.2
5.0	305	315	72.9	75.3
10	248	275	59.3	65.7
15	193	230	46.1	55.0

TABLE 7 (Continued)

Energy requirements				
Adults, moderate activity level, 70 kg body weight[b]				
Years	kJ/kg bw/d		kJ/kg bw/d	
18-29	159	183	38.0	43.7
30-59	148	175	35.4	41.8
Adults, moderate activity level, 50 kg body weight				
Years	kJ/kg bw/d		kJ/kg bw/d	
18–29	180	212	43.0	50.7
30–59	183	212	43.7	50.7
Pregnancy total extra energy requirements				
Trimester	kJ/d		kcal/d	
First	375		90	
Second	1200		287	
Third	1950		466	
Lactation total extra energy requirements				
months	kJ/d		kcal/d	
1-6	2800		669	
>6	1925		460	

Outcomes: Metabolic stability, cognitive outcome, neurological situation, liver disease
Key questions: How can we improve metabolic stability? How can we preserve cognitive function and prevent neurological or liver disease?
Recommendation #13: Deficiencies of energy and/or essential amino acid and other nutrients can cause metabolic instability and morbidity. We strongly recommend involving a specialist metabolic dietitian to balance nutritional requirements with metabolic stability, following the FAO/WHO/UNU 2007 guidelines for protein and energy requirements.
Quality of evidence: moderate (7/12 moderate, 4/12 high, 1/12 low)

5.3 | Supplementation of EAA and of other essential nutrients

EAA supplementation is essential when protein tolerance is too low (less than the safe intake of protein) to provide an overall adequate EAA intake from natural foods and supplements. The use and amount of EAA supplements does, however, vary markedly between centres.[150] If EAA are necessary, a reasonable approach is to provide up to 20% to 30% of the total protein intake. Because of the severe natural protein restriction necessary in ARG1D (see below), up to 50% of the protein supply may be offered as EAA.[150,151]

A subgroup of the EAA, the BCAA, was found decreased in the plasma of patients treated with (high doses of) sodium PBA.[152,153] Thus BCAA supplementation may be useful in these patients.[154,155] EAA supplements should be rich in BCAA but not in tryptophan, phenylalanine, and tyrosine, which are precursors of the neurotransmitters serotonin and dopamine that may be high in hyperammonemia.[155]

EAA are given with meals for maximal use of these amino acids. BCAA supplements can be given as either single amino acids or as a complete mixture.

Outcome: Metabolic stability
Key question: How can we improve metabolic stability?
Recommendation #14: We recommend considering supplementation of essential amino acids, especially of branched-chain amino acids, if natural protein tolerance is very low and/or if the patient receives phenylbutyrate.
Quality of evidence: moderate (7/12 moderate, 4/12 low, 1/12 high)

The low protein diet also places UCD patients at risk of Fe, Zn, Cu, Ca, and cobalamin deficiencies.[148] Furthermore, patients on a very low protein diet or receiving most of the protein as EAA mixtures are at risk of essential fatty acid (EFA) deficiency and may benefit from EFA/LCPUFA-enriched infant formulas or oils (eg, walnut, rapeseed, or sunflower) rich in polyunsaturated fatty acids.[156,157] Vitamin and trace element supplementation may also be required.

5.4 | Practical aspects of dietary management

Since there is no new information regarding *breast feeding*, *bottle feeding*, *weaning*, *nutrition in childhood*, *adolescence*, and *adult patients*, the reader is referred to the first guidelines.[1]

Successful *pregnancies* in UCD patients[158-162] require addressing the special nutritional needs of pregnancy and lactation (Table 7), avoiding undernutrition of protein. Close monitoring during and early after delivery is essential to recognize hyperammonemia within the first 5 days after delivery.

In all patients, the last meal before the night sleep should contain ~25% of the daily intakes of energy, natural protein, EAA, citrulline, and/or arginine (see below) to minimize catabolism during the overnight fast.

Outcomes: Metabolic stability, quality of life
Key questions: How can we improve metabolic stability? How can we reduce the burden of disease?
Recommendation #15: We recommend individualized dietary management, and parents' and patients' training to strengthen their competence for a lifelong dietary treatment.
Quality of evidence: moderate (5/12 moderate, 4/12 low, 3/12 high)

Tube feeding is essential in cases of:

- Inability to suck or swallow due to neurological handicap or severe developmental delay
- Difficulty with the daily administration of EAA and drugs with an unpleasant taste
- Poor appetite and/or food refusal with resultant inadequate energy intake
- Gastrointestinal problems—vomiting, reflux, retching
- Emergency management during intercurrent illnesses

NG tubes are used both in the hospital and at home. In acute episodes, they can expedite the transfer from parenteral to enteral nutrition. Oral food and fluids should be offered unless swallowing is compromised. A gastrostomy is recommended if long-term tube feeding and/or continuous overnight feeds are needed, despite the lack of controlled studies and the risk of tube feeding-dependence.[163] Feed tolerance and patient daily routine should determine the pattern of tube feeding (bolus, continuous, during the day and night). Some hospitals do not support continuous NG feeding at night due to the risk of tube displacement/aspiration.

Outcomes: Metabolic stability, quality of life
Key questions: How can we improve metabolic stability? How can we preserve quality of life? How can we reduce the burden of disease?
Recommendation #16: We recommend early consideration of tube feeding to ensure nutritional adequacy, administration of medications and supplements, prevention of catabolism, and/or maintenance of metabolic stability.
Quality of evidence: low (8/12 low, 4/12 moderate)

5.5 | Pharmacotherapy for long-term treatment

Drugs which are routinely used for long-term treatment of UCDs include nitrogen scavengers (sodium benzoate, sodium PBA or sodium phenylacetate, glycerol phenylbutyrate), L-arginine, L-citrulline, and carbamylglutamate. Some of the medications are available as powder, capsule, tablet, or liquid. This might cause practical problems for the patient if no unambiguous prescription is provided.[93,164]

Outcomes: Metabolic stability, quality of life
Key questions: How can we improve metabolic stability? How can we preserve quality of life? How can we reduce the burden of disease?
Recommendation #17: We suggest providing written drug treatment sheets to parents, pharmacists, and persons involved in patient care.
Quality of evidence: low-moderate (5/12 low, 5/12 moderate, 2/12 high)

Among the *nitrogen scavengers*, *sodium benzoate* is not a registered drug, but it has been used for decades in UCDs. In contrast, *sodium PBA*, the precursor of the active agent phenylacetate, is a licensed drug of more recent introduction. The theoretical superiority of sodium PBA over sodium benzoate in reducing the risk of hyperammonemic episodes because of two nitrogen atoms excreted as PAGN instead of only one in hippurate[110,165] has been questioned.[166] A slow-release more taste-friendly formulation of sodium PBA in the form of taste-masked granules has recently been introduced and approved by the EMA.[167,168] A novel chemical esterified form of PBA, *glycerol phenylbutyrate* (GPB), has been introduced with FDA and EMA approval, after noninferiority to sodium PBA was shown.[169] GPB avoids sodium intake and is a tasteless liquid. In a phase 3, randomized, double-blind, cross-over trial GPB showed good metabolic control and may exhibit more favorable pharmacokinetics than sodium PBA.[170-173] Using the recommended dosing, GPB results in phenylacetic acid plasma concentrations in the therapeutic range in the majority of patients.[174] This new drug formulation thus offers the chance to improve the management of UCD patients.[170]

Outcome: Metabolic stability
Key question: How can we improve metabolic stability?
Recommendation #18: Nitrogen scavengers are a mainstay of therapy in UCD patients. We recommend individualized dosing for each patient.
Quality of evidence: moderate (6/11 moderate, 4/11 high, 1/11 low)

There are no consistent recommendations regarding drug treatment and it should be emphasized that the majority of patients treated in the United States receive sodium or glycerol PBA alone, whereas many centers in Europe consider sodium benzoate as first-line medication because of long-term experience, less side effects, fewer safety concerns, and lower price.

Sodium benzoate is toxic only at a plasma concentrations >2 mmol/L.[175] In theory, both sodium benzoate and sodium PBA could cause acetyl-CoA depletion (both are esterified to CoA), with secondary mitochondrial dysfunction[176] and reduced NAG production (acetyl-CoA is needed to form NAG). PBA is a histone 1,2 deacetylase inhibitor[177] that may have unclear effects on long-term use. Sodium PBA causes amenorrhea or menstrual dysfunction in ~25% of females[134]; less frequently, it can: decrease appetite, cause taste disturbances, or result in a disagreeable body odor. It can deplete BCAA, thereby increasing the risk of endogenous protein catabolism. Decreased leucine and glutamine availability possibly account for the low albumin levels reported in some sodium PBA-treated patients.[152-154,178] Either sodium benzoate and sodium PBA granules, tablets, or undiluted liquid preparations can cause mucositis or gastritis, therefore four dosages with meals and abundant fluids are recommended.[91,164] In the case of GPB, three equally divided daily dosages are recommended. Hypokalemia (resulting from increased renal K^+ loss) can occur after repeated bolus doses but also during long-term treatment. Metabolic acidosis has been observed especially on high doses of one or the other agent.[133,134] Although there are anecdotal reports on successful pregnancies of women taking sodium PBA,[134,160,179] the working group of this guideline regards sodium benzoate to be the safer choice if medication has to continue (expert opinion).

Outcome: Metabolic stability
Key question: What needs to be done during pregnancy?
Recommendation #19: Continuation of treatment with nitrogen scavengers is generally necessary in pregnant UCD patients. Based on biochemical mechanisms, we suggest the use of sodium benzoate. There is insufficient evidence to comment on fetal outcomes after nitrogen scavenger therapy in pregnancy.
Quality of evidence: low (5/11 low, 4/11 moderate, 2/11 high)

L-arginine becomes an essential amino acid in all UCDs because of its impaired synthesis, except in ARG1D, and thus is supplemented (as such or as its precursor *L-citrulline*).[180] These intermediates become limiting particularly when they are massively excreted into the urine as in ASLD and to a lesser extent in ASSD. In these diseases, ASA and citrulline serve as vehicles for nitrogen removal via their excretion in the urine, and thus the provision of arginine reduces the frequency of hyperammonemic episodes.[164,181-183] Fasting plasma arginine concentrations should be about 70 to 120 μmol/L.[164] In NAGSD, CPS1D, OTCD, and the HHH syndrome L-citrulline may be supplemented instead of L-arginine but there are no studies comparing the efficacy of the two.

Outcomes: Metabolic stability, cognitive outcome, neurological situation, liver disease, psychiatric outcome
Key questions: How can we improve metabolic stability? How can we prevent neurological or liver disease, or psychiatric complications?
Recommendation #20: We strongly recommend L-arginine and/or L-citrulline supplementation in UCD patients (may not be required in mild phenotypes, L-arginine is contraindicated in ARG1D). We recommend monitoring plasma arginine levels in all UCD patients.
Quality of evidence: moderate (6/12 moderate, 5/12 high, 1/12 low)

N-carbamyl-L-glutamate (also called carglumic acid or carbamylglutamate) is a deacylase-resistant NAG analogue that is taken up enterally and is able to enter liver mitochondria where it can replace NAG in the activation of CPS1, thus being the specific substitutive therapy for NAGSD.[184-186] Its use has been suggested as a tool for differential diagnosis in unclear neonatal hyperammonemia.[187] Despite the lack of controlled studies, its use should be considered in severe hyperammonemic decompensations of unknown origin. In addition to primary NAGSD, N-carbamyl-L-glutamate is licensed by the EMA for treatment of hyperammonemia in organic acidurias.[188-190] There are no reports on long-term safety or on adverse effects of this drug other than high dose-triggered Chinese restaurant syndrome.[186]

Outcomes: Survival, cognitive outcome, neurological situation, psychiatric outcome
Key questions: Can we improve metabolic stability by using N-carbamyl-L-glutamate? How can we prevent neurological disease or psychiatric complications?
Recommendation #21: We recommend using N-carbamyl-L-glutamate as the first-line medication for treatment of NAGSD and as an emergency drug during acute hyperammonemia of unknown etiology.
Quality of evidence: high (7/11 high, 4/11 moderate)

The plasma carnitine status of all UCD patients should be monitored to detect and treat (in case of severe) secondary carnitine deficiency, related to the low protein diet and to the conjugation of nitrogen scavengers with carnitine.[191-193]

Some members of this guideline group have used neomycin or metronidazole to decrease the load of ammonia producing bacteria in the colon; as there are no controlled studies no recommendation can be made on the use of oral antibiotics.

5.6 | Caring for special situations and emergency regimen in intercurrent illnesses

During intercurrent illness or other events with the risk of hyperammonemia, the *emergency regimen* (Table 6) should be immediately started at home. If there is no prompt improvement the metabolic unit should be contacted urgently.

Despite the potential risks of fever, *vaccinations* have not been identified as relevant causes of metabolic decompensation.[194,195]

Outcome: Metabolic stability
Key question: What needs to be done during special situations?
Recommendation #22: We strongly recommend performing vaccinations following national schedules.
Quality of evidence: moderate (7/12 moderate, 5/12 high)
We suggest antipyretic treatment if temperature exceeds 38°C.
Quality of evidence: low (8/12 low, 4/12 moderate)

Given the risk in UCDs of acute metabolic decompensation during surgery and general anesthesia,[147] elective surgery should only be carried out in centers able and prepared to deal with hyperammonemic decompensations. The patient should be well, with normal preoperative ammonia and amino acid concentrations and without even minor intercurrent illness. The day before surgery, drug treatment should be switched to IV and 10% glucose with appropriate electrolytes (Na^+, K^+) administered IV to ensure anabolism. The patient should be the first on the schedule. Midazolam, s-ketamine, fentanyl, and isoflurane in combination with surgical field infiltration with ropivacaine have been reported[196] to be safe anesthetic agents for these patients. Postsurgical close monitoring of the clinical status and of ammonia is required; the shift to oral medications from IV administration and the termination of IV glucose infusion should only be done in a stable metabolic situation.

Outcome: Metabolic stability
Key question: What needs to be done during surgery and anesthesia?
Recommendation #23: We suggest performing elective surgery in UCD patients in centers with a metabolic expertise and resources including emergency treatment options for hyperammonemia.
Expert opinion

6 | LIVER TRANSPLANTATION FOR UCD PATIENTS

6.1 | The curative nature of liver transplantation

Liver transplantation (LT) has been performed in all UCDs except NAGSD. It provides a practical cure and allows for a

normal diet without taking nitrogen scavengers.[197,198] The same overall posttransplant survival has been found in OTCD and in non-UCD patients.[199,200] Survival rates in large pediatric programs attain now ~95% at 1 year and ~90% at 5 years, with self-reported "good" or "excellent" quality of life posttransplant.[198,200-205] Thus, LT offers severely affected UCD patients a favorable alternative to medical treatment. This procedure may maintain or improve quality of life, which otherwise requires a very disciplined lifestyle, intake of large amounts of drugs, but with permanent impending risk of hyperammonemia and a largely uncertain prognosis.

LT, however, in general will not revert preexisting neurological damage.[206-214] LT has been performed during acute encephalopathy and/or acute liver failure, which are high risk situations requiring case-by-case discussions of the need to transplant. After LT, the lack of citrulline recycling in ASSD and ASLD and of de novo arginine synthesis persists in all UCDs except ARG1D, with arginine becoming an EAA.[215] In most patients these metabolic aberrations have no clinical impact.

