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ADULT-ONSET DIAGNOSIS OF UREA CYCLE DISORDERS: RESULTS OF A FRENCH COHORT OF 71 PATIENTS

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jimd.12403

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Acknowledgments: We thank Grace Stockton-Bliard for English editing.

Competing interests: None of the authors have confilts of interest.

Details of funding: None.

Details of ethics approval: The study was performed in accordance with the Helsinki declaration. Since the study was based on systematic medical data records, as authorized by the French "Commission Nationale Informatique et Liberté" (N° CNIL 1118523) and because no supplementary procedure was performed and no additional biological samples collected, the study did not require approval by an ethic committee according to French legislation on human research (reference methodology CNIL n°2206749, 13/09/2018)."

Abstract

Background: Urea cycle disorders (UCD) are rare diseases that usually affect neonates or young children. During decompensations, hyperammonemia is neurotoxic, leading to severe symptoms and even coma and death if not treated rapidly.

Aims: Description of a cohort of patients with adult onset of UCDs.

Methods: Multicentric, retrospective and descriptive study of French adult patients with a diagnosis after 16 years of age of UCDs due to a deficiency in one of the 6 enzymes (arginase, ASL, ASS, CPS1, NAGS, OTC) or the two transporters (ORNT1 or citrin).

Results: Seventy-one patients were included (68% female, 32% male). The diagnosis was made in the context of (i) a metabolic decompensation (42%), (ii) family history (55%), or (iii) chronic symptoms (3%). The median age at diagnosis was 33 years (range 16-86). Eighty-nine percent of patients were diagnosed with OTC deficiency, 7% CPS1 deficiency, 3% HHH syndrome and 1% argininosuccinic aciduria. For those diagnosed during decompensations (including 23 OTC cases, mostly female), 89% required an admission in intensive care units. Seven deaths were attributed to UCD – 6 decompensations and 1 epilepsy secondary to inaugural decompensation.

Conclusion: This is the largest cohort of UCDs diagnosed in adulthood, which confirms the triad of neurological, gastrointestinal and psychiatric symptoms during hyperammonemic decompensations. We stress that females with OTC deficiency can be symptomatic. With 10% of deaths in this cohort, UCDs in adults remain a life-threatening condition. Physicians working

in adult care must be aware of late-onset presentations given the implications for patients and their families.

Take home message: This large cohort of adult-onset urea cycle disorders underlines the deadly nature of UCD and the need to raise awareness and improve management of these treatable conditions in adult medicine.

Key words: urea cycle disorders, inherited metabolic diseases, hyperammonemia, late-onset diagnosis, adults

Introduction

Urea cycle disorders (UCD) are a group of inherited metabolic diseases caused by a deficiency of one of the six enzymes or two transporters involved in the urea cycle[1]. The urea cycle is necessary to detoxify ammonia, otherwise toxic, and for the endogenous synthesis of arginine[2]. Three of these enzymes are mitochondrial: N-acetylglutamate synthase (NAGS, EC 2.3.1.1), carbamoyl phosphate synthetase I (CPSI, EC 6.3.4.16) and ornithine transcarbamylase (OTC, EC 2.1.3.3); and three cytoplasmic: argininosuccinate synthetase (ASS, EC 6.3.4.5), argininosuccinate lyase (ASL, EC 4.3.2.1) and arginase 1 (ARG1, EC 3.5.3.1). Two transporters are necessary to link the mitochondrial and cytoplasmic compartments: the mitochondrial ornithine transporter 1 (ORNT1 also named solute carrier family 25, member 15 SLC25A15) which carries ornithine into the mitochondria, and the mitochondrial aspartate/glutamate transporter (citrin, transporter SLC25A13).

In most cases, UCDs are diagnosed in the first months of life but late-onset forms have been reported[3-7]. The phenotypic differences between pediatric versus adult onset are not well understood[8]. Furthermore, no cohort exclusively focused on adult-onset patients has been published to date. The aim of this paper is to describe a large French cohort of adult-onset UCD patients.

