Genetics in Medicine (2024) 26, 101039

ELSEVIER

Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ARTICLE

Severity-adjusted evaluation of liver transplantation on health outcomes in urea cycle disorders

Roland Posset[1,*], Sven F. Garbade[1], Florian Gleich[1], Svenja Scharre[1], Jürgen G. Okun[1], Andrea L. Gropman[2], Sandesh C.S. Nagamani[3], Ann-Catrin Druck[1], Friederike Epp[1], Georg F. Hoffmann[1], Stefan Kölker[1], Matthias Zielonka[1,*]; on behalf of the Urea Cycle Disorders Consortium (UCDC) and the European registry and network for Intoxication type Metabolic Diseases (E-IMD) Consortia Study Group

ARTICLE INFO

Article history:
Received 23 August 2023
Received in revised form
29 November 2023
Accepted 30 November 2023
Available online 3 December 2023

Keywords:
Argininosuccinic aciduria
Citrullinemia type 1
Liver transplantation
Ornithine transcarbamylase deficiency
Urea cycle disorders

ABSTRACT

Purpose: Liver transplantation (LTx) is performed in individuals with urea cycle disorders when medical management (MM) insufficiently prevents the occurrence of hyperammonemic events. However, there is a paucity of systematic analyses on the effects of LTx on health-related outcome parameters compared to individuals with comparable severity who are medically managed.

Methods: We investigated the effects of LTx and MM on validated health-related outcome parameters, including the metabolic disease course, linear growth, and neurocognitive outcomes. Individuals were stratified into "severe" and "attenuated" categories based on the genotype-specific and validated *in vitro* enzyme activity.

Results: LTx enabled metabolic stability by prevention of further hyperammonemic events after transplantation and was associated with a more favorable growth outcome compared with individuals remaining under MM. However, neurocognitive outcome in individuals with LTx did not differ from the medically managed counterparts as reflected by the frequency of motor abnormality and cognitive standard deviation score at last observation.

Conclusion: Whereas LTx enabled metabolic stability without further need of protein restriction or nitrogen-scavenging therapy and was associated with a more favorable growth outcome, LTx—as currently performed—was not associated with improved neurocognitive outcomes compared with long-term MM in the investigated urea cycle disorders.

The Article Publishing Charge (APC) for this article was paid by Heidelberg University.

Additional authors and members of the UCDC and E-IMD Consortia Study Group are listed at the end of the manuscript and in Supplemental Table 3.

*Correspondence and requests for materials should be addressed to Roland Posset, Center for Pediatric and Adolescent Medicine, Division of Pediatric Neurology and Metabolic Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany. *Email address:* roland.posset@med.uni-heidelberg.de OR Matthias Zielonka, Center for Pediatric and Adolescent Medicine, Division of Pediatric Neurology and Metabolic Medicine, University Hospital Heidelberg, Im Neuenheimer Feld 430, 69120 Heidelberg, Germany. *Email address:* matthias.zielonka@med.uni-heidelberg.de

Affiliations are at the end of the document.

doi: https://doi.org/10.1016/j.gim.2023.101039

Introduction

Urea cycle disorders (UCDs) are rare inborn errors of metabolism with an estimated cumulative incidence of 1 in 35,000 to 52,000, presenting with a broad phenotypic spectrum ranging from neonatal life-threatening hyperammonemic events (HAEs) to a more variable phenotype with chronic disease burden, such as recurrent vomiting, behavioral, or psychiatric abnormalities and cognitive impairment, with or without HAEs, any time after the neonatal period.[1-3] The principles of long-term (conservative) medical management (MM) consist of a protein-restricted diet—supplemented with essential and/or selective amino acids, such as L-arginine and/or L-citrulline—and alternative pathway medications for nitrogen-scavenging, such as sodium benzoate, sodium phenylbutyrate, and glycerol phenylbutyrate.[4,5] Liver transplantation (LTx) is mainly performed when MM fails to ensure metabolic stability by preventing recurrent hyperammonemic decompensations.[4] Intervention-related outcome parameters in individuals that have undergone LTx have significantly improved with an overall 5-year patient survival rate of over 90% and a 5-year graft survival rate of over 85%.[6,7] Moreover, clinical experiences demonstrate the clear impact of LTx on metabolic stability, prevention of recurrent HAEs, and liberalization of protein intake.[4,8-10]

Currently, there are limited data on the effects of LTx on other health-related outcomes in UCDs, including growth and neurocognitive outcomes. Whereas some data suggest that LTx should be considered for individuals with both a severe and mild (neonatal) phenotype for neuroprotective reasons,[7,11-14] others have not shown a conclusive positive impact of LTx on the neurodevelopmental outcomes.[15,16] Thus far, conducting a systematic analysis on the impact of LTx on health-related outcome parameters in UCDs compared with individuals who undergo MM has been hampered by (1) ethical issues in conducting a randomized controlled trial, (2) an inability to propensity-match between LTx and MM groups because individuals who undergo transplantation might have more severe disease, (3) the lack of available validated methods that can be used to appropriately categorize severity in individuals with UCDs, thus allowing a comparison between individuals with comparable disease severity, (4) use of categorical instead of numerical clinical covariates, which biases intra-individual reassessment, (5) limited numbers of individuals available for comparison given the rarity of UCDs, and (6) variability introduced by varying age ranges of the investigated participants.[7,11-14,16] To overcome some of these challenges, we had previously developed a novel severity-adjusted classification system based on *in vitro* residual enzymatic activities for the 3 most prevalent UCDs, ie, ornithine transcarbamylase-deficiency (OTC-D), citrullinemia type 1 (CTLN1), and argininosuccinic aciduria (ASA).[17-19] Altogether OTC-D, CTLN1, and ASA account for approximately 80% of LTx in UCDs[6,7] reflecting the overall prevalence of those 3 disorders in the untransplanted population.[20] By use of this novel severity-adjusted classification system, we evaluated the impact of LTx on health-related outcome parameters in a pediatric cohort from the United States, Canada, and Europe, which has been longitudinally followed for approximately 2 decades.

Abbreviations

ASA – argininosuccinic aciduria
ASL – argininosuccinate lyase
ASS1 – argininosuccinate synthetase 1
BCAA(s) – branched-chain amino acid(s)
cSDS – cognitive standard deviation score
CTLN1 – citrullinemia type 1
E-IMD – European registry and network for Intoxication type Metabolic Diseases
HAE(s) – hyperammonemic event(s)
IQR – interquartile range
LME – linear mixed effect regression models
LTx – liver transplantation
MM – (conservative) medical management
(m)OTC-D – (male) OTC-deficiency
$NH_4^+{}_{max}$ – initial peak plasma ammonium concentration
OTC – ornithine transcarbamylase
SDS – standard deviation score
UCD(s) – urea cycle disorder(s)
UCDC – Urea Cycle Disorders Consortium

Material and Methods

Eligibility criteria and study sample

Recently, the characterization of the combined cohort of individuals with UCDs prospectively followed by the Urea Cycle Disorders Consortium (UCDC) and the European registry and network for Intoxication type Metabolic Diseases (E-IMD) has been published,[14,21] with a particular focus on the 3 most prevalent UCDs, ie, OTC-D, CTLN1, and ASA.[17-19] The study design and data models of both registries have previously been described.[14,21-23] For analyses presented here, only individuals from the UCDC and E-IMD databases with confirmed diagnosis of male OTC-D (mOTC-D), CTLN1, and ASA on whom information on initial peak plasma ammonium concentration ($NH_4^+{}_{max}$) was available for the first HAE were included. Data were retrieved from the UCDC and E-IMD electronic databases as previously described.[24] A combined and comparative data analysis of both databases was performed according to the 3 principles of Interoperability, Representativeness, and Severity-adjustment.[25]