Outcomes: Survival, metabolic stability, cognitive outcome
Key questions: Can survival be improved by performing liver transplantation? What interventions are appropriate in which situations? How can we improve metabolic stability? How can we preserve cognitive function?
Recommendation #24: We recommend to consider liver transplantation in patients with severe UCDs without sufficient response to standard treatment and with poor quality of life, without severe neurological damage and ideally while in a stable metabolic condition.
Quality of evidence: moderate (8/12 moderate, 4/12 high)

6.2 | Indications and age for liver transplantation

Patient survival rises with increased age at transplantation: the 5-year patient survival rate was 88% for children with UCDs who were <2 years old at transplant and 99% for children who were ≥2 years old at transplant ($P = .006$; total number of patients: 186).[214] If possible, LT in UCD patients should not be performed in children younger than 3 months or below 5 kg bodyweight because of higher rates of complications and lower survival rate in this population.[214,216] The patient should be fully immunized. Regarding the neurological outcome, patients transplanted within the first 12 months of life might benefit more than children who were transplanted later in life.[211]

Outcomes: Survival, metabolic stability, cognitive outcome, quality of life
Key questions: Can survival be improved by performing liver transplantation? What interventions are appropriate in which situations? How can we improve metabolic stability? How can we preserve cognitive function?
Recommendation #25: In patients with neonatal onset UCD (except NAGSD) being considered for liver transplantation, we strongly recommend this is undertaken before the onset of irreversible neurological damage. Transplantation between 3 and 12 months of age and when body weight exceeds 5 kg is associated with a more favorable outcome.
Quality of evidence: moderate (8/12 moderate, 4/12 high)
We strongly recommend considering liver transplantation in patients with severe progressive liver disease and/or with recurrent metabolic decompensations requiring hospitalizations despite standard medical therapy.
Quality of evidence: high (9/12 high, 3/12 moderate)

6.3 | Transplant type, donor, and ethical issues

Orthotopic LT is the standard recommended procedure. Auxiliary LT has been performed in some patients but was associated with a higher rate of complications.[200] The use of liver lobes from living relatives gives comparable results to the use of organs from deceased donors, albeit entailing a small risk to donors.[217] It can reduce waiting times,[205] allows better organ preservation and thorough testing of donor and organ, and it permits the transplant to be performed electively when the recipient is in the best clinical and metabolic condition. In living related LT, heterozygosity seems not to be a problem and even asymptomatic OTC heterozygotes have been successful donors although symptomatic heterozygous donors should not be considered.[218-221]

Decisions on whether or not to perform LT are influenced by ethical considerations which require an individualized process of decision, in particular when the child is already handicapped or when living donor LT is considered.

7 | MONITORING OF PATIENTS WITH UCDS

Medically treated UCD patients require lifelong monitoring by the entire multidisciplinary metabolic team. Clinical and biochemical monitoring depends on age and metabolic stability of the patient. Infants will need more frequent monitoring and adjustment of their diet and treatment than older stable patients.

In practice, young and severely affected patients should be seen at least every 3 months while older or less severely affected patients may only need annual appointments.

Clinical monitoring should record growth and head circumference, include inspection for thin sparse hair or hair loss, skin rashes, and other signs of protein/vitamin deficiency. Neurological and neurocognitive assessments should be performed at regular intervals depending on the clinical situation of the patient. Liver size and structure should be assessed by ultrasound scan, especially in OTCD, ASSD, ASLD, ARG1D, and HHH syndrome. Regular *dietary assessment* (by recall or diet diary) is essential, to review the nutritional adequacy of the diet and when nutritional problems are suspected. Records of the diet, supplements, and drugs taken in the 3 to 5 days preceding the visit are most helpful. Adherence to EAA, vitamin and mineral supplement prescriptions should be checked, and the diet adjusted according to age and growth (see below).[31,54,148] Patients following a low protein diet may have an increased risk for osteoporosis and should be monitored accordingly. However, there are no studies of this in UCDs.

Outcomes: Metabolic stability, auxology
Key questions: How can we improve metabolic stability? How can we achieve normal growth and weight?
Recommendation #26: We strongly recommend regular clinical, biochemical, and nutritional monitoring for all UCD patients by a multidisciplinary metabolic team following individualized schedules.
Expert opinion

Laboratory monitoring must include *plasma ammonia* determination.[222] The goal is a concentration of <80 μmol/L for patients outside the neonatal period (assuming an upper normal limit of 50 μmol/L).[26,133] Venous samples should be used as capillary samples may result in falsely elevated values because of cell content and sweat contamination. Spurious elevations can occur due to poor sample processing, tourniquet use, crying, struggling, or convulsions.[70,223] Sequential ammonia determinations for 24 hours including preprandial, postprandial, and fasting samples are carried out in some centers, but the value of this approach is uncertain. For rapid approximate blood ammonia assessment bed-side methods are available using as little as 20 μL blood and yielding results within 3 to 5 minutes. These methods are recommended only to be used with caution and in the hospital environment, but not in a home setting due to the inaccuracy of the method and the upper limit of detection of some devices of only ~280 μmol/L.

A second pillar of laboratory monitoring is the determination of the *plasma amino acid profile*, to monitor arginine-, citrulline-, and EAA/BCAA supplementation. Plasma arginine should be in the high normal and EAA and BCAA in the normal ranges.[223] Rising plasma glutamine may indicate impending hyperammonemia. Glutamine levels not exceeding 1000 μmol/L are considered tolerable.[26,91,133,224] Since glutamine concentrations change with the fasting/feeding status, they are highest after an overnight fast.[225] Blood sampling should therefore be as standardized as possible, ideally 3 to 4 hours after the last meal and at about the same time of the day.[226,227] UCD patients frequently have their glutamine levels in the higher normal range and their BCAA levels decreased.[226] The latter supports the need for more natural protein or for EAA/BCAA supplements, although decreasing the nitrogen scavenger dose may also increase BCAA values.

Outcomes: Metabolic stability, auxology
Key questions: How can we improve metabolic stability? How can we achieve normal growth and weight?
Recommendation #27: We recommend the following target concentrations for long-term management: of ammonia <80 μmol/L, glutamine <1000 μmol/L, arginine in the high normal range, EAA and BCAA in the normal range.
Quality of evidence: high (8/12 high, 4/12 moderate)

Other blood assays can include determination of vitamins, minerals, trace elements, carnitine, ferritin, cholesterol, and triglycerides in plasma, alpha-fetoprotein, and of EFA in

red blood cells and plasma. Creatine may be assessed especially in patients with OTCD, ASSD, and HHH syndrome, as in these disorders low creatine concentrations were found along with other changes in creatine metabolism[228,229] but the practical consequences of low creatine values are unclear. The blood urea concentration is of little value as it depends mainly on arginine intake, body hydration, and tubular urine flow rate. Blood benzoate and/or PBA/phenylacetate assays may be helpful to prevent toxicity in patients receiving high dosages or repeated boluses.[136] These scavenger determinations are already available in some metabolic centers and their value is being evaluated.

Urine determinations can include ketone body assay (done easily at home) in a spot urine sample to detect catabolism. Hippurate determination in urine can be used to assess compliance with benzoate treatment. Urinary PAGN was shown in patients treated with GPB to be a useful clinical biomarker for determining the dose and for monitoring.[172] Urinary amino acid profiling is not recommended for monitoring. The value of orotate and orotidine excretion measurements (given in relation to creatinine in the second morning void) is dubious, although in principle increasing orotate excretions should reflect greater carbamoylphosphate accumulation indicating an excessive ammonia load.

Neuroimaging in UCDs may reveal brain MRI abnormalities with a variable pattern and extent depending on the stage of disease and correlating with neurological outcome.[230-232] MRI should ideally be performed between days 1 and 4 of a coma- or stroke-like episode, to follow the changes in the apparent diffusion coefficient (ADC). It should be used also in patient monitoring at 2 year-intervals (if patients do not require anesthesia), to correlate motor/language/cognitive development with anatomic changes. MRI sequences should include diffusion tensor imaging, axial T2 and FLAIR, sagittal and axial T1, and magnetic resonance spectroscopy (MRS).[231,233,234] In acute presentations, a diffuse cerebral edema is reflected by abnormal signal intensity with infarct-like aspect and restricted diffusion in areas of cortex and underlying white matter that are often multiple and asymmetrical[235,236] in one or both hemispheres. Basal ganglia involvement is revealed on T2-weighted images by high intensity signals in the caudate nucleus, putamen, and/or globus pallidus. The deep sulci of the insular and perirolandic regions may also display T1 shortening.[235] MRS can reveal highly elevated brain glutamine levels.[237,238] These elevations are helpful to detect subtle changes in OTC females.[233,234,239] The thalamus, brainstem, the occipital region, and the cerebellum tend to be relatively spared, and a few months after acute hyperammonemia a very moderate residual hypersignal on the insula and Rolando region may be visible. Chronic hyperammonemia may be associated with defective myelination and progressive cerebral atrophy, and with nonspecific punctate white matter hyperintensities in some patients.

Outcome: Metabolic stability
Key question: What are prognostic markers for acute and long-term management?
Recommendation #28: We recommend brain MRI, if possible together with spectroscopy, in UCD patients, even in the absence of neurological and/or cognitive impairment, as this may help to adjust treatment. Timing should be decided on a case by case evaluation.
Quality of evidence: moderate (6/12 moderate, 4/12 high, 2/12 low)

8 | COGNITIVE OUTCOMES AND PSYCHOSOCIAL ISSUES IN UCDS

The cognitive outcome of patients with UCDs depends predominantly on the extent and duration of hyperammonemia.[24] Children presenting with neonatal onset have poorer outcome concerning cognitive, adaptive, and behavioral functioning. The relationship between peak blood ammonia levels during the first hyperammonemic crisis and brain involvement seems to be linear: absence of mental retardation and changes in brain images was recorded in 64% of patients with ammonia <180 μmol/L in contrast to 8% of patients with initial ammonia concentrations >360 μmol/L.[18]

Outcome can vary in different UCDs: 33% of ASSD patients had an average or above average IQ compared to 40% in ASLD and 66% in OTCD (81% of the OTCD patients presented with a late-onset).[120] However, even in late-onset OTCD patients with normal IQ, deficits of motor planning and execution were reported.[240] A specific neurocognitive pattern was identified that included weakness in fine motor dexterity/speed and a trend toward weakness in nonverbal intelligence, visual memory, attention/executive skills, and mathematics while verbal intelligence, memory, learning, and reading were preserved.[120] These findings stress the importance of assessing not only IQ and development but also the specific neuropsychological strengths and weaknesses in UCD patients.[120] Behavioral and emotional problems were frequent in 52 patients with UCD from the European Registry and Network of Intoxication-type metabolic diseases (E-IMD) registry and highly associated with intellectual functioning.[241] Clinically asymptomatic OTC heterozygotes outperformed symptomatic heterozygotes, and the performance improved with higher levels of residual urea synthesis activity (assessed by ^{15}N stable isotope studies),

whereas neither the allopurinol loading test results nor the mutation type correlated with neuropsychological performance in these patients.[61]

Outcomes: Cognitive outcome, neurological situation
Key questions: How can we preserve cognitive function and prevent neurological disease?
Recommendation #29: We recommend testing for IQ, development and specific strengths/weaknesses in all patients, including those with milder disease or female OTCD heterozygotes. They may develop specific weaknesses in executive functions even if the IQ is normal.
Quality of evidence: moderate (8/12 moderate, 4/12 high)

Until recently, only little attention has been given to the psychological status of patients and families affected by inborn errors of metabolism including UCD. Studies investigating health-related quality of life (HrQoL), psychological adjustment, and adaptive functioning in UCDs are sparse and their comparability is limited due to the frequent lack of coherence of methodological approaches and assessment instruments.[242]

Patients with UCDs and their parents may have a low HrQoL because the disease is chronic and stressful.[243] This may be amplified by delays in diagnosis and treatment, which, in turn, may be attributed to low awareness toward these rare conditions.[241,244] A cross-sectional study including 10 children and 14 parents with intoxication-type inborn errors including UCDs revealed lower "general well-being" and less leisure activities when compared to leukemia survivors. Parents physical QoL was lower compared to the general population and dietary constraints were a major issue.[245] In patient and caregiver focus groups, dietary restrictions and stigmatization/social exclusion were the factors considered most relevant for HrQoL. Parents and patients stated a need for comprehensive information on disease and treatment mechanisms.[246] Recently, a disease-specific assessment tool for HrQoL in UCDs and other intoxication-type metabolic diseases was developed,[247] which may be a promising option to study aspects specifically relevant to patients with UCDs and their caregivers.

Clearly, psychologists should be involved in the care of the patient of any age from the very moment of diagnosis to cope with initial anxiety and with later-developing psychological problems[248] and also to regularly assess the cognitive level and neuropsychological function of the patient.

Outcome: Quality of life
Key question: How can we reduce the burden of disease?
Recommendation #30: We recommend including psychological monitoring and counseling as an important component of the care of UCD patients and their families since health-related quality of life, anxiety, stress, and psychosocial factors are important outcome parameters.
Quality of evidence: high (6/12 high, 3/12 moderate, 3/12 low)

9 | RECOMMENDATIONS FOR SPECIFIC DISORDERS

Unless indicated otherwise, the diseases described in the following in more detail require the nutritional management given above. Table 8 gives specific recommendations for chronic drug use in each disorder.

9.1 | NAGS and CPS1 deficiency

These two diseases have identical clinical and laboratory manifestations (Figure 2), consisting in primary hyperammonemia with concomitantly elevated plasma glutamine and decreased plasma citrulline and arginine. Orotic acid in urine is not elevated. In neonates, CPS1D can be associated with significant elevation of 3-methylglutaconic acid excretion as detected by urinary organic acid analysis.[249] In NAGSD, carbamylglutamate can substitute the missing NAG, and thus a positive therapeutic response to this compound should be diagnostic for NAGSD.[187] However, the sensitivity and specificity of the initial response to carbamylglutamate is not 100%, since a negative response was reported in a case of NAGSD[250] and a positive response in patients with CPS1D.[251,252] Therefore, the confirmation of NAGSD or CPS1D requires either enzyme or mutation analysis.[102,253] The invasiveness of tissue procurement (liver for both CPS1 and NAGS; or intestinal mucosa for CPS1) for enzyme assay and their technically challenging nature (particularly for NAGSD[254]), as well as lower liver CPS1 activity levels in patients with genetically proven NAGSD[255,256] and HIHA syndrome[257] are the main reasons why enzyme analysis is nowadays used only in individual cases in which genetic diagnosis is inconclusive or not readily available.[258] Figure 3 provides an algorithm on how to

TABLE 8 Dosages of peroral drugs for long-term treatment of UCDs

Disorder	Sodium benzoate[e]	Sodium PBA[a,e], or GPB	L-arginine[e] (hydrochloride and/or free base)	L-citrulline[e]	Carbamyl-glutamate[e]
NAGSD	-	-	-	-	10–100 mg/kg/d
CPS1D	Up to 250 mg/kg/d[b,c] max. 12 g/d	<20 kg: up to 250 mg/kg/d[b,c] >20 kg: 5 g/m^2/d[c] max. 12 g/d	<20 kg: 100-200[b] mg/kg/d or: 0.5-1 mmol/kg/d >20 kg: 2.5-6 g/m^2/d, max. 6 g/d	100-200 mg/kg/d[d] max. 6 g/d	-
OTCD	Same	Same	Same	100–200 mg/kg/d[d] max. 6 g/d	-
ASSD	Same	Same	<20 kg: 100-300[b,c] mg /kg/dor: 0.5-1.5 mmol/kg/d >20 kg: 2.5-6 g/m^2/d[c], max. 8 g/d	-	-
ASLD	Same	Same	<20 kg: 100–300[b,c] mg/kg/d or: 0.5-1.5 mmol/kg/d >20 kg: 2.5-6 g/m^2/d[c], max. 8 g/d	-	-
ARG1D	Same	Same	AVOID	-	-
HHH syndrome	Same	Same	<20 kg: 100–200[b] mg/kg/d >20 kg: 2.5-6 g/m^2/d, max. 6 g/d	100–250 mg/kg/d[d] max. 6 g/d	-

All medications should be divided into three to four doses daily taken with meals and distributed as far as possible throughout the day.

[a]Sodium phenylbutyrate (PBA) was considered of second choice for long-term treatment by most guideline group members. It should be given together with sodium benzoate in patients in which benzoate alone is not enough. GBP, glycerol phenylbutyrate, has essentially the same content (5% more) of phenylbutyrate as sodium phenylbutyrate.

