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Impairment of cognitive function in ornithine transcarbamylase deficiency is global rather than domain-specific and is associated with disease onset, sex, maximum ammonium, and number of hyperammonemic events

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Abstract

Beginning in 2006, the Urea Cycle Disorders Consortium (UCDC) has conducted a longitudinal study of eight inherited deficiencies of enzymes and transporters of the urea cycle, including 444 individuals with ornithine transcarbamylase deficiency (OTCD), of whom 300 (67 males, 233 females) received psychological evaluation. In a cross-sectional study (age range, 3–71 years), analysis of covariance (ANCOVA) determined the association between outcomes in five cognitive domains (global intelligence, executive functions, memory, visuomotor integration, visual perception) and sex, age at testing and timing of disease onset defined as early onset (≤28 days; EO), late onset (LO), or asymptomatic (AS). The dataset of 183 subjects with complete datasets (31 males, 152 females) revealed underrepresentation of EO subjects (2 males, 4 females), who were excluded from the ANCOVA. Although mean scores of LO and AS individuals were within 1 SD of the population norm, AS subjects attained significantly higher scores than LO subjects and males higher scores than females. Correlations between cognitive domains were high, particularly intelligence proved to be a distinguished indicator for cognitive functioning. Maximum plasma

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AUTHOR CONTRIBUTION

P.B., S.K., R.M. done study design; C.B., S.G., P.B. done statistical analysis; C.B., S.G., P.B., S.K., R.M., F.D.A., S.W. done interpretation of results; C.B., P.B., S.K., S.G. wrote the manuscript; all the authors approved the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

CONFLICTS OF INTEREST

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ammonium concentration and intelligence correlated significantly higher in EO ($r = -0.47$) than in LO subjects ($r = 0.04$). Correlation between the number of hyperammonemic events and intelligence scores were similar for EO ($r = -0.30$) and LO ($r = -0.26$) individuals. The number of clinical symptoms was significantly associated with intelligence ($r = -0.28$) but not with scores in other domains. Results suggest that OTCD has a global impact on cognitive functioning rather than a specific effect on distinct cognitive domains.

Keywords

cognitive outcome; ornithine transcarbamylase deficiency; urea cycle disorders

1 | INTRODUCTION

Ornithine transcarbamylase deficiency (OTCD) is the most frequent urea cycle disorder (UCD) with an estimated prevalence of 1:70 000 to 1:56 500 births in North America and Europe.[1–3] The disorder is X-linked, and the *OTC* gene is located on Xp11.4 with more than 400 known variants.[4] Males often present with neonatal hyperammonemic encephalopathy. Overall they have a more severe phenotype than female carriers, since only those females reflecting unfavorable X-inactivation are symptomatic.[5] The key function of the urea cycle is detoxification of ammonium (NH_4^+) to urea. OTCD hampers or abolishes NH_4^+ detoxification, leading to either neonatal fatal hyperammonemia (if untreated) or to recurrent episodes of hyperammonemia if the enzyme deficiency is not complete.[6,7] With increased NH_4^+ levels ATP-dependent glutamine synthesis is increased. This amino acid accumulates in the brain, particularly in astrocytes due to their high glutamine synthetase activity and results in brain edema. Other NH_4^+-induced pathomechanisms include impaired brain energy metabolism, excitotoxicity, altered metabolism of several amino acids and neurotransmitters, and secondary creatine deficiency. NH_4^+-induced neurotoxicity can be acute or chronic reflecting the severity of hyperammonemia as well as age and region-specific vulnerability.[6,9]

Newborns with complete loss or very low residual activity of OTC show early onset (EO) with acute hyperammonemic encephalopathy and coma during the newborn period (≤28 days). Sepsis-like appearance may delay correct diagnosis and treatment initiation, even with early and aggressive treatment neonatal mortality is about 60% in males and 43% in females.[10] The majority of EO survivors suffers from severe intellectual disability and a high risk of recurrent crises.[11,12] Late onset (LO) may occur at any age, presenting with acute clinical symptoms including recurrent vomiting, feeding problems, impaired consciousness, coma, seizures, muscular hypotonia, chronic migraine, and psychiatric symptoms. Long-term manifestations include impairment of cognitive development and motor dysfunction, epilepsy, behavioral and emotional problems, and chronic hepatopathy.[13–16] Acute liver failure can be the initial manifestation in females. Nonspecific signs may delay LO diagnosis, adding to the risk of gradual development of more severe symptoms.[14,17] Some patients have few or no acute decompensations, but can exhibit developmental disabilities, epilepsy, or protein aversion. A few individuals with pathogenic *OTC* gene variations also remained completely asymptomatic.[15,18,19] Treatment consists of a low protein diet

supplemented with citrulline or arginine and, if needed, ammonia scavengers that circumvent the urea cycle, such as sodium or glycerol phenylbutyrate and sodium benzoate. Mild forms of OTCD often require only moderate or even no restriction of protein intake, but prophylactic and intensified measures in emergency situations.