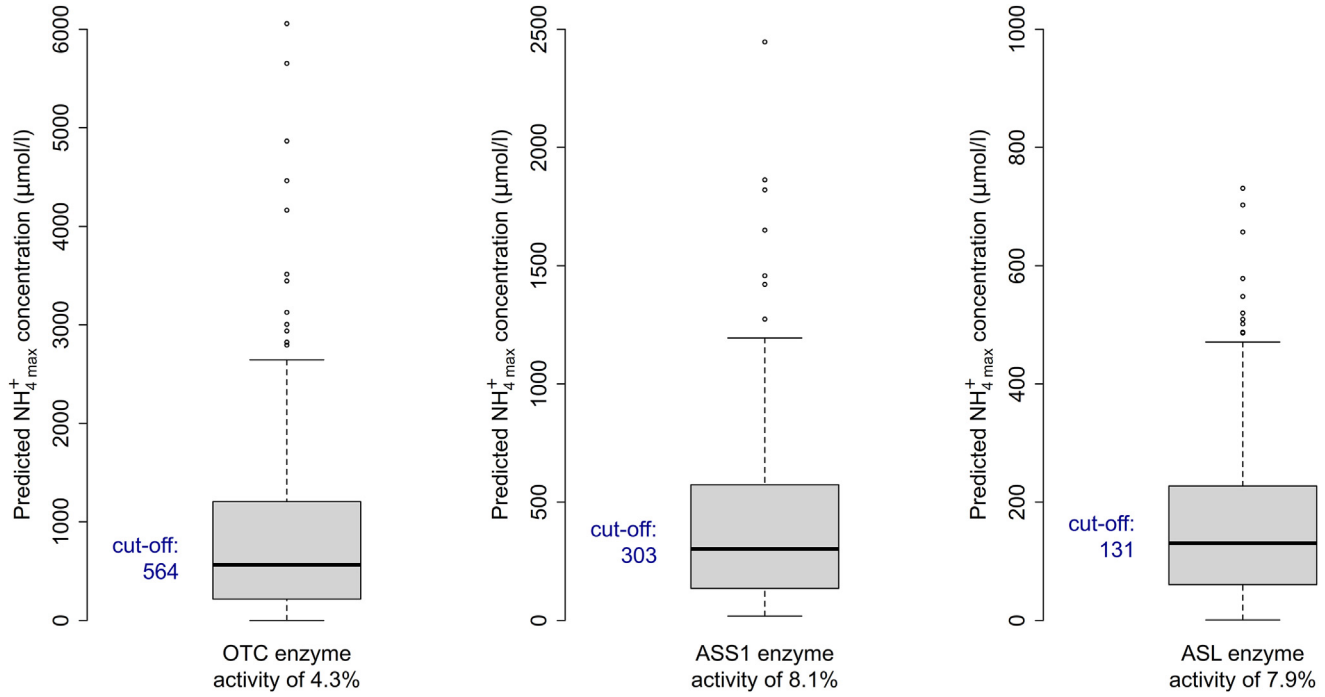

Figure 1 Post-hoc statistical posterior simulation based on the published regression functions between residual enzymatic activity and $NH_4^+{}_{max}$ for the respective UCD subtypes. Predicted $NH_4^+{}_{max}$ (50th centile; in µmol/L) correspond to the disease-specific threshold values of residual enzymatic activities differentiating individuals with a severe or attenuated phenotype with mOTC-D, CTLN1, and ASA. $NH_4^+{}_{max} \geq 564$ µmol/L (mOTC-D), or ≥ 303 µmol/L (CTLN1), or ≥ 131 µmol/L (ASA) are defined as cutoff values corresponding to residual enzymatic acitivity of $\leq 4.3\%$ (mOTC-D), or $\leq 8.1\%$ (CTLN1), or $\leq 7.9\%$ (ASA) and therefore as severe phenotypes. Data are shown as median (black thick line) corresponding to the cutoff value for the surrogate parameter $NH_4^+{}_{max}$, length of the box corresponds to interquartile range (IQR), upper and lower whiskers correspond to max. 1.5 × IQR, each point represents a simulated outlier. ASL, argininosuccinate lyase; ASS1, argininosuccinate synthetase 1; OTC, ornithine transcarbamylase.

Stratification of individuals with mOTC-D, CTLN1, and ASA according to their underlying phenotypic severity

Individuals with mOTC-D, CTLN1, and ASA were stratified into 2 groups according to previously described models reliably predicting individual disease severity based on the underlying pathogenic variant(s) in *OTC*, *ASS1*, or *ASL*.[17-19] The phenotypic severity of individuals with residual enzymatic OTC, ASS1, and ASL activities as assessed by our novel, genotype-specific *in vitro* system below or equal to the threshold values of 4.3% (for mOTC-D), 8.1% (for CTLN1), and 7.9% (for ASA), respectively, were classified as "severe," whereas individuals with residual enzymatic activities above these cutoff values were classified as having an "attenuated" disease course. Given the strong correlation of $NH_4^+{}_{max}$ with the underlying residual enzymatic activities,[17-19] a post-hoc posterior simulation was used by applying the mathematical correlation functions of $NH_4^+{}_{max}$ with the residual enzymatic activities (ie, OTC, ASS1, and ASL) to determine estimated $NH_4^+{}_{max}$ values corresponding to the respective enzymatic cutoff values for phenotype differentiation. For this purpose, 200 predicted $NH_4^+{}_{max}$ values from fitted generalized additive models were drawn. The following $NH_4^+{}_{max}$ values were identified (50th centile): 564 µmol/L for OTC-D (corresponding to residual enzymatic OTC activity of 4.3%), 303 µmol/L for CTLN1 (corresponding to residual enzymatic ASS1 activity of 8.1%), and 131 µmol/L for ASA (corresponding to residual ASL activity of 7.9%; Figure 1). $NH_4^+{}_{max}$ values corresponding to the 75th centile were as follows: 1204 µmol/L for OTC-D, 571 µmol/L for CTLN1, and 228 µmol/L for ASA.

Subsequently, $NH_4^+{}_{max}$ as surrogate marker for residual enzymatic OTC, ASS1, or ASL activity was used for group assignment of probands to their underlying phenotypic severity (ie, severe or attenuated) in order to increase the number of included individuals in these analyses. Moreover, post-hoc statistical analyses excluded additional subgroups within both phenotypic severity groups with regard to HAEs for each UCD subtype.