[b]Serum/plasma levels of benzoate/PBA and plasma levels of arginine should be monitored.

[c]In some patients higher doses are needed (the US FDA studies consider doses up 450-600 mg/kg/d in children weighing less than 20 kg and 9.9 to 13.0 g/m^2/d in children weighing more than 20 kg, adolescents and adults), according to expert advice.

[d]If citrulline is given, there is usually no need for concomitant use of L-arginine.

[e]100 mg equal, respectively, 0.694 mmol sodium benzoate, 0.537 mmol sodium PBA, 0.566 mmol esterified phenylbutyrate in GPB, 0.475 mmol arginine hydrochloride, 0.574 mmol arginine base, 0.571 mmol citrulline, 0.532 mmol carbamylglutamate.

proceed with differentiation between NAGSD and/or CPS1D.

Outcomes: Survival, cognitive outcome
Key questions: How can patients with NAGS and CPS1 deficiencies be identified reliably and early? How can we preserve cognitive function?
Recommendation #31: We strongly recommend genetic analysis for the diagnosis of NAGSD and for CPS1D, since NAGS activity assay is not generally available and enzymatic diagnosis of CPS1D requires liver or intestinal mucosa.
Quality of evidence: high (8/12 high, 4/12 moderate)

Except during metabolic crises *NAGSD is treated* with only carbamylglutamate (available exclusively as oral medication).[37] The recommended oral/enteral loading dose of 100 to 250 mg/kg is followed by an initial daily maintenance dose of 100 to 200 mg/kg divided into (3-)4 doses (based on a T½ = 5-6 hours), which is then titrated down to the minimum required (as low as 10-15 mg/kg).[259,260] Although long-term outcome data are scarce,[259,261] 20 NAGSD patients known to this guideline group members are doing well thus far under this treatment. *CPS1D therapy* is as for other UCDs (Tables 5 and 8) although the use of L-citrulline instead of L-arginine would appear preferable since L-citrulline should allow for incorporation of one extra nitrogen atom in ASA and consequently arginine and urea. However, L-citrulline is more expensive than arginine and not available as an IV drug. Furthermore, its potential superiority to L-arginine has not yet been studied and single reports

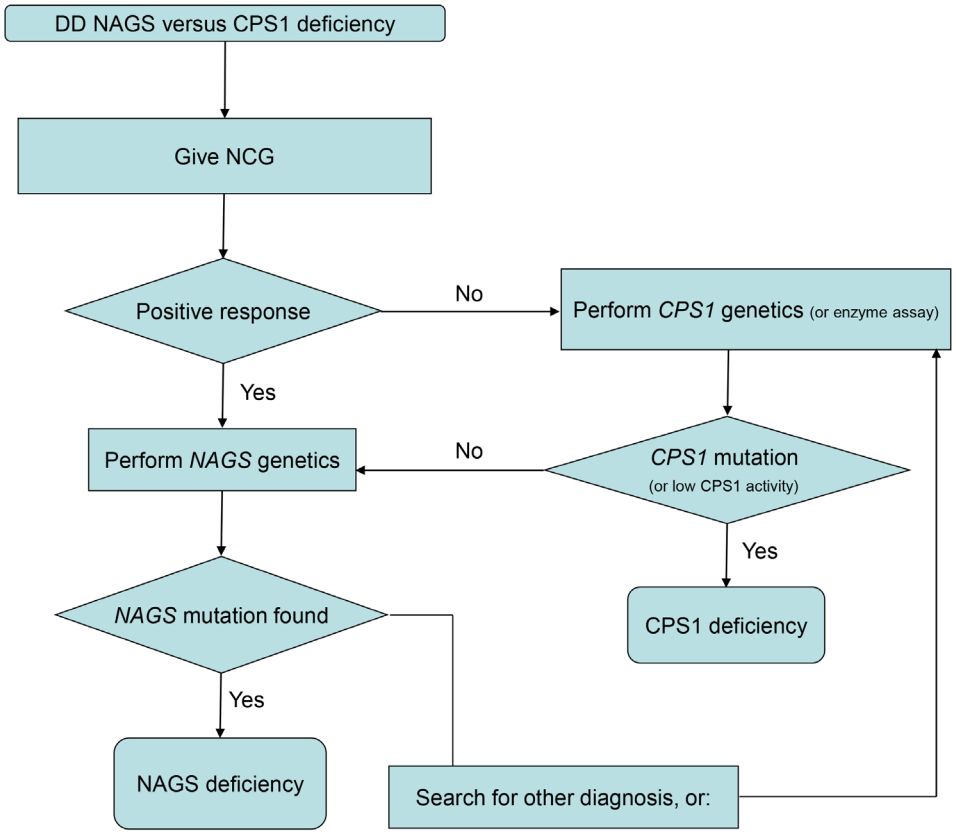

FIGURE 3 Algorithm for the differential diagnosis of NAGSD and CPS1D. CPS1, carbamoylphosphate synthetase 1; DD, differential diagnosis; NAGS, N-acetylglutamate synthase; NCG, N-carbamylglutamate

indicate similar efficacy.[181] Treatment using carbamylglutamate was reviewed[190,262] and was tested in five patients with late-onset CPS1D. In four patients, ureagenesis was improved, and one patient showed marked improvement in nitrogen metabolism.[263]

Outcome: Metabolic stability
Key question: How can we improve metabolic stability in NAGS deficiency?
Recommendation #32: We strongly recommend monotherapy with carbamylglutamate as the treatment of choice outside acute decompensations in NAGSD.
Quality of evidence: high (9/11 high, 2/11 moderate)

9.2 | OTC deficiency

Diagnosis of this X-linked disorder is based on the clinical and family history, hyperammonemia, increased plasma glutamine and alanine, low plasma citrulline, and high urinary orotic acid excretion (Figure 2). OTCD may present with acute liver dysfunction or even acute liver failure, or with chronic liver disease.[45,46,264] In some patients, acute liver failure did resolve with metabolic management of the OTCD but in other patients emergency LT had to be performed.[39] Three OTCD patients with recurrent pancreatitis have been reported. Only one of them had an additional mutation in a gene associated with hereditary pancreatitis and so the relation between recurrent pancreatitis and OTCD is still unexplained.[265] Male OTCD patients mostly present as neonates. They belong to the group of UCD patients with the highest mortality (60%) during initial presentation. In female OTCD, neonatal onset was only 7% in a large meta-analysis.[11]

Mutation identification is the preferred approach to confirm diagnosis, allowing prenatal testing, carrier identification[266] and even genotype-based prognosis.[95,267,268] In ~20% of OTCD patients standard molecular techniques fail to reveal a mutation.[95,269] To increase the detection rate in these patients, liver tissue-derived RNA-based studies[97] or use of oligonucleotide array CGH have been successfully applied.[98] Liver or intestinal mucosa OTC activity assays may be helpful for diagnostic confirmation but may fail in female patients. A recently described LC MS/MS assay of OTC activity in plasma can demonstrate OTCD in hemizygous males and in the majority of symptomatic heterozygous females.[270]

Outcome: Survival
Key question: How can patients with OTC deficiency be identified reliably and early?
Recommendation #33: We strongly recommend genetic analysis for diagnosis of OTCD. We recommend determining OTC enzyme activity assay in plasma, liver, or intestinal mucosa if genetic analysis is inconclusive.
Quality of evidence: high (8/12 high, 4/12 moderate)

When mutation detection, pedigree analysis and enzyme activity assays are uninformative or unavailable, the allopurinol test may be performed to identify female carriers.[271,272] Elevated orotic acid and orotidine concentrations determined in four consecutive 6-hour fractionated urine samples collected after oral intake of allopurinol may be diagnostic but specificity and sensitivity are rather limited.[273] Patients with ASSD, ARG1D, LPI, and the HHH syndrome can also produce positive allopurinol test results, stressing the need for accurate biochemical differential diagnosis of these disorders.[274] For children, age-related reference values have been developed but not for infants below 6 months.[275] Interpretation of the test result based on normalization to the allopurinol dose has been suggested, but again there are only scarce data on newborns and young infants.[276] As an alternative, a modified protein load proved to have increased sensitivity[277] while being less dangerous compared to older protein loading test protocols.[23]

Treatment of OTCD is as for CPS1D. The considerations of a theoretical preference for L-citrulline over L-arginine also apply to OTCD. Although a large fraction of female patients has mild deficiency requiring little or no protein restriction, the risk of decompensation dictates lifelong adoption of preventive measures for emergency situations and monitoring of these patients. Measurement of plasma ammonia and glutamine levels every 6 months is recommended.

9.3 | ASS deficiency (citrullinemia type 1)

The diagnosis is generally straightforward (Figure 2) because of the combination of hyperammonemia and/or elevated plasma glutamine with very strong elevation of plasma citrulline and increased urinary orotic acid, without ASA in plasma or urine. ASSD can also present with signs of liver damage and even with acute liver failure.[43,278] Confirmation with genetic or enzymatic methods is necessary to allow for prenatal testing[279] but sometimes also to rule out citrullinemia type 2 (citrin deficiency). Since enzymatic analysis is complex,[280] mutation identification is preferred and may help to decide on the necessity of dietary therapy.[100,101,279] It is remarkable that certain genotypes are associated with a mild phenotype but these individuals may show up in NBS.[100] In single patients with such a mild phenotype, fatal hyperammonemia occurred in severe catabolic circumstances.[62,63,101,281] Thus, these patients may neither need a protein-restricted diet nor drug therapy but should nevertheless be followed in metabolic centers and have an emergency protocol.

Outcome: Survival
Key questions: How can patients with ASS deficiency be identified reliably and early? What interventions are appropriate in prenatal testing of ASS deficiency?
Recommendation #34: We strongly recommend genetic analysis for diagnostic confirmation and for prenatal testing in citrullinemia type 1.
Quality of evidence: high (8/12 high, 4/12 moderate)

9.4 | ASL deficiency (argininosuccinic aciduria)

The pathognomonic elevation of ASA levels in plasma and urine proves ASLD. Nevertheless, mutation identification and even the determination of the enzyme activity (measured radiometrically in cultured fibroblasts as in ASSD[59]) are desirable. Genetic testing facilitates prenatal testing (although the amniotic fluid ASA level can also be used, see Table 3) and can guide prognosis by associating mutations with clinical severity.[25,282-284] The level of residual ASL activity can influence management decisions.[59,285]

Outcome: Survival
Key questions: How can patients with ASL deficiency be identified reliably and early?
Recommendation #35: We recommend metabolite analysis for confirmation of ASLD since presence of ASA in high concentrations in plasma or urine is diagnostic. We strongly recommend genetic confirmation for family counseling and as method of choice for prenatal testing.
Quality of evidence: high (10/12 high, 2/12 moderate)

The risk of hyperammonemia in ASLD is low with arginine treatment, as two waste nitrogen atoms are excreted

with each ASA molecule. However, a significant proportion of ASLD patients has a poor cognitive outcome even without hyperammonemic decompensation.[28,38,69,286] This raises concerns regarding brain toxicity of ASA,[183,287] of increased guanidinoacetate concentrations,[288] nitric oxide (NO) production, or a role of altered arginine metabolism. This "beyond hyperammonemia" phenotype may in part be explained by the fact that ASL is required for systemic NO production.[289] ASLD patients developed complications that were possibly related to systemic NO deficiency. Particular attention should be given to monitoring blood pressure levels as arterial hypertension was more commonly observed in ASLD.[290,291] One patient showed improvement in some neuropsychological parameters when given a NO donor (isosorbide dinitrate).[292] ASLD can also be associated with progressive liver disease and hepatomegaly, the latter associated with an elevation of plasma triglycerides (Dionisi-Vici C. and Leonard J., personal communications). Hypokalemia (presumably due to tubular loss) is another complication in ASLD requiring monitoring and particular attention at risk circumstances such as gastroenteritis, heat, and other causes of dehydration.

For long-term treatment (Table 8) the same L-arginine dosages as for other UCDs are recommended. Plasma arginine concentrations of <200 μmol/L are targeted.[147,293]

Outcomes: Metabolic stability, cognitive, and hepatic outcome
Key questions: How can we improve metabolic stability in ASL deficiency? How can we preserve cognitive function and how can we prevent significant liver disease in ASL deficiency?
Recommendation #36: We recommend against high-dose L-arginine supplementation in ASLD because of neurological and hepatic complications. We recommend using L-arginine for long-term management at the same dosages as for other UCDs in combination with nitrogen scavengers and protein restriction.
Quality of evidence: moderate (8/11 moderate, 3/11 high)

9.5 | Arginase 1 deficiency (argininemia)

ARG1D markedly differs from other UCDs because patients rarely present in the neonatal period. Between 2 and 4 years of age, progressive spastic paraplegia and developmental delay evolve, with hepatomegaly and in some patients, hyperammonemic episodes.[35,294] Although increased plasma arginine is the disease hallmark, arginine may not always be exceedingly high.[295] The diagnosis can be confirmed by enzymatic assays (in erythrocytes)[94] or by genetic analysis.[296-298] The observation of increased urine orotic acid levels also supports the diagnosis.

Since arginine and its metabolites (eg, guanidinoacetate, also elevated in ARG1D[35,299,300]) are most likely toxic, treatment aims at reducing plasma arginine concentrations below 200 μmol/L.[294] This requires extreme restriction of natural protein[150,295] with up to 50% of the required protein being supplied as an EAA mixture (see above). ARG1D patients are less prone to hyperammonemic crises than patients with other UCDs,[35,297] but they can occur[301-303] and may require use of nitrogen scavengers.[344] It is uncertain whether adherence to treatment, which should halt disease progression,[35,300] can reverse the spastic diplegia.

Outcomes: Metabolic stability, neurological situation of patients, quality of life
Key questions: How can we improve metabolic stability in ARG1 deficiency? How can we prevent neurological disease and how can we reduce the burden of dietary treatment in ARG1 deficiency?
Recommendation #37: We recommend following standard UCD dietary and medical treatment in ARG1D but without the use of L-arginine. We suggest adherence to a strict protein restriction to reduce plasma arginine levels to as low as possible aiming for the upper reference range.
Quality of evidence: moderate (9/12 moderate, 2/12 low, 1/12 high)

10 | HHH SYNDROME

The manifestations of this mitochondrial ornithine transport syndrome differ from those of other UCDs and, as a nearly constant finding, they include spastic paraparesis, which sets in more slowly than in ARG1D, being preceded by hyperreflexia and other pyramidal signs.[29,36,304] While most symptoms are similar to those seen in all other UCDs, liver dysfunction and coagulopathy (with defects of factors VII, IX, and X) are more common in HHH syndrome.[40,304,305] The biochemical profile in this syndrome shows hyperammonemia and homocitrullinuria frequently accompanied by increased orotic acid excretion.[29,306] The disease should be confirmed by assessing isotopically labeled

ornithine incorporation into protein in cultured skin fibroblasts (or liver tissue)[307] or by identifying the mutation[36,308] allowing prenatal testing and carrier detection.

For long-term treatment a low protein diet combined with citrulline supplementation is recommended (Table 8), which prevents hyperammonemia and liver disease but apparently not the spastic paraparesis,[36,162,304,305] although more studies are needed to assess the impact of treatment on the neurological derangements. Since secondary creatine deficiency was reported in this syndrome,[228,305,309] creatine supplementation can be used in patients with low plasma creatine levels.

Outcome: Metabolic stability
Key question: How can we improve metabolic stability in HHH syndrome?
Recommendation #38: We recommend low-protein diet and citrulline or arginine supplementation in HHH syndrome. The impact of these measures on pyramidal dysfunction is unclear.
Quality of evidence: moderate (5/12 moderate, 5/12 low, 2/12 high)

11 | NEW MODELS AND EMERGING THERAPIES

Several animal models with inducible or hypomorphic phenotypes have recently been developed for some of the UCDs. In contrast to previous models with knock-out animals that died early,[310] this strategy allows testing of novel therapeutic strategies.[289,311-313] Such tests for novel therapeutic approaches will further benefit from the recent development of a simplified protocol allowing urea cycle flux studies with stable isotopes and the use of dried blood spots.[314] In addition, the structure of human CPS1[315] and of a robust system for in vitro production of this enzyme[316] have become available, providing models for rationalizing and experimentally testing the effects of the numerous mutations reported in patients with CPS1D,[106] and the efficacy of potential pharmachaperones and artificial activators of CPS1.