Brain damage in OTCD patients has been reported to correlate with the severity and duration of acute hyperammonemia, especially in neonatal patients,[20–22] although this has not been confirmed by all studies.[23] Findings of cognitive impairment and behavioral as well as emotional problems[6,24] are consistent with a pattern of global cerebral atrophy, indicating an impact of NH_4^+-induced neurotoxicity affecting the whole brain. However, focal cerebral abnormalities and specific learning disabilities have also been described.[25,26] Correlations between cognitive status and neurological alterations are not conclusive in every case. Normal IQ can be accompanied by abnormal neuroimaging, and mental deficits can be present despite normal brain scans.[21] Overall, EO patients have poorer intellectual and behavioral outcomes than LO patients, and specific deficits in measures of executive functions, cognitive flexibility and speed, and fine motor dexterity despite intellectual abilities within the average range have been reported in asymptomatic female carriers.[27,28] Selective vulnerability of white matter but better preservation of grey matter has been suggested as an explanation.[25,29]

The aim of the present study is to investigate the associations between timing of disease onset type (EO, LO, and asymptomatic), degree and frequency of hyperammonemia, sex of the individual, and number of clinical findings with results on tests of cognitive functioning in five domains (global intelligence, executive functions, memory, visual-motor integration, and visual perception). The primary question is whether the effect of OTCD on cognitive functioning is global or specific, that is, whether selected cognitive domains will be affected differently. A large sample of individuals with OTCD was available through the longitudinal study conducted by the Rare Diseases Clinical Research Network's Urea Cycle Disorders Consortium (UCDC), comprising 14 research sites at academic centers in the United States, Canada, and Europe.[30,31] Subsets of data and or variables have been published previously.[28,32,33]

2 | MATERIALS AND METHODS

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. The study was approved by the local Instutional Review Boards at all study sites and written informed consent was obtained from all patients or their legal tutors for being included in the study before inclusion. This article does not contain any studies with animal subjects performed by any of the authors.

2.1 | Study sample

Data obtained during study visits between April 2006 and August 2014 were retrieved from the electronic UCDC database. Diagnosis of OTCD at study inclusion was confirmed by at least one of the following criteria: (a) verification of a pathogenic variation in the *OTC* gene, (b) pedigree analysis in an affected family and/or (c) decreased hepatic OTC activity (<20%

of control) and/or (d) elevated urinary orotic acid (>20 mmol/mol creatinine) in a random urine sample or after allopurinol challenge test. Diagnoses of the 16 patients (10 symptomatic, 6 asymptomatic) initially included only because of elevated orotic acid were confirmed as OTCD by their own mutation or enzyme analysis, or by mutation or enzyme analysis in a relative, and/or detailed pedigree analysis. At the time of this study, the UCDC database included 444 individuals with OTCD. Three subjects of unknown onset and 24 patients who underwent liver transplantation were excluded. For another 117 patients no psychological data defining our data model (see paragraph psychological assessment) were available, resulting in a sample size of 300 subjects. The cut-off date for statistical analysis was 12 January 2015.

Following the criteria of the UCDC manual of operations, each metabolic center classified its patients as EO (≤28 days; EO, n = 25, 17 males/8 females) or LO (>28 days; LO, n = 155, 39 males/116 females) after presentation with clinical symptoms during hyperammonemia (plasma NH_4^+ concentration >100 μmol/L) and/or showing at least two of eight clinical symptoms: (a) recurrent vomiting (more than once a month), (b) protein aversion or recurrent symptoms after high protein intake, (c) episodic lethargy, (d) developmental and/or intellectual disability requiring special education or care, (e) abnormal neurological examination, (f) brain edema confirmed by cranial magnetic resonance imaging (MRI) or computer tomography (CT), (g) chronic migraine headaches (more than once a month), or (h) episodic psychosis. Relatives of index patients, confirmed to carry the mutant allele and lacking symptoms or having symptoms too unremarkable to lead to investigation of OTCD were classified as asymptomatic (AS, n = 120, 11 males/109 females). Nine out of 11 asymptomatic males carried variations associated with symptoms apparent in other OTC patients in the UCDC database or in the literature; two were diagnosed because of laboratory results and information available from their family, thereby excluding benign or likely benign variants.

During the course of the study 13 individuals switched from the asymptomatic to the symptomatic group. Patients were grouped according to their onset status (EO, LO, AS) at the time point of psychological testing. Most patients were diagnosed by metabolic investigations after clinical presentation (n = 157) or high-risk family screening with a known index patient (n = 139). One patient was identified by newborn screening. Mode of diagnosis was not reported in three asymptomatic patients. Two male patients (one EO, one LO) died at age 4.5 and 11.9 years, respectively. The cross-sectional sample included data on 233 females (mean age = 24.9 years; SD = 17.3; range: 0.5–71.4) and 67 males (mean age = 15.5 years; SD = 15.2; range: 0.1–68.2).