Biochemical and clinical variables used for data analysis

The following numerical biochemical and clinical variables were included in data analyses: (1) Peak plasma ammonium concentration at initial hyperammonemic decompensation ($NH_4^+{}_{max}$) and (2) number of HAEs ($NH_4^+{}_{max} > 100$ µmol/L) per year during the observation period (ie, time between the date of birth and the last study visit). Anthropometric

data of European probands were compared with growth charts from the United Kingdom, because the ethnic background of the European cohort corresponds well to that of individuals in the United Kingdom.[1,26] For North American probands growth charts from the Center for Disease Control and Prevention (https://www.cdc.gov/growthcharts/cdc_charts.htm) were used. Z-scores for height and weight were calculated at baseline and each scheduled longitudinal research follow-up visit. Preterm infants (< 37th pregnancy week) and z-scores < -3 or > 3 were excluded from data analysis. Motor abnormality was used as superordinate dichotomous variable (yes/no), including several motor variables (dystonia, chorea, ataxia, spasticity, abnormal gross or fine motor function, delayed milestones, and muscular hypotonia or hypertonia) similar to a previously published work.[20] Neurocognitive outcome was assessed using cognitive standard deviation scores (SDS) at the most recent study visit. Cognitive SDS (cSDS) was calculated using full-scale IQ values from Wechsler Abbreviated Scale of Intelligence, Wechsler Intelligence Scale for Children, Wechsler Preschool and Primary Scale of Intelligence, implementing results of the mental developmental index, cognitive scale from the Bayley Scales of Infant Development, and the general adaptive composite from the Adaptive Behavior Assessment System as previously described.[17-19]

Comparative analysis of LTx on evidence-based and validated health-related outcome parameters

The effect of LTx vs MM was systematically assessed within each severe and attenuated phenotypic group. The following validated and previously published outcome parameters were used: (1) metabolic disease course,[17-19,24] (2) linear growth,[27] (3) neurological outcome,[20] and (4) cognitive function.[14,17-19]

Statistical analysis

All statistical analyses were performed using "R," a language for statistical computing and graphics, Version 4.3.1 (Core Team, R: A language and environment for statistical computing. 2024. The R Foundation). For descriptive statistics, mean, standard deviation, median, interquartile range, and range were calculated, until otherwise stated. Count data were analyzed with a χ^2-test. Group means were compared with paired t test or unpaired t test with Welch correction, and Holm's alpha error adjustment was applied when needed. Linear mixed effect regression models (LME) were used to compare an outcome (eg, results from full-scale IQ-tests [cSDS]) among 2 groups (eg, severe and attenuated phenotype) at different points in time (eg, before and after LTx). Post-hoc contrasts in LME were computed with estimated marginal means using Kenward-Roger estimation of degrees of freedom and Holm's alpha error adjustment method. We used R package "lme4" version 1.1-35.1 and package "emmeans" version 1.8.6 to compute LME and post-hoc contrasts.

Results

Description of the study cohort

Overall, 356 individuals with mOTC-D, CTLN1, and ASA were included in this analysis with 231 individuals (64.9%) categorized as severe phenotype and 125 individuals (35.1%) categorized as having an attenuated phenotype. Within the severe phenotype category, 72 individuals (31.1%) had undergone LTx. In the attenuated phenotype category, 10 individuals (8.0%) had undergone LTx. Detailed descriptive characteristics of subsequent analyses are depicted in Supplemental Tables 1 and 2.

LTx is associated with long-term metabolic stability

To investigate whether LTx is an effective intervention to alter the metabolic disease course, we studied the number of HAEs per year of observation in both severe and attenuated severity categories. Although both individuals with a severe and attenuated phenotype who underwent LTx during their disease course suffer more often from HAEs before LTx compared with their medically managed counterparts (severe phenotype before LTx: $n = 56$, mean HAEs: 3.06 vs severe phenotype MM group: $n = 99$, mean HAEs: 0.57; attenuated phenotype before LTx: $n = 9$, mean HAEs: 2.30 vs attenuated phenotype MM group: $n = 63$, mean HAEs: 0.33; each $P < .05$; Welch Two Sample t test), LTx was associated with long-term metabolic stability after the intervention (severe phenotype after LTx: $n = 56$, mean HAEs: 0.00 vs severe phenotype MM group: $n = 99$, mean HAEs: 0.57; attenuated phenotype after LTx: $n = 9$, mean HAEs: 0.00 vs attenuated phenotype MM group: $n = 63$, mean HAEs: 0.33; each $P < .001$; Welch Two Sample t test). To study whether this effect also holds true for a balanced observation period between both groups (LTx vs MM), we calculated the mean age at LTx (severe phenotypic category: 3.1 years, attenuated phenotypic category: 4.8 years) and divided the MM cohort into a group before and another group after the corresponding mean age at LTx. Intriguingly, analyses revealed that for both severe and attenuated phenotypic categories the number of HAEs before LTx is higher in the LTx group than in the respective MM group. Moreover, LTx was associated with sufficient prevention of metabolic decompensations without any subsequent HAEs after transplantation (Figure 2).

LTx is associated with a more favorable growth outcome compared with individuals who received MM

Recently, we have shown that individuals with UCDs are at risk of progressive growth impairment that is directly associated with phenotypic severity and could even be independent from the degree of therapeutic protein

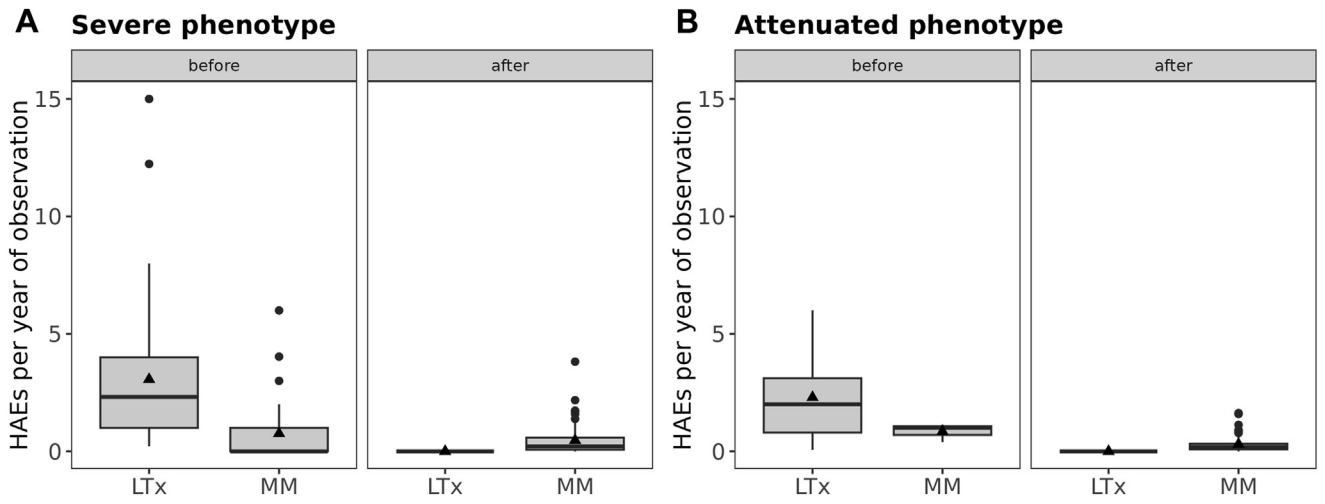

Figure 2 LTx is associated with a sustained metabolic stability without any HAEs after transplantation. A. Boxplot illustrating number of HAEs for individuals with a severe phenotype receiving LTx ($n = 56$) or MM before ($n = 35$) and after ($n = 64$) the respective mean age at transplantation. Data are shown as median (black thick line) and mean (triangle), length of the box corresponds to the interquartile range (IQR), upper and lower whiskers correspond to max. 1.5 × IQR, each point represents an outlier. Welch Two Sample *t* test, each $P < .001$. B. Boxplot illustrating number of HAEs for individuals with an attenuated phenotype receiving LTx ($n = 9$) or MM before ($n = 3$) and after ($n = 60$) the respective mean age at transplantation. Data are shown in analogy to Figure 2A. Welch Two Sample *t* test, $P = .058$ (for LTx vs MM before the respective mean age at transplantation), $P < .001$ (for LTx vs MM after the respective mean age at transplantation). LTx, liver transplantation; MM, medical management; HAE(s), hyperammonemic event(s). For descriptive characteristics see Supplemental Table 2.

restriction.[27] Thus, we assessed linear growth as reflected by height z-scores in individuals who had undergone LTx compared with MM in a severity-adjusted manner. In the severe phenotype category, individuals under MM before receiving a liver graft and those remaining under MM exhibited comparable height z-scores at first observation (mean height SDS: -0.68 LTx vs −0.74 MM; Figure 3). However, although individuals under MM showed a decline of linear growth until the end of the observation period (mean height SDS: −1.31), LTx was associated with a trend toward improved height SDS compared with the MM group at the end of the observation period (mean height SDS: −0.48 LTx vs −1.31 MM; $P = .052$; Welch Two Sample *t* test; Figure 3). Interestingly, weight remained unaffected and in a normal range independent from the therapeutic intervention throughout the whole observation period (Supplemental Figure 1). Comparative analysis of the attenuated phenotype was not possible because of low data density for this cohort.