Advances in the treatment of hyperammonemia were recently reviewed including existing treatment alternatives and novel promising alleys.[317]

11.1 | Experimental therapies

As the brain is the main target of ammonia toxicity,[318] the use of *systemic hypothermia* as a neuroprotective measure was investigated in a pilot study (with historical case controls) in seven acutely encephalopathic, hyperammonemic neonates with UCDs (n = 6) and OAs (n = 1) requiring dialysis.[319] Adjunct whole-body hypothermia was added to standard treatment. Patients' body temperature was maintained at 33.5°C ± 1°C for 72 hours, then rewarmed by 0.5°C every 3 hours over 18 hours. Adjunct therapeutic hypothermia was feasible and safe. This observation adds to an older case report on mild systemic hypothermia (rectal temperature of 34°C for 48 hours together with hemofiltration) which resulted in a striking fall in plasma ammonia in a neonatal patient with hyperammonemic coma.[320] However, no further systematic data are available.

In the field of *cell-based therapies*, hepatocyte transplantation has been suggested in recent years as a therapeutic option for UCDs.[321-325] Since 1997, only a small number of UCD patients are reported who were treated with cell infusions. Some of them experienced serious complications.[325-331] The principle of hepatocyte transplantation was not only considered attractive with respect to organ shortage but also because it offers the option to bridge the time span to a later LT.[323,325]

As an alternative approach to hepatocytes, stem cell transfer has been suggested. Liver derived progenitor cells were reported to have some advantages over stem cells derived from other tissues.[332] The track from the first hepatocyte transplantation in 2000 to products issued from stem cell technology, and the start of EMA approved clinical trials was recently reviewed.[333]

Gene therapy has recently seen many years of preclinical evaluation,[334-339] as reviewed.[340] It has now reached the stage of clinical trials[341] and its value is currently (as of April 2019) being evaluated in adult OTCD (ClinicalTrials.gov Identifier: NCT02991144).

Enzyme replacement therapy (ERT) has been proposed as treatment for ARG1D since the arginase reaction is merely hydrolytic. PEGylated human recombinant arginase 1 (PEG-BCT-100) has been tested in a phase 1 clinical trial for cancer (http://clinicaltrials.gov/ct2/show/NCT00988195). This study was completed in August 2009, but no results have been posted on ClinicalTrials.gov. Currently (as of April 2019), an open-label, multicenter extension study to evaluate the long-term safety, tolerability and effects of intravenous PEGylated human arginase I (pegzilarginase) is open and recruiting ARG1D patients (ClinicalTrials.gov Identifier: NCT03378531).

12 | CLOSING REMARKS

These guidelines aim at delivering the best available level of evidence for any given recommendation. As the field moves forward, the working group of this guideline commits itself to review and revise the work in the future to preserve the achieved quality. Indeed, it is hoped that many of the statements will be substituted in forthcoming years by even more precise and effective recommendations to the benefit of the patients.

ACKNOWLEDGMENTS

This work has been supported by the guideline pool of the Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen (APS). This guideline pool is sponsored by unrestricted grants from several industries that are active in the entire field of inborn errors of metabolism. None of the grant providers were involved at any stage of the guideline development process or during writing of the manuscript. Work on UCDs at University Children's Hospital Zurich is supported by the Swiss National Science Foundation (grant no SNF 320030_176088 to J.H.). V.R. was sponsored by the Center for International Business Education and Research, University of Illinois at Urbana-Champaign-ISCIII and by grants from the Fundación Inocente Inocente and the Spanish Government (MINECO BFU2017-84264-P). The authors want to thank Vassili Valayannopoulos, formerly at Hôpital Necker—Enfants Malades, Paris, for his support of the guideline process until 2016. The authors are also grateful for the support from Elaine Murphy, Consultant Adult Inherited Metabolic Disease at Charles Dent Metabolic Unit, London, for her advice in defining recommendation #10.

CONFLICT OF INTEREST

The authors of this guideline declare no competing interests but disclose the following. J.H. has provided consultancy and/or was involved in educational activities for Aeglea BioTherapeutics, Horizon Pharma, Lucane Pharma, Nutricia Metabolics, Orphan Europe Recordati, and Swedish Orphan Biovitrum AB. D.M. was involved in educational activities for Swedish Orphan Biovitrum AB and has received travel sponsorships and honorariums and provided consultancy for Nutricia Metabolics and Vitaflo. G.P.-M. provided consultancy for Sobi-Biovitrum and Lucane Pharma and received travel grants from Orphan Europe Recordati. R.S. received scientific grants from Nutricia Metabolics; as the chairman of the German Society on Pediatric Inborn Errors of Metabolism (Arbeitsgemeinschaft für Pädiatrische Stoffwechselstörungen, APS) he has sponsor contracts with Nutricia Metabolics, Dr. Schär Medical Nutrition, Swedish Orphan Biovitrum AB, Biomarin, Chiesi, metaX GmbH, Vitaflo, Actelion, Alexion, Amixcus, Cambrooke, CT-RS, Laborservice Onken GmbH, Leadiant GmbH, Lucane Pharma, Orphan Europe Recordati Group, Sanofi Genzyme, Shire, and Ultragenyx. V.R. has provided consultancy and/or was involved in educational activities for Orphan Europe Recordati, Cytonet, and Swedish Orphan Biovitrum AB.

AUTHOR CONTRIBUTIONS

All the authors have been part of the entire guideline process including project planning, methodology training, literature search and evaluation, and drafting and revising the manuscript, tables and figures.

ORCID

Johannes Häberle https://orcid.org/0000-0003-0635-091X

REFERENCES

1. Häberle J, Boddaert N, Burlina A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders. *Orphanet J Rare Dis*. 2012;7:32.
2. Dionisi-Vici C, Rizzo C, Burlina AB, et al. Inborn errors of metabolism in the Italian pediatric population: a national retrospective survey. *J Pediatr*. 2002;140(3):321-327.
3. Summar ML, Koelker S, Freedenberg D, et al. The incidence of urea cycle disorders. *Mol Genet Metab*. 2013;110(1–2):179-180.
4. Häberle J, Huemer M. Evaluation of implementation, adaptation and use of the recently proposed urea cycle disorders guidelines. *JIMD Rep*. 2015;21:65-70.
5. Bachmann C. Outcome and survival of 88 patients with urea cycle disorders: a retrospective evaluation. *Eur J Pediatr*. 2003;162(6):410-416.
6. Nassogne MC, Heron B, Touati G, Rabier D, Saudubray JM. Urea cycle defects: management and outcome. *J Inherit Metab Dis*. 2005;28(3):407-414.
7. Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED. Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. *N Engl J Med*. 1984;310(23):1500-1505.
8. Maestri NE, Clissold D, Brusilow SW. Neonatal onset ornithine transcarbamylase deficiency: a retrospective analysis. *J Pediatr*. 1999;134(3):268-272.
9. Unsinn C, Das A, Valayannopoulos V, et al. Clinical course of 63 patients with neonatal onset urea cycle disorders in the years 2001-2013. *Orphanet J Rare Dis*. 2016;11(1):116.
10. Nettesheim S, Kolker S, Karall D, et al. Incidence, disease onset and short-term outcome in urea cycle disorders -cross-border surveillance in Germany, Austria and Switzerland. *Orphanet J Rare Dis*. 2017;12(1):111.
11. Burgard P, Kölker S, Haege G, Lindner M, Hoffmann GF. Neonatal mortality and outcome at the end of the first year of life in early onset urea cycle disorders-review and meta-analysis of

observational studies published over more than 35 years. *J Inherit Metab Dis*. 2016;39(2):219-229.

12. Harada E, Nishiyori A, Tokunaga Y, et al. Late-onset ornithine transcarbamylase deficiency in male patients: prognostic factors and characteristics of plasma amino acid profile. *Pediatr Int*. 2006;48(2):105-111.
13. Serrano M, Perez-Duenas B, Gomez-Lopez L, et al. Neuropsychiatric manifestations in late-onset urea cycle disorder patients. *J Child Neurol*. 2010;25:352-358.
14. Cavicchi C, Donati M, Parini R, et al. Sudden unexpected fatal encephalopathy in adults with OTC gene mutations-clues for early diagnosis and timely treatment. *Orphanet J Rare Dis*. 2014; 9:105.
15. Enns GM. Neurologic damage and neurocognitive dysfunction in urea cycle disorders. *Semin Pediatr Neurol*. 2008;15(3):132-139.
16. Hediger N, Landolt MA, Diez-Fernandez C, Huemer M, Häberle J. The impact of ammonia levels and dialysis on outcome in 202 patients with neonatal onset urea cycle disorders. *J Inherit Metab Dis*. 2018;41(4):689-698.
17. Nicolaides P, Liebsch D, Dale N, Leonard J, Surtees R. Neurological outcome of patients with ornithine carbamoyltransferase deficiency. *Arch Dis Child*. 2002;86(1):54-56.
18. Kido J, Nakamura K, Mitsubuchi H, et al. Long-term outcome and intervention of urea cycle disorders in Japan. *J Inherit Metab Dis*. 2012;35(5):777-785.
19. Picca S, Dionisi-Vici C, Abeni D, et al. Extracorporeal dialysis in neonatal hyperammonemia: modalities and prognostic indicators. *Pediatr Nephrol*. 2001;16(11):862-867.
20. Erez A. Argininosuccinic aciduria: from a monogenic to a complex disorder. *Genet Med*. 2013;15(4):251-257.
21. van Karnebeek CD, Sly WS, Ross CJ, et al. Mitochondrial carbonic anhydrase VA deficiency resulting from CA5A alterations presents with hyperammonemia in early childhood. *Am J Hum Genet*. 2014;94(3):453-461.
22. Vockley J, Chapman KA, Arnold GL. Development of clinical guidelines for inborn errors of metabolism: commentary. *Mol Genet Metab*. 2013;108(4):203-205.
23. Brusilow S, Horwich A. Urea cycle enzymes. In: Scriver C, Beaudet A, Sly W, Valle D, eds. *The Metabolic & Molecular Bases of Inherited Disease*. 8th ed. New York, NY: McGraw-Hill; 2001:1909-1963.
24. Gropman AL, Summar M, Leonard JV. Neurological implications of urea cycle disorders. *J Inherit Metab Dis*. 2007;30(6): 865-879.
25. Trevisson E, Salviati L, Baldoin MC, et al. Argininosuccinate lyase deficiency: mutational spectrum in Italian patients and identification of a novel ASL pseudogene. *Hum Mutat*. 2007;28(7): 694-702.
26. Leonard JV, Morris AA. Urea cycle disorders. *Semin Neonatol*. 2002;7(1):27-35.
27. Ah Mew N, Simpson KL, Gropman AL, Lanpher BC, Chapman KA, Summar ML. Urea cycle disorders overview. In: Pagon RA, Adam MP, Ardinger HH, et al., eds. *GeneReviews(R)*. Seattle (WA): University of Washington, Seattle; 1993. Last Update: June 22, 2017.
28. Rüegger CM, Lindner M, Ballhausen D, et al. Cross-sectional observational study of 208 patients with non-classical urea cycle disorders. *J Inherit Metab Dis*. 2014;37(1):21-30.
29. Martinelli D, Diodato D, Ponzi E, et al. The hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. *Orphanet J Rare Dis*. 2015;10:29.
30. Deutsch SI, Burket JA, Rosse RB. Valproate-induced hyperammonemic encephalopathy and normal liver functions: possible synergism with topiramate. *Clin Neuropharmacol*. 2009;32(6): 350-352.
31. Smith W, Kishnani PS, Lee B, et al. Urea cycle disorders: clinical presentation outside the newborn period. *Crit Care Clin*. 2005;21 (4 suppl):S9-S17.
32. Kvedar JC, Baden HP, Baden LA, Shih VE, Kolodny EH. Dietary management reverses grooving and abnormal polarization of hair shafts in argininosuccinase deficiency. *Am J Med Genet*. 1991;40(2):211-213.
33. Patel HP, Unis ME. Pili torti in association with citrullinemia. *J Am Acad Dermatol*. 1985;12(1 Pt 2):203-206.
34. Cowley DM, Bowling FG, McGill JJ, van Dongen J, Morris D. Adult-onset arginase deficiency. *J Inherit Metab Dis*. 1998;21(6): 677-678.
35. Crombez EA, Cederbaum SD. Hyperargininemia due to liver arginase deficiency. *Mol Genet Metab*. 2005;84(3):243-251.
36. Salvi S, Santorelli FM, Bertini E, et al. Clinical and molecular findings in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. *Neurology*. 2001;57(5):911-914.
37. Cartagena A, Prasad AN, Rupar CA, et al. Recurrent encephalopathy: NAGS (N-acetylglutamate synthase) deficiency in adults. *Can J Neurol Sci*. 2013;40(1):3-9.
38. Ah Mew N, Krivitzky L, McCarter R, Batshaw M, Tuchman M, Urea Cycle Disorders Consortium of the Rare Diseases Clinical Research Network. Clinical outcomes of neonatal onset proximal versus distal urea cycle disorders do not differ. *J Pediatr*. 2013; 162(2):324-329. e321.
39. Teufel U, Weitz J, Flechtenmacher C, et al. High urgency liver transplantation in ornithine transcarbamylase deficiency presenting with acute liver failure. *Pediatr Transplant*. 2011;15: E110-E115.
40. Fecarotta S, Parenti G, Vajro P, et al. HHH syndrome (hyperornithinaemia, hyperammonaemia, homocitrullinuria), with fulminant hepatitis-like presentation. *J Inherit Metab Dis*. 2006;29 (1):186-189.
41. Ito S, Kurasawa G, Yamamoto K, et al. A pregnant patient with fulminant hepatic failure was found to carry a novel missense mutation in the argininosuccinate synthetase gene. *J Gastroenterol*. 2004;39(11):1115-1117.
42. Mhanni AA, Chan A, Collison M, et al. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome (HHH) presenting with acute fulminant hepatic failure. *J Pediatr Gastroenterol Nutr*. 2008;46(3):312-315.
43. Faghfoury H, Baruteau J, de Baulny HO, Häberle J, Schulze A. Transient fulminant liver failure as an initial presentation in citrullinemia type I. *Mol Genet Metab*. 2011;102(4):413-417.
44. de Groot MJ, Cuppen M, Eling M, et al. Metabolic investigations prevent liver transplantation in two young children with citrullinemia type I. *J Inherit Metab Dis*. 2010;33(suppl 3):S413-S416.
45. Laemmle A, Gallagher RC, Keogh A, et al. Frequency and pathophysiology of acute liver failure in ornithine Transcarbamylase deficiency (OTCD). *PLoS One*. 2016;11(4):e0153358.