2.2 | Psychological assessment

The longitudinal study of the UCDC includes an extensive psychological test battery tapping different cognitive domains. The battery comprises age-appropriate tests for evaluations at 6 and 18 months, and 4, 8, 15, and 18 years or older, as well as adjusted protocols for moderately to severely intellectually disabled subjects (eg, the Bayley cognitive scale was also used for individuals with IQ < 70). This study includes five domains of cognitive function: intelligence, executive functions, memory, visuomotor integration, and visual

perception. All psychological tests used for the measurement of these domains are listed in Supporting Information, Table S1. Psychological assessments were completed at baseline or follow-up visits. Only first assessments were used for the statistical analysis in order to avoid confounding learning effects due to test repetition.

Intelligence was measured with the Bayley cognitive scale or Wechsler tests.[34–40] In 12 individuals (but only one in the ANCOVA sample) no test of general intelligence was available, therefore we used the General Adaptive Composite (GAC) score from the Adaptive Behavior Assessment System-Second Edition (ABAS-II) as a proxy for full-scale IQ. Follow-up for some participants was extremely challenging due to severe intellectual disabilities, far geographical distances from a metabolic center and the time commitments required. The ABAS-II could be completed at home and at the convenience of the family by the parent or primary care taker. Waisbren et al reported that correlations between scores on the ABAS-II and developmental or IQ tests for individuals with UCDs ranged from 0.48 to 0.72 and concordance rates for scores greater than a SD below the normative mean ranged from 69% to 89%.[41] This was also the case in our sample of 159 pairs of IQ and GAC scores ($r = 0.63$, $P < 0.001$). In the general population, scores on the ABAS-II correlate between $r = 0.5$ and 0.7 with IQ, as well.[42]

Executive functions include self-monitoring, cognitive flexibility, working memory, and inhibitory control.[43] Test scores of verbal fluency, focused attention, and working memory have been associated with this domain[44–49] and were used in the analyses for this study. Correlations between scores of different test instruments ranged between $r = 0.40$ and $r = 0.76$.

Memory was measured with tests for verbal and spatial memory.[45,49–52] In our study sample, scores from different memory tests were positively correlated in the range between $r = 0.35$ and $r = 0.50$.

Visuomotor integration was assessed with the Beery-Buktenica Developmental Test of Visual-Motor Integration.[53] This includes a task in which the participant is asked to copy with paper and pencil a sequence of increasingly complex geometric figures.

The *visual perception* task requires the participant to identify which of a series of geometric figures exactly matches a target figure.

Due to different scaling of the diverse tests, all scores were transformed to standard deviation scores (SDS) by subtracting the mean of the age appropriate test norm from the individual test score divided by the SD of the norm (eg, IQ scores were normalized by subtraction of 100, and division by 15). This method was used previously in a study from the UCDC Longitudinal Study[33]. When several tests measuring the same domain were available for a single patient, the mean SDS was calculated. This procedure results in a vector of five domain scores for each individual.

2.3 | Hyperammonemic events

Three indices of hyperammonemic events were defined for each subject. MaxNH_4^+ is the documented maximum plasma NH_4^+ concentration. The number of hyperammonemic events

(NHAE) is the frequency of plasma NH_4^+ measures above 100 μmol/L. For each hyperammonemic event the product of the local peak NH_4^+ level minus 100 and the length of inpatient stay in days was calculated and then divided by two. The sum of all products was used as an estimator for the area under the curve (AUC). The crudeness of the estimator is due to the fact that for most hyperammonemic events only the peak value is recorded in the database. The subtrahend was chosen to measure only the excess AUC in agreement with the UCDC definition of hyperammonemia by levels >100 μM.

2.4 | Clinical phenotype

The clinical phenotype at the last regular visit prior to psychological testing, was calculated for each subject as a summary score from the following 12 items: speech abnormalities, cerebellar findings, contractures, abnormal mental status, reflex abnormalities, other neurological findings; abnormal gait, movement disorders, asymmetries, tone changes, musculoskeletal findings; vision or hearing deficits.

3 | STATISTICAL ANALYSIS

SD scores for the five cognitive domains were analyzed in a repeated analysis of covariance (ANCOVA) with cognitive domain (intelligence, executive functions, memory, visuomotor integration, and visual perception) as the within subject factor, onset type (AS vs LO) and sex (female, male) as between-subjects factors, and age at time of testing as covariate. The model was used to identify interrelationships between cognitive domains, associations between onset type and sex with cognitive outcome, and to analyze a possible effect of age group. Differences between domains and groups were tested post-hoc by Bonferroni-adjusted comparisons. Differences of mean maximum NH_4^+ concentrations, ages at diagnosis, and age at first symptoms between EO and LO groups were tested with *t*-tests. Associations between cognitive function as the critical outcome and clinical parameters as possible predictors were measured by Pearson correlations. Differences between correlation coefficients were tested for significance with Fisher's Z. Significance levels were set at $P = 0.05$ if not indicated otherwise. For all procedures SPSS (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used.