LTx is not associated with reduced frequency of motor abnormality compared with MM

Given the fact, that the neurological long-term outcome in individuals with UCDs depends on the underlying disease severity,[20] we next investigated the effect of LTx in comparison with MM on the frequency of motor abnormalities. Interestingly, the proportion with motor abnormalities did not differ between individuals having undergone LTx and those remaining under MM for both severe ($P = .86$; χ^2-test) and attenuated phenotypes ($P = .31$; χ^2-test). Whereas 46.9% ($n = 23/49$) of individuals with a severe phenotype in the LTx cohort exhibited motor abnormalities at last observation, motor abnormality was present in 48.8% ($n = 20/41$) of individuals receiving MM. Comparable results were obtained for the attenuated phenotype with presence of motor abnormalities in 28.6% ($n = 2/7$) in the LTx and 13.7% ($n = 7/51$) in the MM cohort (Figure 4).

LTx—as currently performed—and long-term MM do not differ with regard to the cognitive outcome

A previous analysis from the UCDC and E-IMD Consortia Study Group suggested that timely LTx might improve cognitive outcomes in individuals with UCDs. However, those preliminary data were neither age-adjusted nor subject to a severity-adjusted classification.[14] We compared age- and severity-adjusted individuals at last observation (severe phenotypic category: LTx vs MM: $P = .34$ for age, Welch Two Sample *t* test; attenuated phenotypic category: LTx vs MM: $P = .58$ for age, Welch Two Sample *t* test) and found that the cognitive outcome did not differ between LTx and long-term MM neither in the severe phenotypic category (mean cSDS for the LTx group: −2.2, mean cSDS for the MM group: −2.2, $P = 1.00$, Welch Two Sample *t* test) nor in the attenuated phenotypic category (mean cSDS for the LTx group: −1.1, mean cSDS for the MM group: −0.5, $P = .61$, Welch Two Sample *t* test). Overall, individuals

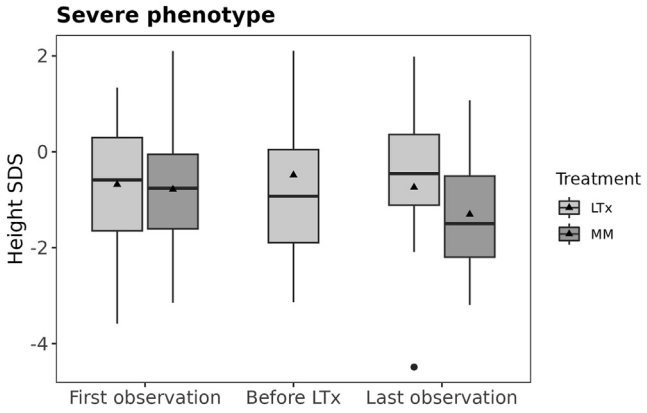

Figure 3 LTx is associated with a more favorable growth outcome when compared with individuals receiving MM. Boxplot illustrating height SDS for individuals with a severe phenotype at first observation (for liver-transplanted [$n = 26$] and medically managed [$n = 24$] individuals), before LTx (for liver-transplanted individuals [$n = 26$]) and at last observation (for liver-transplanted [$n = 26$] and medically managed [$n = 24$] individuals). Mean observation periods between first and last observation correspond to 6.71 years for liver-transplanted and 7.45 years for medically managed individuals. Whereas at first observation, height SDS does not differ between the LTx and MM group ($P = .86$), LTx is associated with a trend toward improved height SDS at last observation when compared with the MM group ($P = .052$). Data are shown as median (black thick line) and mean (triangle), length of the box corresponds to the interquartile range (IQR), upper and lower whiskers correspond to max. 1.5 × IQR, and each point represents an outlier. LTx, liver transplantation; MM, medical management. For descriptive characteristics see Supplemental Table 2.

with a severe phenotype suffer in mean from intellectual disability, whereas those with an attenuated phenotype have in mean normal cognitive function or learning disabilities (Figure 5). Disease-specific analyses revealed analogous results (Supplemental Figures 2-4).

Importantly, repetition of above depicted analyses with $NH_4^+{}_{max}$ cutoff values corresponding to 75th centile (see Materials and Methods) could confirm all presented results with exception of the trend toward improved height SDS associated with LTx in comparison with the MM group at the end of the observation period.

Discussion

Based on a recently established genotype-specific severity classification system for UCDs, we were able to stratify individuals with mOTC-D, CTLN1, and ASA according to the underlying functional disease burden and thus to perform a comparative analysis of the effect of LTx compared with MM on metabolic stability, growth, and neurocognitive outcomes. The present analysis reveals 4 main results: (1) LTx enables sustained metabolic stability with efficient prevention of any subsequent HAEs after transplantation. (2) LTx is associated with a more favorable growth outcome compared with individuals remaining under MM. However, (3) LTx is not associated with reduced frequency of motor abnormality, (4) nor is it associated with an altered cognitive long-term outcome when compared with MM.