46. Gallagher RC, Lam C, Wong D, Cederbaum S, Sokol RJ. Significant hepatic involvement in patients with ornithine transcarbamylase deficiency. *J Pediatr*. 2014;164(4):720-725 e726.
47. Al-Hassnan ZN, Rashed MS, Al-Dirbashi OY, Patay Z, Rahbeeni Z, Abu-Amero KK. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome with stroke-like imaging presentation: clinical, biochemical and molecular analysis. *J Neurol Sci*. 2008;264(1–2):187-194.
48. Choi JH, Kim H, Yoo HW. Two cases of citrullinaemia presenting with stroke. *J Inherit Metab Dis*. 2006;29(1):182-183.
49. Christodoulou J, Qureshi IA, McInnes RR, Clarke JT. Ornithine transcarbamylase deficiency presenting with strokelike episodes. *J Pediatr*. 1993;122(3):423-425.
50. Keegan CE, Martin DM, Quint DJ, Gorski JL. Acute extrapyramidal syndrome in mild ornithine transcarbamylase deficiency: metabolic stroke involving the caudate and putamen without metabolic decompensation. *Eur J Pediatr*. 2003;162(4):259-263.
51. Sperl W, Felber S, Skladal D, Wermuth B. Metabolic stroke in carbamyl phosphate synthetase deficiency. *Neuropediatrics*. 1997;28(4):229-234.
52. Wiltshire EJ, Poplawski NK, Harbord MG, Harrison RJ, Fletcher JM. Ornithine carbamoyltransferase deficiency presenting with chorea in a female. *J Inherit Metab Dis*. 2000;23(8):843-844.
53. Scheuerle AE, McVie R, Beaudet AL, Shapira SK. Arginase deficiency presenting as cerebral palsy. *Pediatrics*. 1993;91(5):995-996.
54. Singh RH, Rhead WJ, Smith W, Lee B, Sniderman King L, Summar M. Nutritional management of urea cycle disorders. *Crit Care Clin*. 2005;21(4 suppl):S27-S35.
55. Snebold NG, Rizzo JF 3rd, Lessell S, Pruett RC. Transient visual loss in ornithine transcarbamoylase deficiency. *Am J Ophthalmol*. 1987;104(4):407-412.
56. Anderson JM, Brodsky MC. Protracted cortical visual loss in a child with ornithine transcarbamylase deficiency. *J Neuroophthalmol*. 2010;30(1):99-101.
57. Gorker I, Tuzun U. Autistic-like findings associated with a urea cycle disorder in a 4-year-old girl. *J Psychiatry Neurosci*. 2005;30(2):133-135.
58. Sedel F, Baumann N, Turpin JC, Lyon-Caen O, Saudubray JM, Cohen D. Psychiatric manifestations revealing inborn errors of metabolism in adolescents and adults. *J Inherit Metab Dis*. 2007;30(5):631-641.
59. Kleijer WJ, Garritsen VH, Linnebank M, et al. Clinical, enzymatic, and molecular genetic characterization of a biochemical variant type of argininosuccinic aciduria: prenatal and postnatal diagnosis in five unrelated families. *J Inherit Metab Dis*. 2002;25(5):399-410.
60. Pascual JC, Matarredona J, Mut J. Acrodermatitis enteropathica-like dermatosis associated with ornithine transcarbamylase deficiency. *Pediatr Dermatol*. 2007;24(4):394-396.
61. Gyato K, Wray J, Huang ZJ, Yudkoff M, Batshaw ML. Metabolic and neuropsychological phenotype in women heterozygous for ornithine transcarbamylase deficiency. *Ann Neurol*. 2004;55(1):80-86.
62. Enns GM, O'Brien WE, Kobayashi K, Shinzawa H, Pellegrino JE. Postpartum "psychosis" in mild argininosuccinate synthetase deficiency. *Obstet Gynecol*. 2005;105(5 Pt 2):1244-1246.
63. Häberle J, Vilaseca MA, Meli C, et al. First manifestation of citrullinemia type I as differential diagnosis to postpartum psychosis in the puerperal period. *Eur J Obstet Gynecol Reprod Biol*. 2010;149(2):228-229.
64. Fassier T, Guffon N, Acquaviva C, D'Amato T, Durand DV, Domenech P. Misdiagnosed postpartum psychosis revealing a late-onset urea cycle disorder. *Am J Psychiatry*. 2011;168(6):576-580.
65. Ahrens MJ, Berry SA, Whitley CB, Markowitz DJ, Plante RJ, Tuchman M. Clinical and biochemical heterogeneity in females of a large pedigree with ornithine transcarbamylase deficiency due to the R141Q mutation. *Am J Med Genet*. 1996;66(3):311-315.
66. Ausems MG, Bakker E, Berger R, et al. Asymptomatic and late-onset ornithine transcarbamylase deficiency caused by a A208T mutation: clinical, biochemical and DNA analyses in a four-generation family. *Am J Med Genet*. 1997;68(2):236-239.
67. Klaus V, Vermeulen T, Minassian B, et al. Highly variable clinical phenotype of carbamylphosphate synthetase 1 deficiency in one family: an effect of allelic variation in gene expression? *Clin Genet*. 2009;76(3):263-269.
68. Vergano SA, Crossette JM, Cusick FC, Desai BR, Deardorff MA, Sondheimer N. Improving surveillance for hyperammonemia in the newborn. *Mol Genet Metab*. 2013;110(1–2):102-105.
69. Martin-Hernandez E, Aldamiz-Echevarria L, Castejon-Ponce E, et al. Urea cycle disorders in Spain: an observational, cross-sectional and multicentric study of 104 cases. *Orphanet J Rare Dis*. 2014;9:187.
70. Barsotti RJ. Measurement of ammonia in blood. *J Pediatr*. 2001;138(1 suppl):S11-S19;discussion S19-20.
71. Häberle J. Clinical practice: the management of hyperammonemia. *Eur J Pediatr*. 2011;170(1):21-34.
72. Marin-Valencia I, Vilaseca MA, Thio M, Garcia-Cazorla A, Artuch R, Campistol J. Assessment of the perimortem protocol in neonates for the diagnosis of inborn errors of metabolism. *Eur J Paediatr Neurol*. 2010;14(2):125-130.
73. Burton BK. Inborn errors of metabolism in infancy: a guide to diagnosis. *Pediatrics*. 1998;102(6):E69.
74. Leonard JV, Morris AA. Diagnosis and early management of inborn errors of metabolism presenting around the time of birth. *Acta Paediatr*. 2006;95(1):6-14.
75. Ellaway CJ, Wilcken B, Christodoulou J. Clinical approach to inborn errors of metabolism presenting in the newborn period. *J Paediatr Child Health*. 2002;38(5):511-517.
76. Saudubray JM, Sedel F, Walter JH. Clinical approach to treatable inborn metabolic diseases: an introduction. *J Inherit Metab Dis*. 2006;29(2–3):261-274.
77. Clay AS, Hainline BE. Hyperammonemia in the ICU. *Chest*. 2007;132(4):1368-1378.
78. Häberle J. Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. *Arch Biochem Biophys*. 2013;536(2):101-108.
79. Hudak ML, Jones MD Jr, Brusilow SW. Differentiation of transient hyperammonemia of the newborn and urea cycle enzyme defects by clinical presentation. *J Pediatr*. 1985;107(5):712-719.
80. Tuchman M, Georgieff MK. Transient hyperammonemia of the newborn: a vascular complication of prematurity? *J Perinatol*. 1992;12(3):234-236.

81. Ballard RA, Vinocur B, Reynolds JW, et al. Transient hyperammonemia of the preterm infant. *N Engl J Med*. 1978;299 (17):920-925.
82. Bachmann C. Inherited hyperammonemias. In: Blau N, Duran M, Blaskovic ME, Gibson KM, eds. *Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases*. Berlin, Germany: Springer; 2003:261-276.
83. Summar ML, Barr F, Dawling S, et al. Unmasked adult-onset urea cycle disorders in the critical care setting. *Crit Care Clin*. 2005;21(4 suppl):S1-S8.
84. Felig DM, Brusilow SW, Boyer JL. Hyperammonemic coma due to parenteral nutrition in a woman with heterozygous ornithine transcarbamylase deficiency. *Gastroenterology*. 1995;109(1): 282-284.
85. Trivedi M, Zafar S, Spalding MJ, Jonnalagadda S. Ornithine transcarbamylase deficiency unmasked because of gastrointestinal bleeding. *J Clin Gastroenterol*. 2001;32(4):340-343.
86. Summar ML, Dobbelaere D, Brusilow S, Lee B. Diagnosis, symptoms, frequency and mortality of 260 patients with urea cycle disorders from a 21-year, multicentre study of acute hyperammonaemic episodes. *Acta Paediatr*. 2008;97(10):1420-1425.
87. Jensen V. The TURP syndrome. *Can J Anaesth*. 1991;38(1): 90-96.
88. Kenzaka T, Kato K, Kitao A, et al. Hyperammonemia in urinary tract infections. *PLoS One*. 2015;10(8):e0136220.
89. Hsu KH, Cheng CH, Tseng MH, Hsu JF, Lien R, Yang PH. Hyperammonemia in distal renal tubular acidosis: a new case and review of the literature. *Pediatr Neonatol*. 2015;56(6):432-434.
90. Brusilow SW, Maestri NE. Urea cycle disorders: diagnosis, pathophysiology, and therapy. *Adv Pediatr Infect Dis*. 1996;43: 127-170.
91. Ahrens M, Barsotti R, Batshaw M, et al. Consensus statement from a conference for the management of patients with urea cycle disorders. *J Pediatr*. 2001;138:S1-S5.
92. Summar M, Tuchman M. Proceedings of a consensus conference for the management of patients with urea cycle disorders. *J Pediatr*. 2001;138(1 suppl):S6-S10.
93. Summar M. Current strategies for the management of neonatal urea cycle disorders. *J Pediatr*. 2001;138(1 suppl):S30-S39.
94. Tomlinson S, Westall RG. Argininosuccinic aciduria. Argininosuccinase and arginase in human blood cells. *Clin Sci*. 1964;26:261-269.
95. Yamaguchi S, Brailey LL, Morizono H, Bale AE, Tuchman M. Mutations and polymorphisms in the human ornithine transcarbamylase (OTC) gene. *Hum Mutat*. 2006;27(7):626-632.
96. Cohen YH, Bargal R, Zeigler M, Markus-Eidlitz T, Zuri V, Zeharia A. Hyperargininemia: a family with a novel mutation in an unexpected site. *JIMD Rep*. 2012;5:83-88.
97. Engel K, Nuoffer JM, Muhlhausen C, et al. Analysis of mRNA transcripts improves the success rate of molecular genetic testing in OTC deficiency. *Mol Genet Metab*. 2008;94(3):292-297.
98. Shchelochkov OA, Li FY, Geraghty MT, et al. High-frequency detection of deletions and variable rearrangements at the ornithine transcarbamylase (OTC) locus by oligonucleotide array CGH. *Mol Genet Metab*. 2009;96(3):97-105.
99. Wang J, Shchelochkov OA, Zhan H, et al. Molecular characterization of CPS1 deletions by array CGH. *Mol Genet Metab*. 2011; 102(1):103-106.
100. Häberle J, Pauli S, Schmidt E, Schulze-Eilfing B, Berning C, Koch HG. Mild citrullinemia in Caucasians is an allelic variant of argininosuccinate synthetase deficiency (citrullinemia type 1). *Mol Genet Metab*. 2003;80(3):302-306.
101. Gao HZ, Kobayashi K, Tabata A, et al. Identification of 16 novel mutations in the argininosuccinate synthetase gene and genotype-phenotype correlation in 38 classical citrullinemia patients. *Hum Mutat*. 2003;22(1):24-34.
102. Sancho-Vaello E, Marco-Marin C, Gougeard N, et al. Understanding N-acetyl-L-glutamate synthase deficiency: mutational spectrum, impact of clinical mutations on enzyme functionality, and structural considerations. *Hum Mutat*. 2016;37(7):679-694.
103. Ah Mew N, Caldovic L. N-acetylglutamate synthase deficiency: an insight into genetics, epidemiology, pathophysiology, and treatment. *Appl Clin Genet*. 2011;4:127-135.
104. Kretz R, Hu L, Wettstein V, Leiteritz D, Häberle J. Phytohemagglutinin stimulation of lymphocytes improves mutation analysis of carbamoylphosphate synthetase 1. *Mol Genet Metab*. 2012; 106(3):375-378.
105. Rapp B, Häberle J, Linnebank M, et al. Genetic analysis of carbamoylphosphate synthetase I and ornithine transcarbamylase deficiency using fibroblasts. *Eur J Pediatr*. 2001;160(5):283-287.
106. Häberle J, Shchelochkov OA, Wang J, et al. Molecular defects in human carbamoyl phosphate synthetase I: mutational spectrum, diagnostic and protein structure considerations. *Hum Mutat*. 2011;32(6):579-589.
107. Heibel SK, Ah Mew N, Caldovic L, Daikhin Y, Yudkoff M, Tuchman M. N-carbamylglutamate enhancement of ureagenesis leads to discovery of a novel deleterious mutation in a newly defined enhancer of the NAGS gene and to effective therapy. *Hum Mutat*. 2011;32(10):1153-1160.
108. Williams M, Burlina A, Rubert L, et al. N-Acetylglutamate synthase deficiency due to a recurrent sequence variant in the N-acetylglutamate synthase enhancer region. *Sci Rep*. 2018;8(1): 15436.
109. Leonard JV, Ward Platt MP, Morris AA. Hypothesis: proposals for the management of a neonate at risk of hyperammonaemia due to a urea cycle disorder. *Eur J Pediatr*. 2008;167(3):305-309.
110. Maestri NE, Hauser ER, Bartholomew D, Brusilow SW. Prospective treatment of urea cycle disorders. *J Pediatr*. 1991;119(6): 923-928.
111. Sniderman King L, Singh RH, Rhead WJ, Smith W, Lee B, Summar ML. Genetic counseling issues in urea cycle disorders. *Crit Care Clin*. 2005;21(4 suppl):S37-S44.
112. Häberle J, Koch HG. Genetic approach to prenatal diagnosis in urea cycle defects. *Prenat Diagn*. 2004;24(5):378-383.
113. Kamoun P, Fensom AH, Shin YS, et al. Prenatal diagnosis of the urea cycle diseases: a survey of the European cases. *Am J Med Genet*. 1995;55(2):247-250.
114. Chadefaux-Vekemans B, Rabier D, Chabli A, et al. Improving the prenatal diagnosis of citrullinemia using citrulline/ornithine +arginine ratio in amniotic fluid. *Prenat Diagn*. 2002;22(6): 456-458.
115. Mandell R, Packman S, Laframboise R, et al. Use of amniotic fluid amino acids in prenatal testing for argininosuccinic aciduria and citrullinaemia. *Prenat Diagn*. 1996;16(5):419-424.
116. Miller MJ, Soler-Alfonso CR, Grund JE, et al. Improved standards for prenatal diagnosis of citrullinemia. *Mol Genet Metab*. 2014;112(3):205-209.