4 | RESULTS

4.1 | Sample characteristics

Age at diagnosis ranged from postnatal days 1 to 41 in EO males (mean = 8.4 days, SD = 10.6; n = 17) and between day 1 and 3.5 years in EO females (mean = 1.53 years, SD = 1.5; n = 8); means were significantly different ($t(23) = 4.25$, $P = 0.0002$). Age at diagnosis for LO males (mean = 7.7 years, SD = 11.5; range = 28 days to 55 years; n = 39) vs LO females (mean = 8.27 years, SD = 11.1; range = 1 day to 49 years: n = 116) was not different ($t(153) = 0.27$, $P = 0.39$). Similarly for AS subjects, there was no significant difference ($t(117) = 1.18$, $P = 0.24$) in age at diagnosis for males (mean = 21.0 years, SD = 19.3; range = day 44 to 58 years; n = 11) compared to females (mean = 27.0 years, SD = 15.6; range = day 1 to 63 years; n = 108).

Comparison of the total OTCD sample (n = 417) with the subsample who received psychological testing (n = 300) revealed an underrepresentation of EO male subjects ($\chi^2(5) = 32.13$; $P < 0.0019$). Exclusion of liver-transplanted boys contributed to this underrepresentation. Additionally, difficulties for successfully accomplishing the comprehensive psychological test battery due to lower cognitive functioning may have contributed to selection bias. From the 300 patients with psychological data, only 183 had complete data sets for all five cognitive domains (Table 1). Since only two male and four female EO patients had complete datasets, patients with EO were excluded from the ANCOVA but included in other analyses.

4.2 | Cognitive domains

Main effects were significant for cognitive domain (Huynh-Feldt $F(2.7, 468.6) = 7.72$, $P < 0.001$), LO vs AS ($F(1, 172) = 10.77$, $P = 0.001$), and sex ($F(1, 172) = 9.91$, $P = 0.002$). Across all domains and onset types, males (mean = 0.266, SD = 0.937) scored significantly higher than females (mean = −0.320, SD = 0.803). The interaction of domain with onset type was significant (Huynh-Feldt $F(2.7, 468.6) = 2.715$, $P = 0.05$), as well as the interaction of domain with age at testing (Huynh-Feldt $F(2.7, 468.6) = 4.465$, $P < 0.01$). Neither the triple interaction of cognitive domain, onset type, and sex (Huynh-Feldt $F(2.72, 468.6) = 0.392$, $P = 0.74$), nor any other interaction was significant. The interaction of domain with onset type revealed significantly better test results for the AS group in all cognitive domains except for visual perception (Figure 1 and Table S2). AS individuals scored between 0.6 and 1.2 SDS higher than LO patients in intelligence, executive functions, memory, and visuomotor integration. In the visual perception domain the difference of 0.3 SDS was nonsignificant. In cross-sectional analyses, the interaction of domain with age showed similar scores across all ages for intelligence (SDS = 0.2) and visual perception (SDS = −0.6), but memory scores were found to be inversely correlated with age, ranging from 0.2 SDS at 3.2 years to −0.7 SDS in the seventh decade, and scores for visuomotor integration started from −0.8 SDS at 3.2 years and were −1.5 SDS in the oldest cohort. Conversely, scores for EF were at a mean of −0.2 SDS at 3.2 years increased across age groups by 0.5 SDS.

Correlations of SD scores of the five cognitive domains revealed that the domains were not independent from each other. Intelligence correlated with all other domains (0.67 EF; 0.56 M; 0.55 VMI; 0.51 VP; all $P < 0.001$). The correlational pattern was similar after pairwise inclusion of tests as well as for the sample of LO and AS individuals with complete test profiles (Table S3). For the sample with complete tests (n = 177) using Fisher's Z-transformation the mean correlation of all other tests (EF, M, VMI, VP) with IQ was 0.6, with EF (IQ, M, VMI, VP) was 0.5, with M 0.46, with VMI 0.48, and with VP 0.43. IQ correlated significantly higher with all other tests than M ($P = 0.04$) and VP ($P = 0.02$). The comparison of the correlation for IQ vs VMI showed a trend ($P = 0.06$), and the comparison of the correlations with IQ vs EF was not significant ($P = 0.10$). Mean correlations of EF, M, VMI, VP with all other tests respectively were not significantly different. These findings support the use of IQ as an appropriate single parameter to represent the five cognitive domains measured in this study. Therefore, for all further analyses we used intelligence as an indicator of cognitive performance.