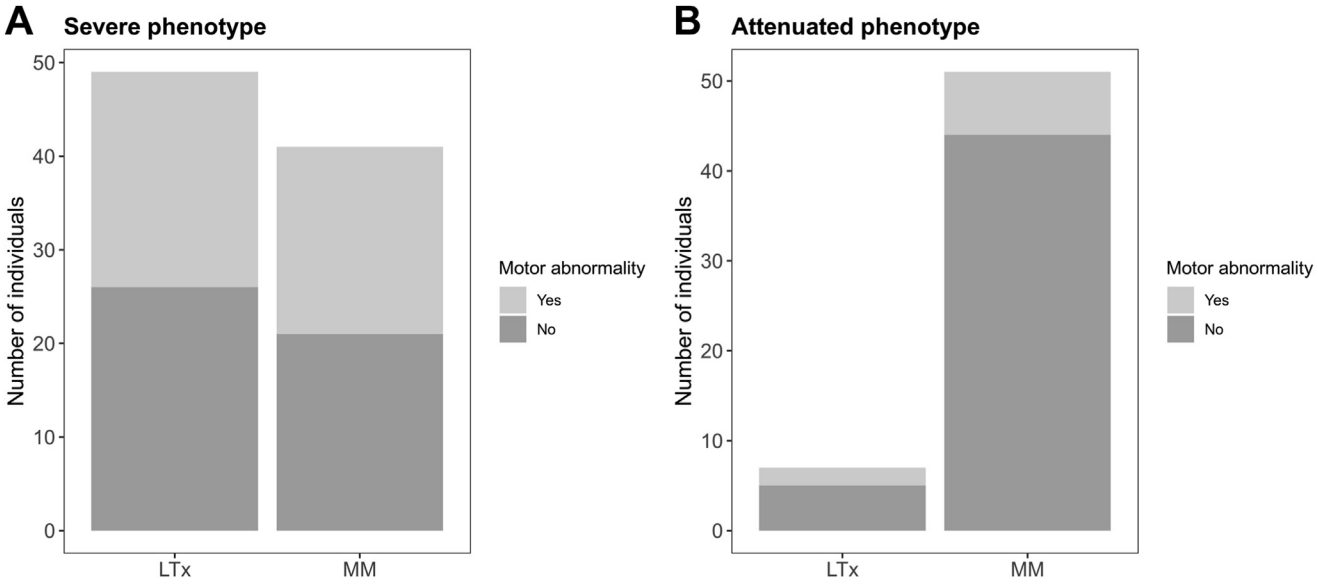

Figure 4 Motor abnormality is independent from the therapeutic intervention but is determined by the underlying disease severity. Boxplot illustrating the presence and absence of motor abnormality for individuals with a severe (A) or attenuated (B) phenotype. Bright shading corresponds to the presence, dark shading corresponds to the absence of motor abnormalities at last observation. Both severe (LTx: $n = 23/49$; MM: $n = 20/41$; $P = .86$, χ^2-test) and attenuated (LTx: $n = 2/7$; MM: $n = 7/51$; $P = .31$, χ^2-test) phenotypes are unaffected by the therapeutic intervention with regard to the presence of motor abnormalities. LTx, liver transplantation; MM, medical management. For descriptive characteristics see Supplemental Table 2.

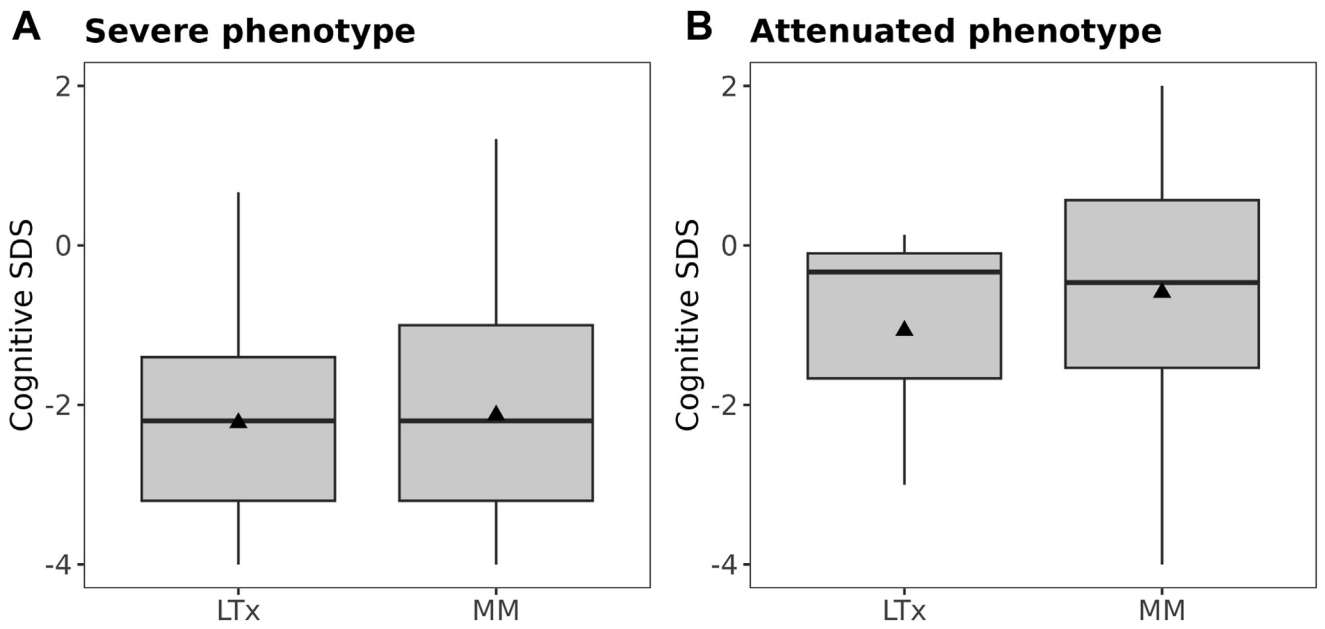

Figure 5 LTx and long-term MM do not differ with regard to the cognitive outcome. A. Boxplot depicting cognitive SDS at last follow-up testing for age-adjusted individuals with a severe phenotype receiving LTx ($n = 37$) or MM ($n = 34$). Data are shown as median (black thick line) and mean (triangle), length of the box corresponds to the interquartile range (IQR), upper and lower whiskers correspond to max. 1.5 × IQR, and each point represents an outlier. Welch Two Sample t test, $P = 1.00$. B. Boxplot depicting cognitive SDS at last follow-up testing for age-adjusted individuals with an attenuated phenotype receiving LTx ($n = 3$) or MM ($n = 53$). Data are shown in analogy to Figure 5A. Welch Two Sample t test, $P = .61$. LTx, liver transplantation; MM, medical management. For descriptive characteristics see Supplemental Table 2.

Quality of data analysis strategy

Previous analyses aiming to evaluate the impact of LTx on the health-related outcomes in individuals with UCDs often faced the challenge of using categorical instead of numerical (cognitive) variables, and limitations both in sample size and intra-individual reassessment. Moreover, methodological challenges regarding age and especially severity adjustment—recently carried out by arbitrary clinical, biochemical, or mathematical classification systems—resulted in the unavailability of a long-term medically managed group of comparable severity for systematic comparative analyses.[12-14,16] By establishment of 3 disease prediction models based on the genotypes for mOTC-D, CTLN1, and ASA and by identifying cutoff values for severe and attenuated phenotypes,[17-19] we were able to establish a novel severity-adjusted classification system reflecting the intrinsic disease burden that served as pivotal tool for the establishment of a validated severity-adjusted control group that was medically managed and comparable to individuals who received a liver graft. Furthermore, longitudinal data from the UCDC and E-IMD Consortia Study Group enabled analysis of a relevant number of age-adjusted individuals with numerical (cognitive) outcome parameters, longitudinally and individually reassessed, if possible. This research strategy allowed to reliably assess the health-related outcome parameters.

From bench to bedside and back—clues for disease modifiers with regard to number of HAEs alongside the disease course

The effect of LTx on the metabolic disease course has been extensively investigated in individuals with UCDs with unequivocal and conclusive results showing that LTx leads to sustained metabolic stability with efficient prevention of recurrent hyperammonemic decompensations without need of further protein restriction or nitrogen-scavenger therapy after transplantation.[4,8-10,13,16] This study confirms those results. However, equal observation periods applying the mean age at transplantation for dividing the MM group in respective cohorts before and after transplantation revealed that individuals receiving a liver graft during the disease course exhibited a higher number of HAEs before transplantation in comparison with individuals remaining under long-term MM independent from the underlying phenotypic severity. Given the fact, that individuals within a severity group (severe or attenuated) in general are confronted with an equal number of HAEs as shown by post-hoc analysis, the present results imply that in a specific subset of individuals (ie, receiving a liver graft) the number of HAEs is not associated with the degree of enzymatic dysfunction but is rather determined by additional modifying pathomechanistic factors making those individuals prone to metabolic decompensations (eg, during catabolism).

Future research is needed to identify those (potentially genetic) disease modifiers, eg, by exome, transcriptome, or proteome analyses, in order to evaluate whether those can be therapeutically addressed at all (before a potential transplantation).