117. Wilcken B, Haas M, Joy P, et al. Expanded newborn screening: outcome in screened and unscreened patients at age 6 years. *Pediatrics*. 2009;124(2):e241-e248.
118. Posset R, Garcia-Cazorla A, Valayannopoulos V, et al. Age at disease onset and peak ammonium level rather than interventional variables predict the neurological outcome in urea cycle disorders. *J Inherit Metab Dis*. 2016;39(5):661-672.
119. Bachmann C. Long-term outcome of patients with urea cycle disorders and the question of neonatal screening. *Eur J Pediatr*. 2003;162(suppl 1):S29-S33.
120. Krivitzky L, Babikian T, Lee HS, Thomas NH, Burk-Paull KL, Batshaw ML. Intellectual, adaptive, and behavioral functioning in children with urea cycle disorders. *Pediatr Res*. 2009;66(1): 96-101.
121. Merritt JL 2nd, Brody LL, Pino G, Rinaldo P. Newborn screening for proximal urea cycle disorders: current evidence supporting recommendations for newborn screening. *Mol Genet Metab*. 2018;124(2):109-113.
122. Cavicchi C, Malvagia S, la Marca G, et al. Hypocitrullinemia in expanded newborn screening by LC-MS/MS is not a reliable marker for ornithine transcarbamylase deficiency. *J Pharm Biomed Anal*. 2009;49(5):1292-1295.
123. Naylor EW, Chace DH. Automated tandem mass spectrometry for mass newborn screening for disorders in fatty acid, organic acid, and amino acid metabolism. *J Child Neurol*. 1999;14(suppl 1):S4-S8.
124. Rashed MS. Clinical applications of tandem mass spectrometry: ten years of diagnosis and screening for inherited metabolic diseases. *J Chromatogr B Biomed Sci Appl*. 2001;758(1):27-48.
125. Sander J, Janzen N, Sander S, et al. Neonatal screening for citrullinaemia. *Eur J Pediatr*. 2003;162(6):417-420.
126. Mercimek-Mahmutoglu S, Moeslinger D, Häberle J, et al. Long-term outcome of patients with argininosuccinate lyase deficiency diagnosed by newborn screening in Austria. *Mol Genet Metab*. 2010;100(1):24-28.
127. Barends M, Pitt J, Morrissy S, Tzanakos N, Boneh A. Biochemical and molecular characteristics of patients with organic acidaemias and urea cycle disorders identified through newborn screening. *Mol Genet Metab*. 2014;113(1–2):46-52.
128. Sokoro AA, Lepage J, Antonishyn N, et al. Diagnosis and high incidence of hyperornithinemia-hyperammonemia-homocitrullinemia (HHH) syndrome in northern Saskatchewan. *J Inherit Metab Dis*. 2010;33(suppl 3):S275-S281.
129. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *N Engl J Med*. 2007;356(22): 2282-2292.
130. Schaefer F, Straube E, Oh J, Mehls O, Mayatepek E. Dialysis in neonates with inborn errors of metabolism. *Nephrol Dial Transplant*. 1999;14(4):910-918.
131. Nakamura K, Kido J, Mitsubuchi H, Endo F. Diagnosis and treatment of urea cycle disorder in Japan. *Pediatr Int*. 2014;56(4): 506-509.
132. De Bie I, Lemyre E, Lambert M. Favorable long-term outcome following severe neonatal hyperammonemic coma in a patient with argininosuccinate synthetase deficiency. *JIMD Rep*. 2011;1: 83-88.
133. Feillet F, Leonard JV. Alternative pathway therapy for urea cycle disorders. *J Inherit Metab Dis*. 1998;21(suppl 1):101-111.
134. Batshaw ML, MacArthur RB, Tuchman M. Alternative pathway therapy for urea cycle disorders: twenty years later. *J Pediatr*. 2001;138(1 suppl):S46-S54; discussion S54-45.
135. Burlina AB, Ogier H, Korall H, Trefz FK. Long-term treatment with sodium phenylbutyrate in ornithine transcarbamylase-deficient patients. *Mol Genet Metab*. 2001;72(4):351-355.
136. MacArthur RB, Altincatal A, Tuchman M. Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers. *Mol Genet Metab*. 2004;81(suppl 1): S67-S73.
137. Praphanphoj V, Boyadjiev SA, Waber LJ, Brusilow SW, Geraghty MT. Three cases of intravenous sodium benzoate and sodium phenylacetate toxicity occurring in the treatment of acute hyperammonaemia. *J Inherit Metab Dis*. 2000;23(2):129-136.
138. Picca S, Bartuli A, Dionisi-Vici C. Medical management and dialysis therapy for the infant with an inborn error of metabolism. *Semin Nephrol*. 2008;28(5):477-480.
139. Sadowski RH, Harmon WE, Jabs K. Acute hemodialysis of infants weighing less than five kilograms. *Kidney Int*. 1994;45 (3):903-906.
140. Tsai IJ, Hwu WL, Huang SC, et al. Efficacy and safety of intermittent hemodialysis in infants and young children with inborn errors of metabolism. *Pediatr Nephrol*. 2014;29(1):111-116.
141. Hiroma T, Nakamura T, Tamura M, Kaneko T, Komiyama A. Continuous venovenous hemodiafiltration in neonatal onset hyperammonemia. *Am J Perinatol*. 2002;19(4):221-224.
142. Spinale JM, Laskin BL, Sondheimer N, Swartz SJ, Goldstein SL. High-dose continuous renal replacement therapy for neonatal hyperammonemia. *Pediatr Nephrol*. 2013;28(6):983-986.
143. Boneh A. Dietary protein in urea cycle defects: how much? Which? How? *Mol Genet Metab*. 2014;113(1–2):109-112.
144. Rodney S, Boneh A. Amino acid profiles in patients with urea cycle disorders at admission to hospital due to metabolic decompensation. *JIMD Rep*. 2013;9:97-104.
145. WHO Technical Report Series. *Protein and amino acid requirement in human Nutrition*; 2007.
146. Shchelochkov OA, Dickinson K, Scharschmidt BF, Lee B, Marino M, Le Mons C. Barriers to drug adherence in the treatment of urea cycle disorders: assessment of patient, caregiver and provider perspectives. *Mol Genet Metab Rep*. 2016;8:43-47.
147. Berry GT, Steiner RD. Long-term management of patients with urea cycle disorders. *J Pediatr*. 2001;138(1 suppl):S56-S60. discussion S60-51.
148. Dixon M. Disorders of amino acid metabolism, organic acidemias and urea cycle defects. Organic acidemias and urea cycle disorders. In: Shaw V, Lawson M, eds., Wiley & Sons Ltd, Oxford, UK. *Clinical Pediatric Dietetics*; 2007:357-389.
149. Leonard JV. The nutritional management of urea cycle disorders. *J Pediatr*. 2001;138(1 suppl):S40-S44;discussion S44-45.
150. Adam S, Almeida MF, Assoun M, et al. Dietary management of urea cycle disorders: European practice. *Mol Genet Metab*. 2013; 110(4):439-445.
151. Adam S, Champion H, Daly A, et al. Dietary management of urea cycle disorders: UK practice. *J Hum Nutr Diet*. 2012;25(4): 398-404.
152. Scaglia F, Carter S, O'Brien WE, Lee B. Effect of alternative pathway therapy on branched chain amino acid metabolism in

urea cycle disorder patients. *Mol Genet Metab*. 2004;81(suppl 1): S79-S85.
153. Burrage LC, Jain M, Gandolfo L, Lee BH, Members of the Urea Cycle Disorders Consortium, Nagamani SC. Sodium phenylbutyrate decreases plasma branched-chain amino acids in patients with urea cycle disorders. *Mol Genet Metab*. 2014;113 (1–2):131-135.
154. Scaglia F. New insights in nutritional management and amino acid supplementation in urea cycle disorders. *Mol Genet Metab*. 2010;100(suppl 1):S72-S76.
155. Bachmann C. Hyperammonaemia: review of current treatment strategies. In: Bachmann C, Häberle J, Leonard JV, eds. *Pathophysiology and management of hyperammonemia*. Heilbronn, Germany: SPS Publications; 2006:157-173.
156. Vlaardingerbroek H, Hornstra G, de Koning TJ, et al. Essential polyunsaturated fatty acids in plasma and erythrocytes of children with inborn errors of amino acid metabolism. *Mol Genet Metab*. 2006;88(2):159-165.
157. Sanjurjo P, Ruiz JI, Montejo M. Inborn errors of metabolism with a protein-restricted diet: effect on polyunsaturated fatty acids. *J Inherit Metab Dis*. 1997;20(6):783-789.
158. Mendez-Figueroa H, Lamance K, Sutton VR, Aagaard-Tillery K, Van den Veyver I. Management of ornithine transcarbamylase deficiency in pregnancy. *Am J Perinatol*. 2010;27(10):775-784.
159. Worthington S, Christodoulou J, Wilcken B, Peat B. Pregnancy and argininosuccinic aciduria. *J Inherit Metab Dis*. 1996;19(5): 621-623.
160. Ituk U, Constantinescu OC, Allen TK, Small MJ, Habib AS. Peripartum management of two parturients with ornithine transcarbamylase deficiency. *Int J Obstet Anesth*. 2012;21(1):90-93.
161. Langendonk JG, Roos JC, Angus L, et al. A series of pregnancies in women with inherited metabolic disease. *J Inherit Metab Dis*. 2012;35(3):419-424.
162. Kim SZ, Song WJ, Nyhan WL, Ficicioglu C, Mandell R, Shih VE. Long-term follow-up of four patients affected by HHH syndrome. *Clin Chim Acta*. 2012;413(13–14):1151-1155.
163. Singh RH. Nutrition management of patients with inherited disorders of urea cycle enzymes. In: Acosta PB, ed. *Nutrition management of patients with inherited metabolic disorders*. Sudbury: Jones & Bartlett Learning; 2009:405-429.
164. Wilcken B. Problems in the management of urea cycle disorders. *Mol Genet Metab*. 2004;81(suppl 1):S86-S91.
165. Maestri NE, Brusilow SW, Clissold DB, Bassett SS. Long-term treatment of girls with ornithine transcarbamylase deficiency. *N Engl J Med*. 1996;335(12):855-859.
166. Comte B, Kasumov T, Pierce BA, et al. Identification of phenylbutyrylglutamine, a new metabolite of phenylbutyrate metabolism in humans. *J Mass Spectrom*. 2002;37(6):581-590.
167. Guffon N, Kibleur Y, Copalu W, Tissen C, Breitkreutz J. Developing a new formulation of sodium phenylbutyrate. *Arch Dis Child*. 2012;97(12):1081-1085.
168. Kibleur Y, Dobbelaere D, Barth M, Brassier A, Guffon N. Results from a Nationwide cohort temporary utilization authorization (ATU) survey of patients in France treated with Pheburane ((R)) (sodium phenylbutyrate) taste-masked granules. *Paediatr Drugs*. 2014;16(5):407-415.
169. Lichter-Konecki U, Diaz GA, Merritt JL 2nd, et al. Ammonia control in children with urea cycle disorders (UCDs); phase 2 comparison of sodium phenylbutyrate and glycerol phenylbutyrate. *Mol Genet Metab*. 2011;103(4):323-329.
170. Berry SA, Lichter-Konecki U, Diaz GA, et al. Glycerol phenylbutyrate treatment in children with urea cycle disorders: pooled analysis of short and long-term ammonia control and outcomes. *Mol Genet Metab*. 2014;112(1):17-24.
171. Diaz GA, Krivitzky LS, Mokhtarani M, et al. Ammonia control and neurocognitive outcome among urea cycle disorder patients treated with glycerol phenylbutyrate. *Hepatology*. 2013;57(6): 2171-2179.
172. Mokhtarani M, Diaz GA, Rhead W, et al. Urinary phenylacetylglutamine as dosing biomarker for patients with urea cycle disorders. *Mol Genet Metab*. 2012;107(3):308-314.
173. Smith W, Diaz GA, Lichter-Konecki U, et al. Ammonia control in children ages 2 months through 5 years with urea cycle disorders: comparison of sodium phenylbutyrate and glycerol phenylbutyrate. *J Pediatr*. 2013;162(6):1228-1234, 1234.e1.
174. Mokhtarani M, Diaz GA, Rhead W, et al. Elevated phenylacetic acid levels do not correlate with adverse events in patients with urea cycle disorders or hepatic encephalopathy and can be predicted based on the plasma PAA to PAGN ratio. *Mol Genet Metab*. 2013;110(4):446-453.
175. Tremblay GC, Qureshi IA. The biochemistry and toxicology of benzoic acid metabolism and its relationship to the elimination of waste nitrogen. *Pharmacol Ther*. 1993;60(1):63-90.
176. Griffith AD, Cyr DM, Egan SG, Tremblay GC. Inhibition of pyruvate carboxylase by sequestration of coenzyme A with sodium benzoate. *Arch Biochem Biophys*. 1989;269(1):201-207.
177. Monneret C. Histone deacetylase inhibitors. *Eur J Med Chem*. 2005;40(1):1-13.
178. Tuchman M, Lee B, Lichter-Konecki U, et al. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. *Mol Genet Metab*. 2008;94(4):397-402.
179. Lamb S, Aye CY, Murphy E, Mackillop L. Multidisciplinary management of ornithine transcarbamylase (OTC) deficiency in pregnancy: essential to prevent hyperammonemic complications. *BMJ Case Rep*. 2013; https://doi.org/10.1136/bcr-2012-007416.
180. Brusilow SW, Batshaw ML. Arginine therapy of argininosuccinase deficiency. *Lancet*. 1979;1(8108):124-127.
181. Brusilow SW. Arginine, an indispensable amino acid for patients with inborn errors of urea synthesis. *J Clin Invest*. 1984;74(6): 2144-2148.
182. Nagasaka H, Yorifuji T, Murayama K, et al. Effects of arginine treatment on nutrition, growth and urea cycle function in seven Japanese boys with late-onset ornithine transcarbamylase deficiency. *Eur J Pediatr*. 2006;165(9):618-624.
183. Keskinen P, Siitonen A, Salo M. Hereditary urea cycle diseases in Finland. *Acta Paediatr*. 2008;97(10):1412-1419.
184. Kim S, Paik WK, Cohen PP. Ammonia intoxication in rats: protection by N-carbamoyl-L-glutamate plus L-arginine. *Proc Natl Acad Sci USA*. 1972;69(12):3530-3533.
185. Caldovic L, Morizono H, Daikhin Y, et al. Restoration of ureagenesis in N-acetylglutamate synthase deficiency by N-carbamylglutamate. *J Pediatr*. 2004;145(4):552-554.
186. Bachmann C, Colombo JP, Jaggi K. N-acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observations and treatment. *Adv Exp Med Biol*. 1982;153:39-45.