4.3 | Parameters of hyperammonemia

Relative to LO subjects, EO subjects were found to score significantly higher on measures of $MaxNH_4^+$ and AUC, while scoring significantly lower on intellectual tests. There were no significant differences in NHAE between the EO and LO groups (Table 2). There was a significantly stronger negative correlation between intelligence and $MaxNH_4^+$ for the EO group than for the LO group (−0.47 vs −0.04; $Z = -1.88$, $P = 0.03$). The EO and LO groups did not differ significantly in their correlations between intelligence and NHAE (−0.30 vs −0.26; $Z = -0.17$; $P = 0.43$) and in their correlations between intelligence and AUC (−0.47 vs −0.21; $Z = -1.11$, $P = 0.13$). For the combined group of EO and LO subjects IQ was negatively correlated with all three metabolic parameters: $MaxNH_4^+$ ($r = -0.29$, $P = 0.001$, n = 135), NHAE ($r = -0.25$, $P < 0.001$, n = 165), and AUC ($r = -0.33$, $P < 0.001$, n = 127). In contrast, for the combined EO/LO group, correlations of the three hyperammonemia parameters ($MaxNH_4^+$, NHAE, AUC) with the other four cognitive domains (EF, M, VMI, VP) were found to be nonsignificant as well as significantly lower (correlation coefficients −0.14 to +0.18; $P < 0.05$ for all Z).

For EO males, $MaxNH_4^+$ was higher ($P < 0.01$), and IQ was lower ($P = 0.001$) than for EO females. NHAE ($P = 0.068$) and AUC ($P = 0.066$) showed trends for higher values in EO males. None of the differences between LO males and females were statistically significant (Table S4).

4.4 | Clinical findings

A univariate 3×2 (onset type; sex) ANOVA of frequencies of abnormal clinical findings (see Table S5) revealed a significant main effect of onset group ($F(2, 294) = 10.08$, $P > 0.001$). Differences between males and females ($F(1, 294) = 0.70$, $P = 0.41$) as well as the interaction of onset type by sex were nonsignificant ($F(2, 294) = 0.43$, $P = 0.65$). Bonferroni adjusted post-hoc comparisons of onset groups showed significantly more clinical findings in EO patients (mean = 1.84, SD = 2.135) compared with LO (mean = 0.67, SD = 1.11; $P < 0.001$) and AS individuals (mean = 0.45, SD = 0.818; $P < 0.001$). The difference between the LO and AS groups was not significant ($P = 0.33$). The correlation between numbers of clinical findings and IQ was significant ($r = -0.284$; $P = 0.001$; n = 269) with higher IQ scores being associated with fewer clinical signs. Correlations between all other cognitive domains and clinical findings were nonsignificant as well as significantly lower (coefficients between −0.121 and −0.08).

5 | DISCUSSION

This study presents an analysis of the cognitive phenotype and clinical manifestations in 300 patients with OTCD including onset type, sex, and parameters of hyperammonemia. Our results indicate that the impact of OTCD on intellectual outcome is global rather than specific, affecting all five domains: intelligence, executive functions, memory, visuomotor integration, and visual perception. The profiles for these five cognitive domains appear to be similar for females and males and for EO, LO, and AS individuals. Likewise results have also been found in a study of symptomatic and asymptomatic adult females with OTCD.[27] This study also revealed main effects between symptomatic and asymptomatic subjects as

well as between neonatal vs LO mutation types (asymptomatic outperformed symptomatic individuals and adult females with LO mutations outperformed those with neonatal mutations), but a similar neuropsychological phenotype. Another study analyzing 43 individuals with OTCD from the UCDC sample[28] hypothesized that OTCD results in a specific impairment of attention and executive functions, but acknowledged the need for closer examination of the pattern of cognitive domains. This hypothesis has not been corroborated by the present study.

Another hypothesis suggests that deficits in OTCD may be related to difficulties in fine motor control.[32] We found a significant interaction of cognitive domains with onset type. Compared with tests results in other domains VMI scores were relatively lower for AS subjects than for LO subjects (see Figure 1). However, it is not plausible that difficulties in fine motor control should be more prominent in AS than in LO subjects. Particularly the test for VMI does not require the individual to draw smooth lines, which would require well-developed fine motor control. A possible alternative interpretation is a relative floor effect. Copying increasingly complex geometric patterns may simply be too difficult rather than a specific OTCD effect. In a study of 5 year old very-low-birthweight children,[54] VMI scores were about 1 SD lower than IQ scores, with VP scores in between, and in a sample of 155 children from 7 to 10 years of a primarily middle-class elementary school VMI scores were 0.5 SDS lower than VP scores.[55] Even if hyperammonemia due to OTCD would cause exclusive impairment of a single domain, this would not allow defining a specific OTCD profile as this domain might be sensitive to any kind of neurotoxic event.[56] Remarkably a similar pattern for IQ, memory and VMI has recently been found for patients with three other UCDs.[33] The only test for a selective deficit would be the investigation of a healthy control group with the same test battery.