LTx is associated with a more favorable growth outcome when compared with individuals who underwent long-term MM

Recently, it was shown that individuals with UCDs under medical long-term therapy suffer from relevant postnatal linear growth retardation, which was independent from the degree of therapeutic protein restriction but was associated with the underlying phenotypic severity.[27] Moreover, reduced to borderline-low plasma branched-chain amino acid (BCAA) concentrations were observed in those individuals that are prone to postnatal growth impairment.[27] Reduced BCAA concentrations are a biochemical hallmark in acute and/or chronic hyperammonemic conditions and maintaining stable plasma BCAA concentrations is crucial to stimulate growth.[28,29] Furthermore, therapy with sodium phenylbutyrate is also associated with decreased plasma BCAA concentrations.[30] Previous data suggest that LTx appears to have a beneficial effect on growth and is associated with normal plasma BCAA concentrations by prevention of recurrent or chronic hyperammonemic conditions after transplantation.[24] However, the effect of LTx on postnatal growth was not yet compared with a medically managed and severity-adjusted control cohort. The current analysis reveals that LTx is associated with a more favorable postnatal growth compared with individuals otherwise receiving MM, which was already evident after an observation period of only 7 years. This positive effect of LTx on postnatal linear growth might be (partially) explained by the normalization of BCAA concentrations after LTx[24] because normalization of plasma ammonium concentration (after LTx) stops enhanced consumption of BCAAs via increased propionate oxidation to supply the tricarboxylic acid cycle with carbon backbones otherwise present under acute and chronic hyperammonemic conditions.[31-35] Moreover, cessation of nitrogen scavenging therapy (ie, especially sodium phenylbutyrate) after LTx might add on the normalization of BCAA concentrations. Importantly, LTx is not only associated with cessation of postnatal linear growth impairment but is also associated with the maintenance of growth parameters within the normal range.

LTx—as currently performed—and long-term MM do not differ with regard to the neurocognitive outcome as reflected by cSDS

This study reveals that LTx (currently performed at a mean age of 3.1 year for the severe phenotypic category and 4.8 years for the attenuated phenotypic category) was not associated with better neurocognitive outcomes in comparison with MM as reflected by the unaltered frequency of motor abnormality and cognitive SDS at last observation. These results are consistent with recently reported data from a questionnaire-based nationwide study from Japan, which could not find a difference in cognitive outcome in individuals with UCDs affected by a severe neonatal disease manifestation, and with a Patient-Centered Outcome Research Institute study from the US.[15,16] However, previous analyses implied a positive effect of LTx on the cognitive outcome, but data were neither appropriately severity- nor age-adjusted and relied on categorical variables, which might have introduced bias into those investigations.[7,11-14]

The observation of unaltered cognitive outcome might be caused by the long-term deleterious impact of hyperammonemic encephalopathy at initial disease manifestation, which usually is the most severe hyperammonemic decompensation during the disease course. Neurocognitive impairment might therefore be a long-term sequelae of $NH_4^+{}_{max}$ and/or subsequent metabolic decompensations that might not be sufficiently altered by any of the currently applicable therapeutic strategies. Moreover, cytosolic UCDs (ie, CTLN1 and ASA) are characterized by a neurodegenerative disease course with progressive cognitive decline in a subset of individuals. In this regard, it was recently shown that individuals with CTLN1 and ASA may exhibit cognitive impairment independent from the initial $NH_4^+{}_{max}$,[14] implying additional pathomechanisms underlying cognitive dysfunction, such as neurotoxicity of argininosuccinic acid and guanidinosuccinic acid in ASA.[36,37] Recently, ASL was shown to be expressed in the locus coeruleus and dopaminergic neurons of the substantia nigra of the mammalian CNS with key functions in the regulation of catecholamine synthesis.[38,39] Tissue-specific ASL deficiency in the locus coeruleus is associated with reduced tyrosine hydroxylase activity with consecutively diminished catecholamine synthesis causing increased seizure reactivity.[38] Moreover, loss of ASL in $ALDH1A1^+$ neurons of the substantia nigra, a subpopulation that is pivotal for the pathogenesis of Parkinson disease, resulted in the formation of tyrosine aggregates and elevated alpha-synuclein levels.[39] Intriguingly, ASS1, the deficient enzyme in CTLN1, has been identified to exert RNA-binding properties involved in post-transcriptional gene regulation; thus, pathogenic variants in the *ASS1* gene could potentially interfere with RNA metabolism.[40] Those cerebral ammonia-independent pathomechanisms contributing to the neurodegenerative disease course in cytosolic UCDs are likely not addressed by LTx, which might provide an additional explanation for the unaltered neurocognitive outcome despite prevention of subsequent hyperammonemic episodes after transplantation.

Whether LTx at the earliest (technically) possible time point might improve the neurocognitive outcome by

prevention of neurological sequelae of recurrent hyperammonemic decompensations during the most vulnerable phase in brain development needs to be elucidated in future research.

Limitations and directions for future research

This study has some inherent limitations. First, the effect of LTx on mortality rates in mOTC-D, CTLN1, and ASA could not be investigated because of the low number of deceased individuals in the study sample. Moreover, because of the lack of appropriate disease prediction models, further UCD subtypes could not yet be included in this analysis. Second, systematic longitudinal studies involving cerebral MRI are indispensable to assess the long-term effect of LTx on further outcome parameters, such as morphological signs of brain damage or spectroscopic alterations *in vivo*. Furthermore, the lack of important covariates, such as availability of medical care, varying medication protocols, or adherence to dietary management, which could not be assessed in this study might have had an impact on overall outcomes. Importantly, it was not the aim of this study to evaluate intervention-related outcome (survival rates relating to the transplantation intervention, frequency of graft failure, complications, etc.) or quality of life. Moreover, important covariates such as presence, duration, and severity of coma during metabolic decompensation(s), as well as epilepsy, could not be included in this study because of low data density and quality of entered data elements in both registries. Their potential association with the underlying phenotypic severity and effect on the cognitive outcome remain a subject of future research optimally performed in the context of prospective clinical trials. In perspective, future analyses will be able to replace $NH_4^+{}_{max}$ as surrogate parameter for residual enzymatic activity once genetic testing is carried out for all individuals with UCDs followed by determination of residual enzymatic activity.

Conclusion

This age- and severity-adjusted comparative evaluation of LTx vs MM for the 3 most prevalent UCDs, ie, mOTC-D, CTLN1, and ASA, shows that LTx is associated with sustained metabolic stability without occurrence of any metabolic decompensations after transplantation. Interestingly, results imply additional disease modifiers leading to a higher number of HAEs for individuals receiving a liver graft before the intervention when compared with medically managed counterparts. LTx is associated with a more favorable growth outcome when compared with individuals under long-term MM. However, LTx —as currently performed— and long-term MM do not differ with regard to the neurocognitive outcome.

Data Availability

The UCDC database is registered at the US National Library of Medicine (https://clinicaltrials.gov), whereas the E-IMD registry is recorded on the German Clinical Trials Register (https://www.drks.de). Because of existing data protection laws, the data sets generated and analyzed during this study are not publicly available. Data ownership is retained by the members of the UCDC and E-IMD Consortia Study Group. Data availability is subject to the consent of both consortia upon request.