187. Guffon N, Schiff M, Cheillan D, Wermuth B, Häberle J, Vianey-Saban C. Neonatal hyperammonemia: the N-carbamoyl-L-glutamic acid test. *J Pediatr*. 2005;147(2):260-262.
188. Tuchman M, Caldovic L, Daikhin Y, et al. N-carbamylglutamate markedly enhances ureagenesis in N-acetylglutamate deficiency and propionic acidemia as measured by isotopic incorporation and blood biomarkers. *Pediatr Res*. 2008;64(2):213-217.
189. Häberle J. Role of carglumic acid in the treatment of acute hyperammonemia due to N-acetylglutamate synthase deficiency. *Ther Clin Risk Manag*. 2011;7:1-6.
190. Häberle J. Carglumic acid for the treatment of N-acetylglutamate synthase deficiency and of acute hyperammonemia. *Expert Rev Endocrinol Metab*. 2012;7:263-271.
191. Feoli-Fonseca JC, Lambert M, Mitchell G, et al. Chronic sodium benzoate therapy in children with inborn errors of urea synthesis: effect on carnitine metabolism and ammonia nitrogen removal. *Biochem Mol Med*. 1996;57(1):31-36.
192. Mayatepek E, Kurczynski TW, Hoppel CL, Gunning WT. Carnitine deficiency associated with ornithine transcarbamylase deficiency. *Pediatr Neurol*. 1991;7(3):196-199.
193. Ohtani Y, Ohyanagi K, Yamamoto S, Matsuda I. Secondary carnitine deficiency in hyperammonemic attacks of ornithine transcarbamylase deficiency. *J Pediatr*. 1988;112(3):409-414.
194. Häberle J, Lachman R. Diagnosis and treatment of OTC in females. Paper presented at: EMG Workshop Proceedings 20102010; Lisboa.
195. Morgan TM, Schlegel C, Edwards KM, et al. Vaccines are not associated with metabolic events in children with urea cycle disorders. *Pediatrics*. 2011;127(5):e1147-e1153.
196. Schmidt J, Kroeber S, Irouschek A, Birkholz T, Schroth M, Albrecht S. Anesthetic management of patients with ornithine transcarbamylase deficiency. *Paediatr Anaesth*. 2006;16(3):333-337.
197. Whitington PF, Alonso EM, Boyle JT, et al. Liver transplantation for the treatment of urea cycle disorders. *J Inherit Metab Dis*. 1998;21(suppl 1):112-118.
198. Kim IK, Niemi AK, Krueger C, et al. Liver transplantation for urea cycle disorders in pediatric patients: a single-center experience. *Pediatr Transplant*. 2013;17(2):158-167.
199. Leonard JV, McKiernan PJ. The role of liver transplantation in urea cycle disorders. *Mol Genet Metab*. 2004;81(suppl 1):S74-S78.
200. Morioka D, Kasahara M, Takada Y, et al. Current role of liver transplantation for the treatment of urea cycle disorders: a review of the worldwide English literature and 13 cases at Kyoto University. *Liver Transpl*. 2005;11(11):1332-1342.
201. Campeau PM, Pivalizza PJ, Miller G, et al. Early orthotopic liver transplantation in urea cycle defects: follow up of a developmental outcome study. *Mol Genet Metab*. 2010;100(suppl 1):S84-S87.
202. Bucuvalas JC, Britto M, Krug S, et al. Health-related quality of life in pediatric liver transplant recipients: a single-center study. *Liver Transpl*. 2003;9(1):62-71.
203. Alonso EM, Neighbors K, Mattson C, et al. Functional outcomes of pediatric liver transplantation. *J Pediatr Gastroenterol Nutr*. 2003;37(2):155-160.
204. Yu L, Rayhill SC, Hsu EK, Landis CS. Liver transplantation for urea cycle disorders: analysis of the united network for organ sharing database. *Transplant Proc*. 2015;47(8):2413-2418.
205. Bourdeaux C, Darwish A, Jamart J, et al. Living-related versus deceased donor pediatric liver transplantation: a multivariate analysis of technical and immunological complications in 235 recipients. *Am J Transplant*. 2007;7(2):440-447.
206. Kawagishi N, Satoh K, Enomoto Y, et al. Improved quality of life and unchanged magnetic resonance brain imaging after living donor liver transplantation for late-onset ornithine transcarbamylase deficiency: report of a case. *Surg Today*. 2005;35(12):1087-1091.
207. Busuttil AA, Goss JA, Seu P, et al. The role of orthotopic liver transplantation in the treatment of ornithine transcarbamylase deficiency. *Liver Transpl Surg*. 1998;4(5):350-354.
208. Fletcher JM, Couper R, Moore D, Coxon R, Dorney S. Liver transplantation for citrullinaemia improves intellectual function. *J Inherit Metab Dis*. 1999;22(5):581-586.
209. Santos Silva E, Martins E, Cardoso ML, Barbot C, Vilarinho L, Medina M. Liver transplantation in a case of argininaemia. *J Inherit Metab Dis*. 2001;24(8):885-887.
210. Ensenauer R, Tuchman M, El-Youssef M, et al. Management and outcome of neonatal-onset ornithine transcarbamylase deficiency following liver transplantation at 60 days of life. *Mol Genet Metab*. 2005;84(4):363-366.
211. McBride KL, Miller G, Carter S, Karpen S, Goss J, Lee B. Developmental outcomes with early orthotopic liver transplantation for infants with neonatal-onset urea cycle defects and a female patient with late-onset ornithine transcarbamylase deficiency. *Pediatrics*. 2004;114(4):e523-e526.
212. Newnham T, Hardikar W, Allen K, et al. Liver transplantation for argininosuccinic aciduria: clinical, biochemical, and metabolic outcome. *Liver Transpl*. 2008;14(1):41-45.
213. Stevenson T, Millan MT, Wayman K, et al. Long-term outcome following pediatric liver transplantation for metabolic disorders. *Pediatr Transplant*. 2010;14(2):268-275.
214. Perito ER, Rhee S, Roberts JP, Rosenthal P. Pediatric liver transplantation for urea cycle disorders and organic acidemias: united network for organ sharing data for 2002-2012. *Liver Transpl*. 2014;20(1):89-99.
215. Rabier D, Narcy C, Bardet J, Parvy P, Saudubray JM, Kamoun P. Arginine remains an essential amino acid after liver transplantation in urea cycle enzyme deficiencies. *J Inherit Metab Dis*. 1991;14(3):277-280.
216. Sundaram SS, Alonso EM, Whitington PF. Liver transplantation in neonates. *Liver Transpl*. 2003;9(8):783-788.
217. Hashikura Y, Ichida T, Umeshita K, et al. Donor complications associated with living donor liver transplantation in Japan. *Transplantation*. 2009;88(1):110-114.
218. Nagasaka H, Yorifuji T, Egawa H, Kikuta H, Tanaka K, Kobayashi K. Successful living-donor liver transplantation from an asymptomatic carrier mother in ornithine transcarbamylase deficiency. *J Pediatr*. 2001;138(3):432-434.
219. Morioka D, Takada Y, Kasahara M, et al. Living donor liver transplantation for noncirrhotic inheritable metabolic liver diseases: impact of the use of heterozygous donors. *Transplantation*. 2005;80(5):623-628.
220. Kasahara M, Sakamoto S, Horikawa R, et al. Living donor liver transplantation for pediatric patients with metabolic disorders: the Japanese multicenter registry. *Pediatr Transplant*. 2014;18(1):6-15.

221. Wakiya T, Sanada Y, Mizuta K, et al. Living donor liver transplantation for ornithine transcarbamylase deficiency. *Pediatr Transplant*. 2011;15(4):390-395.
222. Lee B, Diaz GA, Rhead W, et al. Blood ammonia and glutamine as predictors of hyperammonemic crises in patients with urea cycle disorder. *Genet Med*. 2015;17(7):561-568.
223. Bachmann C. Preanalytical effects on biochemical results for medical decisions in urea cycle defects. *J Pediatr Biochem*. 2014; 4(1):17-22.
224. Maestri NE, McGowan KD, Brusilow SW. Plasma glutamine concentration: a guide in the management of urea cycle disorders. *J Pediatr*. 1992;121(2):259-261.
225. Brusilow SW. Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion. *Pediatr Res*. 1991;29(2): 147-150.
226. Bachmann C. Interpretation of plasma amino acids in the follow-up of patients: the impact of compartmentation. *J Inherit Metab Dis*. 2008;31(1):7-20.
227. Duran M. Amino acids. In: Blau N, Duran M, Gibson KM, eds. *Laboratory Guide to the Methods in Biochemical Genetics*. Springer, Heidelberg; 2008:53-89.
228. Boenzi S, Pastore A, Martinelli D, et al. Creatine metabolism in urea cycle defects. *J Inherit Metab Dis*. 2012;35(4):647-653.
229. Arias A, Garcia-Villoria J, Ribes A. Guanidinoacetate and creatine/creatinine levels in controls and patients with urea cycle defects. *Mol Genet Metab*. 2004;82(3):220-223.
230. Bireley WR, Van Hove JL, Gallagher RC, Fenton LZ. Urea cycle disorders: brain MRI and neurological outcome. *Pediatr Radiol*. 2012;42(4):455-462.
231. Gropman A. Brain imaging in urea cycle disorders. *Mol Genet Metab*. 2010;100(suppl 1):S20-S30.
232. Gunz AC, Choong K, Potter M, Miller E. Magnetic resonance imaging findings and neurodevelopmental outcomes in neonates with urea-cycle defects. *Int Med Case Rep J*. 2013;6:41-48.
233. Gropman AL, Fricke ST, Seltzer RR, et al. 1H MRS identifies symptomatic and asymptomatic subjects with partial ornithine transcarbamylase deficiency. *Mol Genet Metab*. 2008;95(1–2): 21-30.
234. Gropman AL, Gertz B, Shattuck K, et al. Diffusion tensor imaging detects areas of abnormal white matter microstructure in patients with partial ornithine transcarbamylase deficiency. *AJNR Am J Neuroradiol*. 2010;31(9):1719-1723.
235. Takanashi J, Barkovich AJ, Cheng SF, et al. Brain MR imaging in neonatal hyperammonemic encephalopathy resulting from proximal urea cycle disorders. *AJNR Am J Neuroradiol*. 2003;24 (6):1184-1187.
236. Majoie CB, Mourmans JM, Akkerman EM, Duran M. Poll-The BT. Neonatal citrullinemia: comparison of conventional MR, diffusion-weighted, and diffusion tensor findings. *AJNR Am J Neuroradiol*. 2004;25(1):32-35.
237. Connelly A, Cross JH, Gadian DG, Hunter JV, Kirkham FJ, Leonard JV. Magnetic resonance spectroscopy shows increased brain glutamine in ornithine carbamoyl transferase deficiency. *Pediatr Res*. 1993;33(1):77-81.
238. Choi CG, Yoo HW. Localized proton MR spectroscopy in infants with urea cycle defect. *AJNR Am J Neuroradiol*. 2001;22(5): 834-837.
239. Gropman AL, Shattuck K, Prust MJ, et al. Altered neural activation in ornithine transcarbamylase deficiency during executive cognition: an fMRI study. *Hum Brain Mapp*. 2013;34(4): 753-761.
240. Drogari E, Leonard JV. Late onset ornithine carbamoyl transferase deficiency in males. *Arch Dis Child*. 1988;63(11):1363-1367.
241. Jamiolkowski D, Kolker S, Glahn EM, et al. Behavioural and emotional problems, intellectual impairment and health-related quality of life in patients with organic acidurias and urea cycle disorders. *J Inherit Metab Dis*. 2016;39(2):231-241.
242. Zeltner NA, Huemer M, Baumgartner MR, Landolt MA. Quality of life, psychological adjustment, and adaptive functioning of patients with intoxication-type inborn errors of metabolism - a systematic review. *Orphanet J Rare Dis*. 2014;9:159.
243. Hatzmann J, Heymans HS, Ferrer-i-Carbonell A, van Praag BM, Grootenhuis MA. Hidden consequences of success in pediatrics: parental health-related quality of life--results from the care project. *Pediatrics*. 2008;122(5):e1030-e1038.
244. Cederbaum JA, LeMons C, Rosen M, Ahrens M, Vonachen S, Cederbaum SD. Psychosocial issues and coping strategies in families affected by urea cycle disorders. *J Pediatr*. 2001;138(1 suppl):S72-S80.
245. Fabre A, Baumstarck K, Cano A, et al. Assessment of quality of life of the children and parents affected by inborn errors of metabolism with restricted diet: preliminary results of a cross-sectional study. *Health Qual Life Outcomes*. 2013;11:158.
246. Zeltner NA, Landolt MA, Baumgartner MR, et al. Living with intoxication-type inborn errors of metabolism: a qualitative analysis of interviews with paediatric patients and their parents. *JIMD Rep*. 2017;31:1-9.
247. Zeltner NA, Baumgartner MR, Bondarenko A, et al. Development and psychometric evaluation of the MetabQoL 1.0: a quality of life questionnaire for paediatric patients with intoxication-type inborn errors of metabolism. *JIMD Rep*. 2017;37:27-35.
248. Feillet F, MacDonald A, Hartung Perron D, Burton B. Outcomes beyond phenylalanine: an international perspective. *Mol Genet Metab*. 2010;99(suppl 1):S79-S85.
249. Rokicki D, Pajdowska M, Trubicka J, et al. 3-Methylglutaconic aciduria, a frequent but underrecognized finding in carbamoyl phosphate synthetase I deficiency. *Clin Chim Acta*. 2017;471: 95-100.
250. Nordenstrom A, Halldin M, Hallberg B, Alm J. A trial with N-carbamylglutamate may not detect all patients with NAGS deficiency and neonatal onset. *J Inherit Metab Dis*. 2007;30(3):400.
251. Ah Mew N, Daikhin E, Payan I, et al. N-carbamylglutamate increases ureagenesis in patients with CPSI deficiency (abstract). *Mol Genet Metab*. 2010;99:207.
252. Williams M, Huijmans JG, van Diggelen OP, van der Louw EJTM, De Klerk JBC, Häberle J. Carbamoylphosphate synthase I (CPS 1) deficiency: treatment with carglumic acid (Carbaglu) (abstract). *J Inherit Metab Dis*. 2010;33:S118.
253. Caldovic L, Morizono H, Tuchman M. Mutations and polymorphisms in the human N-acetylglutamate synthase (NAGS) gene. *Hum Mutat*. 2007;28(8):754-759.
254. Heckmann M, Wermuth B, Häberle J, Koch HG, Gortner L, Kreuder JG. Misleading diagnosis of partial N-acetylglutamate synthase deficiency based on enzyme measurement corrected by mutation analysis. *Acta Paediatr*. 2005;94(1):121-124.
255. Caldovic L, Morizono H, Panglao MG, Cheng SF, Packman S, Tuchman M. Null mutations in the N-acetylglutamate synthase

gene associated with acute neonatal disease and hyperammonemia. *Hum Genet*. 2003;112(4):364-368.
256. Caldovic L, Morizono H, Panglao MG, et al. Late onset N-acetylglutamate synthase deficiency caused by hypomorphic alleles. *Hum Mutat*. 2005;25(3):293-298.
257. Ihara K, Miyako K, Ishimura M, et al. A case of hyperinsulinism/hyperammonaemia syndrome with reduced carbamoyl-phosphate synthetase-1 activity in liver: a pitfall in enzymatic diagnosis for hyperammonaemia. *J Inherit Metab Dis*. 2005;28(5):681-687.
258. Gautschi M, Eggimann S, Nuoffer JM. Current role of enzyme analysis for urea cycle disorders. *J Pediatr Biochem*. 2014;4(1): 23-32.
259. Gessler P, Buchal P, Schwenk HU, Wermuth B. Favourable long-term outcome after immediate treatment of neonatal hyperammonemia due to N-acetylglutamate synthase deficiency. *Eur J Pediatr*. 2010;169(2):197-199.
260. Belanger-Quintana A, Martinez-Pardo M, Garcia MJ, et al. Hyperammonaemia as a cause of psychosis in an adolescent. *Eur J Pediatr*. 2003;162(11):773-775.
261. Guffon N, Gessler P, Galloway P, et al. Treatment of NAGS deficiency: retrospective data on 23 patients treated with carglumic acid over 16 years (abstract). *Mol Genet Metab*. 2011;102(3): 286-287.
262. Daniotti M, la Marca G, Fiorini P, Filippi L. New developments in the treatment of hyperammonemia: emerging use of carglumic acid. *Int J Gen Med*. 2011;4:21-28.
263. Ah Mew N, McCarter R, Daikhin Y, et al. Augmenting ureagenesis in patients with partial carbamyl phosphate synthetase 1 deficiency with N-carbamyl-L-glutamate. *J Pediatr*. 2014; 165(2):401-403.e403.
264. Ihara K, Yoshino M, Hoshina T, et al. Coagulopathy in patients with late-onset ornithine transcarbamylase deficiency in remission state: a previously unrecognized complication. *Pediatrics*. 2013;131(1):e327-e330.
265. Prada CE, Kaul A, Hopkin RJ, et al. Recurrent pancreatitis in ornithine transcarbamylase deficiency. *Mol Genet Metab*. 2012; 106(4):482-484.
266. Tuchman M. The clinical, biochemical, and molecular spectrum of ornithine transcarbamylase deficiency. *J Lab Clin Med*. 1992; 120(6):836-850.
267. Numata S, Harada E, Maeno Y, et al. Paternal transmission and slow elimination of mutant alleles associated with late-onset ornithine transcarbamylase deficiency in male patients. *J Hum Genet*. 2008;53(1):10-17.
268. Brassier A, Gobin S, Arnoux JB, et al. Long-term outcomes in ornithine transcarbamylase deficiency: a series of 90 patients. *Orphanet J Rare Dis*. 2015;10:58.
269. Caldovic L, Abdikarim I, Narain S, Tuchman M, Morizono H. Genotype-phenotype correlations in ornithine transcarbamylase deficiency: a mutation update. *J Genet Genomics*. 2015;42(5): 181-194.
270. Krijt J, Sokolova J, Jesina P, et al. Activity of the liver enzyme ornithine carbamoyltransferase (OTC) in blood: LC-MS/MS assay for non-invasive diagnosis of ornithine carbamoyltransferase deficiency. *Clin Chem Lab Med*. 2017;55(8):1168-1177.
271. Hauser ER, Finkelstein JE, Valle D, Brusilow SW. Allopurinol-induced orotidinuria. A test for mutations at the ornithine carbamoyltransferase locus in women. *N Engl J Med*. 1990;322 (23):1641-1645.
272. Maestri NE, Lord C, Glynn M, Bale A, Brusilow SW. The phenotype of ostensibly healthy women who are carriers for ornithine transcarbamylase deficiency. *Medicine (Baltimore)*. 1998; 77(6):389-397.
273. Grunewald S, Fairbanks L, Genet S, et al. How reliable is the allopurinol load in detecting carriers for ornithine transcarbamylase deficiency? *J Inherit Metab Dis*. 2004;27(2): 179-186.
274. Tuchman M. Allopurinol-induced orotidinuria. *N Engl J Med*. 1990;323(19):1352-1353.
275. Burlina AB, Ferrari V, Dionisi-Vici C, Bordugo A, Zacchello F, Tuchman M. Allopurinol challenge test in children. *J Inherit Metab Dis*. 1992;15(5):707-712.
276. Riudor E, Arranz JA, Rodes M, Rubio V, Sentis M, Burlina AB. Influence of dose and age on the response of the allopurinol test for ornithine carbamoyltransferase deficiency in control infants. *J Inherit Metab Dis*. 2000;23(7):662-668.
277. Potter M, Hammond JW, Sim KG, Green AK, Wilcken B. Ornithine carbamoyltransferase deficiency: improved sensitivity of testing for protein tolerance in the diagnosis of heterozygotes. *J Inherit Metab Dis*. 2001;24(1):5-14.
278. de Groot MJ, Cuppen M, Eling M, et al. Metabolic investigations prevent liver transplantation in two young children with citrullinemia type I. *J Inherit Metab Dis*. 2010;33(Suppl3): S413–416.
279. Engel K, Höhne W, Häberle J. Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. *Hum Mutat*. 2009;30(3):300-307.
280. Kleijer WJ, Thoomes R, Galjaard H, Wendel U, Fowler B. First-trimester (chorion biopsy) diagnosis of citrullinaemia and methylmalonicaciduria. *Lancet*. 1984;2(8415):1340.
281. Berning C, Bieger I, Pauli S, et al. Investigation of citrullinemia type I variants by in vitro expression studies. *Hum Mutat*. 2008; 29(10):1222-1227.
282. Engel K, Vuissoz JM, Eggimann S, et al. Bacterial expression of mutant argininosuccinate lyase reveals imperfect correlation of in-vitro enzyme activity with clinical phenotype in argininosuccinic aciduria. *J Inherit Metab Dis*. 2012;35(1): 133-140.
283. Trevisson E, Burlina A, Doimo M, et al. Functional complementation in yeast allows molecular characterization of missense argininosuccinate lyase mutations. *J Biol Chem*. 2009;284(42): 28926-28934.
284. Balmer C, Pandey AV, Rüfenacht V, et al. Mutations and polymorphisms in the human Argininosuccinate Lyase (ASL) gene. *Hum Mutat*. 2014;35(1):27-35.
285. Hu L, Pandey AV, Balmer C, et al. Unstable argininosuccinate lyase in variant forms of the urea cycle disorder argininosuccinic aciduria. *J Inherit Metab Dis*. 2015;38(5):815-827.
286. Nagamani SC, Erez A, Lee B. Argininosuccinate lyase deficiency. *Genet Med*. 2012;14(5):501-507.
287. Kölker S, Sauer SW, Surtees RA, Leonard JV. The aetiology of neurological complications of organic acidaemias--a role for the blood-brain barrier. *J Inherit Metab Dis*. 2006;29(6):701-704. discussion 705-706.
288. van Spronsen FJ, Reijngoud DJ, Verhoeven NM, Sooranni-Lunsing RJ, Jakobs C, Sijens PE. High cerebral guanidinoacetate