Older subjects in the study attained scores that were similar or somewhat higher than younger subjects on tests of intelligence, visual perception, and executive functions. On the other hand, scores on tests of memory and visuomotor integration decreased with age as indicated by ANCOVA. This might be due to the very large age range of the whole sample (Table 1). The number of repeated measurements in the UCDC study is not yet large enough to allow a longitudinal developmental analysis.

Mean results of EO and LO subjects were within one SD from zero, that is, the normal range. However, the significant main effect of better results for males than for females across all cognitive domains is difficult to explain. Overall, diagnostic delays were longer for EO and LO females than for males, but this cannot explain the difference in male and female AS subjects. Post-hoc tests for a possible influence of sociodemographic features (patients' and parents' income or years of education) were all nonsignificant.

Intelligence measures were associated positively with all other cognitive domains and negatively with indices of hyperammonemia and clinical findings. None of the other cognitive domains showed a comparable correlational pattern. Furthermore, patients with a higher number of clinical findings achieved lower scores on tests of intelligence, which supports the conclusion of a global effect of OTCD. MaxNH_4^+ was most highly associated with intelligence scores in EO patients, whereas NHAE and AUC appeared to be equally

associated with IQ in EO and LO subjects. EO males showed significantly higher MaxNH_4^+ levels and AUC scores than EO females, while the latter showed a trend for higher NHAE. Male EO patients also showed significantly lower intelligence scores. No sex differences were found in the LO group (Table S5).

Our results indicate that intelligence may be the best single marker for the evaluation of cognitive outcome. Measures of intelligence correlated highly with scores on the other domains tested, and even more important, correlations with measures of plasma NH_4^+ were higher than for the scores of other domains. The interpretation of intelligence as a global concept fits well with current research on intelligence.[57] In our analysis, we mainly used Wechsler instruments defining intelligence as the global capacity of the individual to act purposefully, to think rationally and to deal effectively with his/her environment.[58] The concept of intelligence is also attractive due to its predictive value for educational attainment and is appropriate to be included in guidelines for diagnosis and management.[59,60] The results of this study provide evidence for an abbreviated test battery and the use of intelligence tests for the evaluation of cognitive outcome in OTCD. Overall, including IQ data from individuals with incomplete tests show mean results in the normal band for the AS and the LO subgroups (however, with relatively large standard deviations for the latter), and even for EO females, while the outcome for EO males is rather poor (Table S2). On the other hand, for the evaluation of the short-term impact of metabolic variation such as hyperammonemia and/or treatment effects in clinical trials, outcome measures such as reaction time, interference control, working memory, and cognitive flexibility may be more appropriate.[61] This approach would allow for a differentiated investigation of the relationship between a proximal metabolic and/or neurological event and a distal behavioral effect.

6 | LIMITATIONS

A limitation of studies covering a large age range is the necessity to change test instruments with age. An age- and sex-matched control group would have mitigated this issue, but was not available in the present study. The search for selective effects of OTCD in the UCDC longitudinal study a priori required a comprehensive test battery placing considerable demands on participants. From the 441 patients with known onset, only 300 underwent psychological testing and 183 had complete datasets. EO patients with psychological data were underrepresented, therefore the data added descriptively to Figure 1 should be regarded cautiously. In all onset groups, mean intelligence scores were higher for individuals completing all tests in the study protocol than for those with incomplete results (Table S2). On the one hand having a complete dataset may indicate a better outcome. On the other hand, it may indicate an ascertainment bias excluding low functioning individuals. It has been previously shown for large samples that homogeneous intellectual functioning is particularly true for low functioning individuals,[62] this however needs to be verified for individuals with OTCD.

Our result that IQ is a distinguished outcome measure is based on a cross-sectional approach, but may not be effective for longitudinal research, developmental monitoring or clinical trials. Onset type was associated with age at the time of testing, that is, EO, LO, and

AS subjects comprise three different age groups (childhood, adolescence, and adulthood, respectively). Among these three cohorts, age-related variable exposure to the metabolic perturbations and treatments may add confounding elements. However, at least for EO individuals it has been shown in a meta-analysis[10] that no improvement of survival was reported in publications between 1978 and 2014. Although there are elaborated sequential designs to solve these problems,[63] they are beyond the scope of this particular study and of research of rare diseases in general. Nonetheless, patterns of results are highly consistent for males and females in all onset groups, which support our conclusions.