Acknowledgments

The authors thank the following study coordinators: Saima Ali, Kia Bryan, Liora Caspi, Kiaira Coles, Sara Elsbecker, Debbie Fu, Florian Gleich, Seishu Horikoshi, Elijah Kravets, Genya Kisin, Rhonda Jones, Levonne Phillip, Thu Quan, Kara Simpson, Julia Sloan, Tamar Stricker, Hayden Vreugdenhil, Ashley Wilson, and Melissa Zerofsky—and study neuropsychologists—Fabienne Dietrich Alber, Elizabeth Kerr, Casey Krueger, Eva Mamak, Ami Norris-Brilliant, David Schwartz, Arianna K. Stefanatos, Rachel Tangen, and Magdalena E. Walter. The authors would also like to acknowledge the contributions of (former) longitudinal study PIs: Mark L. Batshaw, Stephen Cederbaum, Annette Feigenbaum, Brendan Lee, Uta Lichter-Konecki, Margretta R. Seashore, and Marshall L. Summar. Moreover, the authors thank Dr Carmen Capito, and Dr Florence Lacaille for their contribution. The authors are grateful for the contributions and leadership of the late Cynthia LeMons. Ms LeMons served as a Co-PI for the UCDC and the Executive Director for the National Urea Cycle Disorders Foundation. In particular, we are indebted to all our individuals with UCDs and their families for their trust, patience, and participation in both longitudinal registry studies for many years.

Funding

The Urea Cycle Disorders Consortium (UCDC; U54HD061221) is part of the National Institutes of Health Rare Disease Clinical Research Network, supported through collaboration between the Office of Rare Diseases Research, the National Center for Advancing Translational Science and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The UCDC is also supported by the O'Malley Foundation, the Rotenberg Family Fund, the Dietmar Hopp Foundation, the Kettering Fund, and the National Urea Cycle Disorders Foundation. In addition, support for neuropsychological testing is provided by a National Institutes of Health grant for

Intellectual and Developmental Disability Research Centers (U54HD090257). This work was also supported in part by the Clinical Translational Core at Baylor College of Medicine, which is supported by the IDDRC grant number P50 HD103555 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The E-IMD patient registry has received funding by the European Union (E-IMD; EAHC no 2010 12 01; coordinator: Stefan Kölker), in the framework of the Health Programme. After the end of the EU funding period the E-IMD patient registry has been sustained by funding from the Kindness-for-Kids Foundation (Munich, Germany) and the Kettering Fund. This work was supported by a Trainee Research Fellowship Award 2022-2023 provided by the UCDC. S.K. and G.F.H. received funding from the Dietmar Hopp Foundation for coordinating the study "Newborn Screening and Metabolic Medicine 2020 (NBS2020)", which included individuals with UCDs.

Author Information

Supplemental Table 3 Conceptualization: R.P., M.Z.; Data Curation: S.F.G., F.G., UCDC, E-IMD; Formal Analysis: R.P., S.F.G., F.G., S.S., J.G.O., A.L.G., S.C.S.N., A.-C.D., F.E., G.F.H., S.K., M.Z., UCDC, E-IMD; Methodology: R.P., S.F.G., F.G., S.S., J.G.O., A.L.G., S.C.S.N., A.-C.D., F.E., M.Z.; Investigation: R.P., S.F.G., F.G., F.G., S.S., J.G.O., A.L.G., S.C.S.N., A.-C.D., F.E., G.F.H., S.K., M.Z., UCDC, E-IMD; Resources: J.G.O., A.L.G., S.C.S.N., G.F.H., S.K.; Validation: R.P., S.F.G., F.G., M.Z.; Visualization: S.F.G., F.G.; Writing-original draft: R.P., M.Z.; Writing-review and editing: R.P., S.F.G., F.G., F.G., S.S., J.G.O., A.L.G., S.C.S.N., A.-C.D., F.E., G.F.H., S.K., M.Z., UCDC, E-IMD; Supervision: S.C.S.N., S.K.

Ethics Declaration

All procedures complied with the ethical standards of the Helsinki Declaration of 1975, as revised in 2013. Before enrollment to this study, written informed consent was given by the probands or their legal representatives. The respective UCDC and E-IMD study sites received written approval from an Institutional Review Board or Research Ethics Committee.

Conflict of Interest

Roland Posset receives consultancy fees from Immedica Pharma AB. Stefan Kölker receives funding from Immedica Pharma AB for the European Post-Authorization Registry for Ravicti (glycerol phenylbutyrate) oral liquid in partnership with the European registry and network for Intoxication type Metabolic Diseases (E-IMD) (EU PAS Register number EUPAS17267; http://www.encepp.eu/). Georg F. Hoffmann received lecture fees from Swedish Orphan Biovitrum GmbH. The sponsors have in no way influenced the design, conductance, analysis, and report of this study. All other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2023.101039) contains supplementary material, which is available to authorized users.

Members of UCDC consortium (to be listed in PubMed in alphabetical order)

Nicholas Ah Mew, Matthias R. Baumgartner, Gerard T. Berry, Susan A. Berry, Margo Breilyn, Lindsay C. Burrage, Curtis R. Coughlin 2nd, George A. Diaz, Gregory M. Enns, Can Ficicioglu, Renata C. Gallagher, Cary O. Harding, Laura Konczal, Christina Lam, Shawn E. McCandless, Andreas Schulze, Jennifer Seminara, Tamar Stricker, Greta Wilkening, and Derek Wong.

Members of E-IMD registry (to be listed in PubMed in alphabetical order)

Adrijan Sarajlija, Alberto B. Burlina, Allan M. Lund, Anastasia Skouma, Andrew A. Morris, Anupam Chakrapani, Carlo Dionisi-Vici, Chris Mühlhausen, Consuelo Pedrón-Giner, Daniela Karall, Dries Dobbelaere, Elisenda Cortès-Saladelafont, Francois Eyskens, Frits A. Wijburg, Gwendolyn Gramer, Ivo Barić, Javier Blasco-Alonso, Jiri Zeman, Jolanta Sykut-Cegielska, Margreet Wagenmakers, Maria L. Couce, Persephone Augoustides-Savvopoulou, Peter Freisinger, Peter M. van Hasselt, Roshni Vara, Ute Spiekerkoetter, Yin-Hsiu Chien.

Affiliations

[1]Heidelberg University, Medical Faculty Heidelberg, and Center for Pediatric and Adolescent Medicine, Department I, Division of Pediatric Neurology and Metabolic Medicine, University Hospital Heidelberg, Heidelberg, Germany; [2]Children's National Health System and The George Washington School of Medicine, Washington, DC; [3]Department of Molecular and Human Genetics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX

References

1. Kölker S, Garcia-Cazorla A, Valayannopoulos V, et al. The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the

initial presentation. *J Inherit Metab Dis*. 2015;38(6):1041-1057. http://doi.org/10.1007/s10545-015-9839-3
2. Lopes FF, Sitta A, de Moura Coelho D, et al. Clinical findings of patients with hyperammonemia affected by urea cycle disorders with hepatic encephalopathy. *Int J Dev Neurosci*. 2022;82(8):772-788. http://doi.org/10.1002/jdn.10229
3. Nettesheim S, Kölker 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. http://doi.org/10.1186/s13023-017-0661-x
4. 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(6):1192-1230. http://doi.org/10.1002/jimd.12100
5. Ribas GS, Lopes FF, Deon M, Vargas CR. Hyperammonemia in inherited metabolic diseases. *Cell Mol Neurobiol*. 2022;42(8):2593-2610. http://doi.org/10.1007/s10571-021-01156-6
6. 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. http://doi.org/10.1016/j.transproceed.2015.09.020
7. Ziogas IA, Wu WK, Matsuoka LK, et al. Liver transplantation in children with urea cycle disorders: the importance of minimizing waiting time. *Liver Transpl*. 2021;27(12):1799-1810. http://doi.org/10.1002/lt.26186
8. 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. http://doi.org/10.1002/lt.20587
9. Sood V, Squires JE, Mazariegos GV, Vockley J, McKiernan PJ. Living related liver transplantation for metabolic liver diseases in children. *J Pediatr Gastroenterol Nutr*. 2021;72(1):11-17. http://doi.org/10.1097/MPG.0000000000002952
10. 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. http://doi.org/10.1023/a:1005317909946
11. García Vega M, Andrade JD, Morais A, et al. Urea cycle disorders and indications for liver transplantation. *Front Pediatr*. 2023;11:1103757. http://doi.org/10.3389/fped.2023.1103757
12. Kido J, Matsumoto S, Mitsubuchi H, Endo F, Nakamura K. Early liver transplantation in neonatal-onset and moderate urea cycle disorders may lead to normal neurodevelopment. *Metab Brain Dis*. 2018;33(5):1517-1523. http://doi.org/10.1007/s11011-018-0259-6
13. Kido J, Matsumoto S, Momosaki K, et al. Liver transplantation may prevent neurodevelopmental deterioration in high-risk patients with urea cycle disorders. *Pediatr Transplant*. 2017;21(6). http://doi.org/10.1111/petr.12987
14. Posset R, Gropman AL, Nagamani SCS, et al. Impact of diagnosis and therapy on cognitive function in urea cycle disorders. *Ann Neurol*. 2019;86(1):116-128. http://doi.org/10.1002/ana.25492
15. Ah Mew NM R, Izem R, et al. *Comparing Treatment Options for Urea Cycle Disorders*. Patient-Centered Outcomes Research Institute (PCORI); 2020.
16. Kido J, Matsumoto S, Häberle J, et al. Role of liver transplantation in urea cycle disorders: report from a nationwide study in Japan. *J Inherit Metab Dis*. 2021;44(6):1311-1322. http://doi.org/10.1002/jimd.12415
17. Scharre S, Posset R, Garbade SF, et al. Predicting the disease severity in male individuals with ornithine transcarbamylase deficiency. *Ann Clin Transl Neurol*. 2022;9(11):1715-1726. http://doi.org/10.1002/acn3.51668
18. Zielonka M, Garbade SF, Gleich F, et al. From genotype to phenotype: early prediction of disease severity in argininosuccinic aciduria. *Hum Mutat*. 2020;41(5):946-960. http://doi.org/10.1002/humu.23983
19. Zielonka M, Kölker S, Gleich F, et al. Early prediction of phenotypic severity in citrullinemia type 1. *Ann Clin Transl Neurol*. 2019;6(9):1858-1871. http://doi.org/10.1002/acn3.50886
20. 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. http://doi.org/10.1007/s10545-016-9938-9
21. Posset R, Garbade SF, Boy N, et al. Transatlantic combined and comparative data analysis of 1095 patients with urea cycle disorders-A successful strategy for clinical research of rare diseases. *J Inherit Metab Dis*. 2019;42(1):93-106. http://doi.org/10.1002/jimd.12031
22. Kölker S, Dobbelaere D, Häberle J, et al. Networking across borders for individuals with organic acidurias and urea cycle disorders: the E-IMD consortium. *JIMD Rep*. 2015;22:29-38. http://doi.org/10.1007/8904_2015_408
23. Seminara J, Tuchman M, Krivitzky L, et al. Establishing a consortium for the study of rare diseases: the Urea Cycle Disorders Consortium. *Mol Genet Metab*. 2010;100(suppl 1):S97-S105. http://doi.org/10.1016/j.ymgme.2010.01.014
24. Posset R, Kölker S, Gleich F, et al. Severity-adjusted evaluation of newborn screening on the metabolic disease course in individuals with cytosolic urea cycle disorders. *Mol Genet Metab*. 2020;131(4):390-397. http://doi.org/10.1016/j.ymgme.2020.10.013
25. Posset R, Zielonka M, Gleich F, et al. The challenge of understanding and predicting phenotypic diversity in urea cycle disorders. *J Inherit Metab Dis*. 2023;46(6):1007-1016. http://doi.org/10.1002/jimd.12678
26. Cole TJ, Williams AF, Wright CM, RCPCH Growth Chart Expert Group. Revised birth centiles for weight, length, and head circumference in the UK-WHO growth charts. *Ann Hum Biol*. 2011;38(1):7-11. http://doi.org/10.3109/03014460.2011.544139
27. Posset R, Garbade SF, Gleich F, et al. Long-term effects of medical management on growth and weight in individuals with urea cycle disorders. *Sci Rep*. 2020;10(1):11948. http://doi.org/10.1038/s41598-020-67496-3
28. Lui JC, Garrison P, Baron J. Regulation of body growth. *Curr Opin Pediatr*. 2015;27(4):502-510. http://doi.org/10.1097/MOP.0000000000000235
29. Semba RD, Shardell M, Sakr Ashour FA, et al. Child stunting is associated with low circulating essential amino acids. *EBiomedicine*. 2016;6:246-252. http://doi.org/10.1016/j.ebiom.2016.02.030
30. Burrage LC, Jain M, Gandolfo L, et al. Sodium phenylbutyrate decreases plasma branched-chain amino acids in patients with urea cycle disorders. *Mol Genet Metab*. 2014;113(1–2):131-135. http://doi.org/10.1016/j.ymgme.2014.06.005
31. Kanamatsu T, Tsukada Y. Effects of ammonia on the anaplerotic pathway and amino acid metabolism in the brain: an ex vivo 13C NMR spectroscopic study of rats after administering [2-13C]] glucose with or without ammonium acetate. *Brain Res*. 1999;841(1-2):11-19. http://doi.org/10.1016/s0006-8993(99)01772-2
32. Probst J, Kölker S, Okun JG, et al. Chronic hyperammonemia causes a hypoglutamatergic and hyperGABAergic metabolic state associated with neurobehavioral abnormalities in zebrafish larvae. *Exp Neurol*. 2020;331:113330. http://doi.org/10.1016/j.expneurol.2020.113330
33. Shen J, Petersen KF, Behar KL, et al. Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo 13C NMR. *Proc Natl Acad Sci U S A*. 1999;96(14):8235-8240. http://doi.org/10.1073/pnas.96.14.8235
34. Zielonka M, Breuer M, Okun JG, Carl M, Hoffmann GF, Kölker S. Pharmacologic rescue of hyperammonemia-induced toxicity in zebrafish by inhibition of ornithine aminotransferase. *PLoS One*. 2018;13(9):e0203707. http://doi.org/10.1371/journal.pone.0203707
35. Zielonka M, Probst J, Carl M, Hoffmann GF, Kölker S, Okun JG. Bioenergetic dysfunction in a zebrafish model of acute hyperammonemic decompensation. *Exp Neurol*. 2019;314:91-99. http://doi.org/10.1016/j.expneurol.2019.01.008
36. Baruteau J, Jameson E, Morris AA, et al. Expanding the phenotype in argininosuccinic aciduria: need for new therapies. *J Inherit Metab Dis*. 2017;40(3):357-368. http://doi.org/10.1007/s10545-017-0022-x
37. Erez A, Nagamani SC, Shchelochkov OA, et al. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med*. 2011;17(12):1619-1626. http://doi.org/10.1038/nm.2544

38. Lerner S, Anderzhanova E, Verbitsky S, et al. ASL metabolically regulates tyrosine hydroxylase in the nucleus locus coeruleus. *Cell Rep*. 2019;29(8):2144-2153.e7. http://doi.org/10.1016/j.celrep.2019.10.043
39. Lerner S, Eilam R, Adler L, et al. ASL expression in ALDH1A1^{+} neurons in the substantia nigra metabolically contributes to neurodegenerative phenotype. *Hum Genet*. 2021;140(10):1471-1485. http://doi.org/10.1007/s00439-021-02345-5
40. Castello A, Fischer B, Eichelbaum K, et al. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell*. 2012;149(6):1393-1406. http://doi.org/10.1016/j.cell.2012.04.031