and variable creatine concentrations in argininosuccinate synthetase and lyase deficiency: implications for treatment? *Mol Genet Metab*. 2006;89(3):274-276.

289. Erez A, Nagamani SC, Shchelochkov OA, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med*. 2011;17(12):1619-1626.
290. Brunetti-Pierri N, Erez A, Shchelochkov O, Craigen W, Lee B. Systemic hypertension in two patients with ASL deficiency: a result of nitric oxide deficiency? *Mol Genet Metab*. 2009;98 (1–2):195-197.
291. Erez A, Nagamani SC, Lee B. Argininosuccinate lyase deficiency-argininosuccinic aciduria and beyond. *Am J Med Genet C Semin Med Genet*. 2011;157C(1):45-53.
292. Nagamani SC, Campeau PM, Shchelochkov OA, et al. Nitric-oxide supplementation for treatment of long-term complications in argininosuccinic aciduria. *Am J Hum Genet*. 2012;90(5): 836-846.
293. Nagamani SC, Shchelochkov OA, Mullins MA, et al. A randomized controlled trial to evaluate the effects of high-dose versus low-dose of arginine therapy on hepatic function tests in argininosuccinic aciduria. *Mol Genet Metab*. 2012;107(3): 315-321.
294. Schlune A, Vom Dahl S, Haussinger D, Ensenauer R, Mayatepek E. Hyperargininemia due to arginase I deficiency: the original patients and their natural history, and a review of the literature. *Amino Acids*. 2015;47(9):1751-1762.
295. Cederbaum SD, Moedjono SJ, Shaw KN, Carter M, Naylor E, Walzer M. Treatment of hyperargininaemia due to arginase deficiency with a chemically defined diet. *J Inherit Metab Dis*. 1982; 5(2):95-99.
296. Ash DE, Scolnick LR, Kanyo ZF, Vockley JG, Cederbaum SD, Christianson DW. Molecular basis of hyperargininemia: structure-function consequences of mutations in human liver arginase. *Mol Genet Metab*. 1998;64(4):243-249.
297. Scaglia F, Lee B. Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. *Am J Med Genet C Semin Med Genet*. 2006;142C(2):113-120.
298. Diez-Fernandez C, Rüfenacht V, Gemperle C, Fingerhut R, Häberle J. Mutations and common variants in the human arginase 1 (ARG1) gene: impact on patients, diagnostics, and protein structure considerations. *Hum Mutat*. 2018;39(8):1029-1050.
299. Brosnan JT, Brosnan ME. Creatine metabolism and the urea cycle. *Mol Genet Metab*. 2010;100(suppl 1):S49-S52.
300. Huemer M, Carvalho DR, Brum JM, et al. Clinical phenotype, biochemical profile, and treatment in 19 patients with arginase 1 deficiency. *J Inherit Metab Dis*. 2016;39(3):331-340.
301. Jain-Ghai S, Nagamani SC, Blaser S, Siriwardena K, Feigenbaum A. Arginase I deficiency: severe infantile presentation with hyperammonemia: more common than reported? *Mol Genet Metab*. 2011;104(1–2):107-111.
302. Scholl-Bürgi S, Sigl SB, Häberle J, et al. Amino acids in CSF and plasma in hyperammonaemic coma due to arginase1 deficiency. *J Inherit Metab Dis*. 2008;31(suppl 2):S323-S328.
303. Zhang Y, Landau YE, Miller DT, Marsden D, Berry GT, Kellogg MD. Recurrent unexplained hyperammonemia in an adolescent with arginase deficiency. *Clin Biochem*. 2012;45(18): 1583-1586.
304. Debray FG, Lambert M, Lemieux B, et al. Phenotypic variability among patients with hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome homozygous for the delF188 mutation in SLC25A15. *J Med Genet*. 2008;45(11):759-764.
305. Dionisi Vici C, Bachmann C, Gambarara M, Colombo JP, Sabetta G. Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome: low creatine excretion and effect of citrulline, arginine, or ornithine supplement. *Pediatr Res*. 1987;22(3): 364-367.
306. Palmieri F. Diseases caused by defects of mitochondrial carriers: a review. *Biochim Biophys Acta*. 2008;1777(7–8): 564-578.
307. Shih VE, Mandell R, Herzfeld A. Defective ornithine metabolism in cultured skin fibroblasts from patients with the syndrome of hyperornithinemia, hyperammonemia and homocitrullinuria. *Clin Chim Acta*. 1982;118(2–3):149-157.
308. Camacho JA, Obie C, Biery B, et al. Hyperornithinaemia-hyperammonaemia-homocitrullinuria syndrome is caused by mutations in a gene encoding a mitochondrial ornithine transporter. *Nat Genet*. 1999;22(2):151-158.
309. Morini C, Capozzi P, Boenzi S, Rizzo C, Santorelli FM, Dionisi-Vici C. Retinal degeneration. *Ophthalmology*. 2009;116(8):1593-1593.e1.
310. Deignan JL, Cederbaum SD, Grody WW. Contrasting features of urea cycle disorders in human patients and knockout mouse models. *Mol Genet Metab*. 2008;93(1):7-14.
311. Kasten J, Hu C, Bhargava R, et al. Lethal phenotype in conditional late-onset arginase 1 deficiency in the mouse. *Mol Genet Metab*. 2013;110(3):222-230.
312. Perez CJ, Jaubert J, Guenet JL, et al. Two hypomorphic alleles of mouse Ass1 as a new animal model of citrullinemia type I and other hyperammonemic syndromes. *Am J Pathol*. 2010;177(4): 1958-1968.
313. Sin YY, Ballantyne LL, Mukherjee K, et al. Inducible arginase 1 deficiency in mice leads to hyperargininemia and altered amino acid metabolism. *PLoS One*. 2013;8(11):e80001.
314. Allegri G, Deplazes S, Grisch-Chan HM, et al. A simple dried blood spot-method for in vivo measurement of ureagenesis by gas chromatography-mass spectrometry using stable isotopes. *Clin Chim Acta*. 2017;464:236-243.
315. de Cima S, Polo LM, Diez-Fernandez C, et al. Structure of human carbamoyl phosphate synthetase: deciphering the on/off switch of human ureagenesis. *Sci Rep*. 2015;5:16950.
316. Diez-Fernandez C, Martinez AI, Pekkala S, et al. Molecular characterization of carbamoyl-phosphate synthetase (CPS1) deficiency using human recombinant CPS1 as a key tool. *Hum Mutat*. 2013;34(8):1149-1159.
317. Matoori S, Leroux JC. Recent advances in the treatment of hyperammonemia. *Adv Drug Deliv Rev*. 2015;90:55-68.
318. Braissant O. Current concepts in the pathogenesis of urea cycle disorders. *Mol Genet Metab*. 2010;100(suppl 1):S3-S12.
319. Lichter-Konecki U, Nadkarni V, Moudgil A, et al. Feasibility of adjunct therapeutic hypothermia treatment for hyperammonemia and encephalopathy due to urea cycle disorders and organic acidemias. *Mol Genet Metab*. 2013;109(4):354-359.
320. Whitelaw A, Bridges S, Leaf A, Evans D. Emergency treatment of neonatal hyperammonaemic coma with mild systemic hypothermia. *Lancet*. 2001;358(9275):36-38.
321. Dhawan A, Mitry RR, Hughes RD. Hepatocyte transplantation for liver-based metabolic disorders. *J Inherit Metab Dis*. 2006;29 (2–3):431-435.

322. Enns GM, Millan MT. Cell-based therapies for metabolic liver disease. *Mol Genet Metab*. 2008;95(1–2):3-10.
323. Meyburg J, Das AM, Hoerster F, et al. One liver for four children: first clinical series of liver cell transplantation for severe neonatal urea cycle defects. *Transplantation*. 2009;87(5): 636-641.
324. Meyburg J, Hoffmann GF. Liver, liver cell and stem cell transplantation for the treatment of urea cycle defects. *Mol Genet Metab*. 2010;100(suppl 1):S77-S83.
325. Meyburg J, Opladen T, Spiekerkotter U, et al. Human heterologous liver cells transiently improve hyperammonemia and ureagenesis in individuals with severe urea cycle disorders. *J Inherit Metab Dis*. 2018;41(1):81-90.
326. Strom SC, Fisher RA, Rubinstein WS, et al. Transplantation of human hepatocytes. *Transplant Proc*. 1997;29(4):2103-2106.
327. Horslen SP, McCowan TC, Goertzen TC, et al. Isolated hepatocyte transplantation in an infant with a severe urea cycle disorder. *Pediatrics*. 2003;111(6 pt 1):1262-1267.
328. Stephenne X, Najimi M, Sibille C, Nassogne MC, Smets F, Sokal EM. Sustained engraftment and tissue enzyme activity after liver cell transplantation for argininosuccinate lyase deficiency. *Gastroenterology*. 2006;130(4):1317-1323.
329. Stephenne X, Najimi M, Smets F, Reding R, de Ville de Goyet J, Sokal EM. Cryopreserved liver cell transplantation controls ornithine transcarbamylase deficient patient while awaiting liver transplantation. *Am J Transplant*. 2005;5(8):2058-2061.
330. Meyburg J, Hoffmann GF. Liver cell transplantation for the treatment of inborn errors of metabolism. *J Inherit Metab Dis*. 2008; 31(2):164-172.
331. Puppi J, Tan N, Mitry RR, et al. Hepatocyte transplantation followed by auxiliary liver transplantation--a novel treatment for ornithine transcarbamylase deficiency. *Am J Transplant*. 2008;8 (2):452-457.
332. Sokal EM. From hepatocytes to stem and progenitor cells for liver regenerative medicine: advances and clinical perspectives. *Cell Prolif*. 2011;44(suppl 1):39-43.
333. Sokal EM. Treating inborn errors of liver metabolism with stem cells: current clinical development. *J Inherit Metab Dis*. 2014;37 (4):535-539.
334. Cunningham SC, Kok CY, Dane AP, et al. Induction and prevention of severe hyperammonemia in the spfash mouse model of ornithine transcarbamylase deficiency using shRNA and rAAV-mediated gene delivery. *Mol Ther*. 2011;19(5):854-859.
335. Cunningham SC, Siew SM, Hallwirth CV, et al. Modeling correction of severe urea cycle defects in the growing murine liver using a hybrid recombinant adeno-associated virus/piggyBac transposase gene delivery system. *Hepatology*. 2015;62(2): 417-428.
336. Lee EK, Hu C, Bhargava R, et al. AAV-based gene therapy prevents neuropathology and results in normal cognitive development in the hyperargininemic mouse. *Gene Ther*. 2013;20(8): 785-796.
337. Wang L, Bell P, Morizono H, et al. AAV gene therapy corrects OTC deficiency and prevents liver fibrosis in aged OTC-knock out heterozygous mice. *Mol Genet Metab*. 2017;120(4):299-305.
338. Wang L, Wang H, Morizono H, et al. Sustained correction of OTC deficiency in spf(ash) mice using optimized self-complementary AAV2/8 vectors. *Gene Ther*. 2012;19(4): 404-410.
339. Wang L, Morizono H, Lin J, et al. Preclinical evaluation of a clinical candidate AAV8 vector for ornithine transcarbamylase (OTC) deficiency reveals functional enzyme from each persisting vector genome. *Mol Genet Metab*. 2012;105(2):203-211.
340. Viecelli HM, Thöny B. Challenges of experimental gene therapy for urea cycle disorders. *J Pediatr Biochem*. 2014;4(1):65-73.
341. Zhong L, Li S, Li M, et al. Vector sequences are not detected in tumor tissue from research subjects with ornithine transcarbamylase deficiency who previously received adenovirus gene transfer. *Hum Gene Ther*. 2013;24(9):814-819.
342. Häberle J, Rubio V. Hyperammonemias and related disorders. In: Blau N, Duran M, Gibson KM, Dionisi-Vici C, eds. *Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases*. Springer, Heidelberg; 2014:47-62.
343. Dixon M, MacDonald A, White F, Stafford J. *Disorders of amino acids metabolism, organic acidaemias and urea cycle disorders*. In: Shaw V, Dip Dietetics P (eds) Clinical Paediatric Dietetics. John Wiley & Sons, Ltd, Oxfaord, UK, 2015:381–525
344. Qureshi IA, Letarte J, Ouellet R, Batshaw ML, Brusilow S (1984) Treatment of hyperargininemia with sodium benzoate and arginine-restricted diet. *J Pediatr* 104: 473–476.

How to cite this article: Häberle J, Burlina A, Chakrapani A, et al. Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. *J Inherit Metab Dis*. 2019;42: 1192–1230. https://doi.org/10.1002/jimd.12100