7 | CONCLUSION

OTCD is associated with global functional impairment across different cognitive domains and clinical signs rather than a pattern of deficits in specific domains. The cognitive phenotype of OTCD patients with EO is associated with the maximum NH_4^+ level, whereas the cognitive phenotype of OTCD patients with LO is associated with the number of hyperammonemic events and AUC. Intelligence appears to be an appropriate marker for the evaluation and monitoring of cognitive outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ABAS-II	Adaptive Behavior Assessment System II
ANCOVA	analysis of covariance
AS	asymptomatic
AUC	area under the curve

CMS	Children's Memory Scale
CT	computer tomography
CVLT	California Verbal Learning Test
D-KEFS	Delis-Kaplan Executive Function System
EF	executive function
EO	early onset (first symptoms during the newborn period; ≤ 28 days)
GAC	Global Adaptive Composite Score (ABAS II)
IQ	intelligence quotient
K-ABC	Kaufman Assessment Battery for Children
LO	late onset (first symptoms after the newborn period)
M	memory
MaxNH4$^+$	patient's maximum ammonium level
MRI	magnetic resonance imaging
NEPSY	A Developmental Neuropsychological Assessment
NH4$^+$	ammonium
NHAE	number of hyperammonemic events
OTCD	ornithine transcarbamylase deficiency
RWT	Regensburger Wortflüssigkeits-test
SDS	Standard deviation score
TEA-Ch	Test of everyday attention for children
UCD	urea cycle disorder
VLMT	Verbaler Lern- und Merkfähigkeitstest (verbal learning and memory test)
VMI	visual-motor integration
VP	visual perception
WAIS	Wechsler adult intelligence scale
WASI	Wechsler abbreviated scale of intelligence
WISC	Wechsler intelligence scale for children
WMS	Wechsler memory scale

WPPSI	Wechsler preschool and primary scale of intelligence
Z	Fisher's Z

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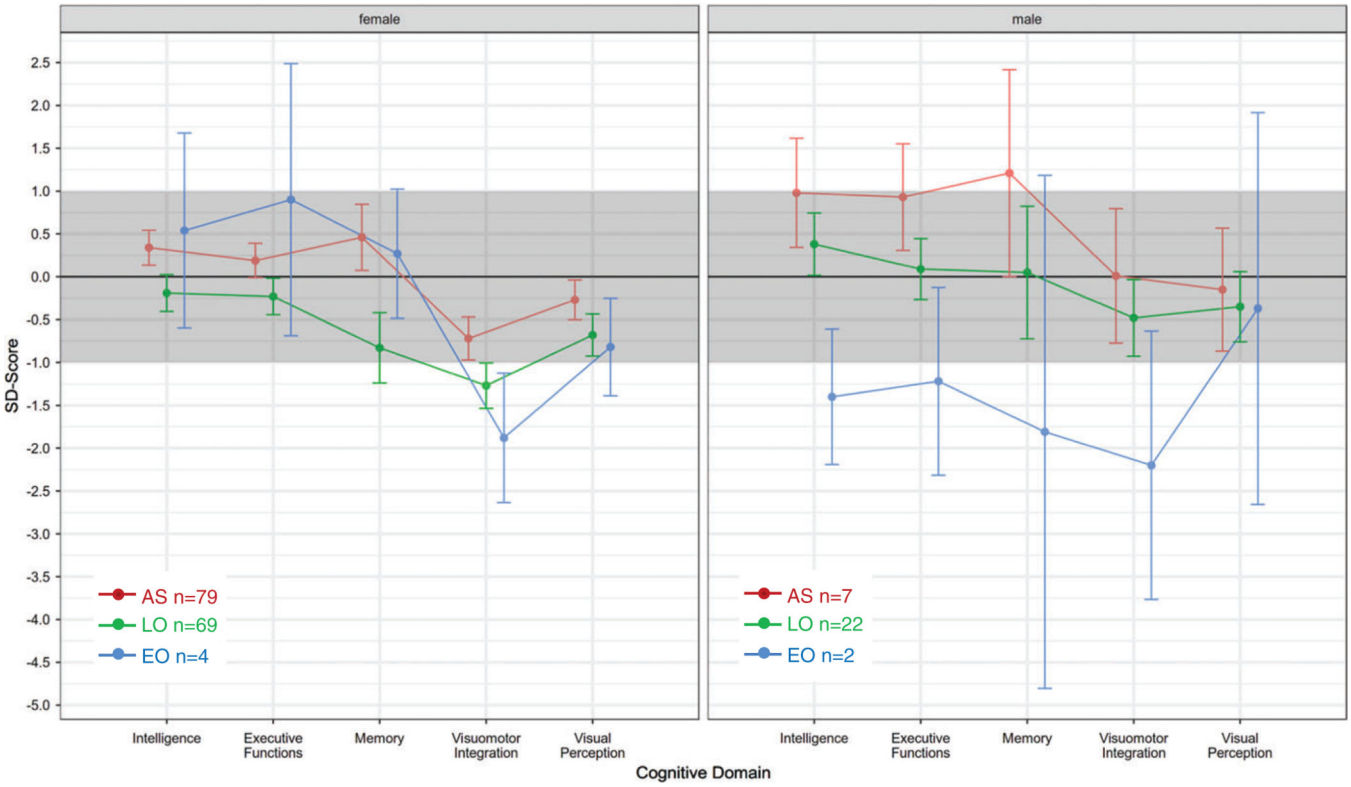

FIGURE 1.

Means and SDs of patients with complete scores in all domains. Data of LO and AS subjects are corrected for age by ANCOVA analysis. Data for EO patients are added for descriptive purposes

TABLE 1

Distributions of onset type and sex in the whole UCDC sample and representativeness of the sample with psychological tests

	EO		LO		AS	
Total sample n = 417	**Male n = 71**	**Female n = 10**	**Male n = 56**	**Female n = 153**	**Male n = 12**	**Female n = 115**
Age (years) mean (SD) [min-max]	7.1 (7.6) [0–33]	12.8 (10.6) [1–35]	20.8 (13.0) [1–74]	23.3 (14.9) [1–79]	26.0 (23.1) [0–69]	37.3 (17.2) [2–73]
Sample with psychological tests n = 300	n = 17 {51}[a]	n = 8 {7}[a]	n = 39 {40}[a]	n = 116 {110}[a]	n = 11 {9}[a]	n = 109 {83}[a]
Age at test (years) mean (SD) [min-max]	5.8 (9.0) [0–29]	11.6 (9.2) [2–29]	17.3 (12.0) [0–68]	17.6 (13.7) [1–71]	24.3(22.1) [3–67]	33.5 (17.2) [1–68]
Age at first symptoms (days) mean (SD) [min-max]	3.1 (2.4) [0–8]	4.0 (4.9) [0–14]	2486 (4211) [122–24 837]	1835 (2872) [61–14 245]		
Age diagnosis (days) mean (SD) [min-max]	8.4 (10.6) [0–41]	560 (547) [0–1278]	2803 (4180) [28–20 089]	3019 (4042) [0–17 897]	7663 (7046) [42–21 185]	9847 (5702) [0–23 011]
Sample with tests for all five psychological domains n = 183	n = 2	n = 4	n = 22	n = 69	n = 7	n = 79
Age at test (years) mean (SD) [min-max]	7.9 (1.7) [6.6–9.1]	15.1 (10.7) [3.2–28.5]	21.1 (14.2) [6.6–68.2]	19.4 (14.5) [3.7–71.4]	35.2 (20.6) [11.3–66.6]	37.5 (15.3) [5.7–68.4]
Age at first symptoms (days) mean (SD) [min-max]	5.0 (4.2) [2.0–8.0]	5.5 (6.0) [0.0–14.0]	3560.4 (5242.8) [121.0–24837.0]	1970.5 (3095.0) [42.0–14244.8]		
Age diagnosis (days) mean (SD) [min-max]	11.5 (3.5) [9.0–14.0]	640.8 (735.9) [0.0–1278.4]	4222.2 (5115.9) [395.7–20088.8]	3302.9 (4288.6) [60.9–17897.3]	11 470.4 (5990.9) [4017.8–21184.5]	11 095.6 (5247.1) [0.0–23010.8]

[a]Expected frequencies in curly brackets based on the distribution of the total sample of 417. $\chi^2(5) = 32.18$; $P < 0.001$. Male EO patients are significantly underrepresented. Based on the distribution of the total OTCD sample 51 male EO OTCD patients would be expected, leading to a cell $\chi^2(1)$ of 22.74; $P < 0.001$.

TABLE 2

Association of maximum plasma ammonium levels ($MaxNH_4^+$), number of hyperammonaemic events (NHAE) and area under the curve (AUC) of all hyperammonaemic events with intelligence scores in early (EO) and late onset (LO) patients

		$MaxNH_4^+$ µmol/l	**NHAE**	**AUC**	**$Intelligence_{sds}$**
EO	N	21	25	19	24
	Mean (SD)	734.4 (596.4)	3.6 (4.8)	2656.5 (2075.2)	−1.37 (1.65)
LO	N	121	155	114	141
	Mean (SD)	288.6 (154.3)	3.9 (5.4)	598.8 (878.7)	−0.44 (1.38)
	t Test	t^* = 3.41 (df = 20); P = 0.003	t = −0.26 (df = 178); P = 0.78	t^* = 3.02 (df = 19); P = 0.007	t = −2.99 (df = 163); P = 0.003
EO	*r* with intelligence (n, p)	−0.47 (21, 0.032)	−0.30 (24, 0.155)	−0.47 (19, 0.043)	/
LO	*r* with intelligence (n, p)	−0.04 (114, 0.67)	−0.26 (141, 0.002)	−0.21 (108, 0.033)	/
	Fisher' Z (p)	−1.88 (0.03)	−0.17 (0.43)	−1.11(0.13)	

t^* = *t* test for inhomogeneous variances.