Materials and methods

Patients

This study was multicentric, retrospective and observational. French metabolic specialists were contacted by e-mail through the mailing list of the Adult group of the French Society for the

Study of Inherited Metabolic Diseases (SFEIMA) and data were collected through a questionnaire. Inclusion criteria were: 1) patients with UCD : NAGS deficiency (OMIM 237310), CPSI deficiency (OMIM 237300), OTC deficiency, (OTCD, OMIM 311250), citrullinemia type I or ASS deficiency (OMIM 215700), argininosuccinic aciduria or ASL deficiency (OMIM 207900), argininemia or ARGI deficiency (OMIM 207800) or diseases caused by deficiency in one of the two transporters envolved in HHH syndrome (Hyperornithinemia-Hyperammonemia-Homocitrullinuria syndrome, OMIM 238970), either ORNT1 deficiency or citrullinemia type II (OMIM 605814, 603471) due to citrin deficiency; and 2) diagnosis after sixteen years of age.

Collected data included diagnosis, clinical signs (neurological, psychiatric or gastrointestinal symptoms), previous clinical symptoms that could be related to former decompensations (noticed by the patient or family and reported in the patient's chart), biological analyses (ammonia, plasma aminoacids, urinary orotic acid), long-term treatment, diets and genetic pathogenic variants. Data were collected by metabolic specialists using patients' records as the source of information. An anonymized database was constituted in accordance with the reference methodology MR004 of the "Commission Nationale de l'Informatique et Libertés" (N° 2206749, 13/09/2018)

Statistics

Collected data were expressed as mean, median and range for quantitative variables and as percentage for qualitative variables.

Results

Eighty-five patients were screened for this study: they were diagnosed between 1997 and 2017, except two mothers of OTC deficient patients diagnosed in 1973 and 1980. Four patients were excluded because diagnosis was made during childhood; in 9 cases the data were missing and in one case the final diagnosis was not confirmed (Figure 1). We included 71 patients described in Tables I[9-12], II[9-12], III and IV[9], from 12 centers: Paris (17 patients), Lille (16), Tours (10), Rennes (9), Bordeaux (7), Lyon (4), Strasbourg (3) and one patient from each of the other centers (Angers, Montpellier, Reims, Grenoble). Forty-eight patients were female (68%) and 23 were male (32%). The diagnosis was made at the time of an acute decompensation for 30 patients (42%), in the context of chronic symptoms for 2 patients (3%) or through family screening for 39 patients (55%). Mean age at diagnosis was 37 years (median age 33 years, range 16-86). Sixty-three patients were diagnosed with OTC deficiency (89%), 5 with CPS1 deficiency (7%), 2 with HHH syndromes (3%), and one with argininosuccinic aciduria (1%). At inclusion, 9 patients were already deceased: 7 deaths were related to UCD (6 patients from hyperammonemic decompensations and one neurologic sequelea following inaugural crisis), whereas one patient died during follow-up (unknown cause). All patients who died from hyperammonemia were diagnosed concomitantly to metabolic decompensations: 3 patients had OTC deficiency (2 men and one woman), 2 had CPS1 deficiency, and one had HHH syndrome.

Patients diagnosed during acute decompensation

This group comprises 30 patients (Table I and II)[9-12]: 16 were female (53%) and 14 were male (47%). Mean age at diagnosis was 39 years (median age 35 years, range 18-69). Ten patients did not have any comorbidity (missing data for one patient). Medical history was otherwise notable for 2 patients with surgical procedures, 2 patients treated for depression and 8 patients

with neurological symptoms (developmental delay, migraines, seizures, episodes of confusion and capsulo-thalamic stroke). During this hyperammonemic decompensation, 16 patients (53%) displayed gastrointestinal problems: 15 (50%) with nausea or vomiting, 4 (13%) with anorexia and 4 (13%) with abdominal pain. Ten patients (34%) displayed psychiatric symptoms: delirium, puerperal psychosis, catatonic depression, behavioural disturbance or visual hallucinations. Twenty-eight patients (93%) had neurological symptoms: coma (19 patients, 63%), lethargy or drowsiness (14 patients, 47%), confusion (14 patients, 47%), agitation (8 patients, 27%), seizures (8 patients, 27%), ataxia (7 patients, 23%), slurred speech (6 patients, 20%), increased or decreased muscle tone (6 patients, 20%), headaches (5 patients, 17%), brain oedema (5 patients, 17%), pseudo strokes (3 patients, 10%), and acute loss of vision (1 patient, 3%). Four patients (13%) died during this first decompensation including one female with OTC deficiency, whereas 2 other patients died after diagnosis during a subsequent decompensation. One patient died because of neurologic sequelae following his first decompensation (status epilepticus without hyperammonemia) whereas another patient died of hepatocarcinoma.

Twenty-three patients (77%) were diagnosed with OTC deficiency (14 women and 9 men), 5 (17%) with CPS1 deficiency, one (3%) with HHH and one (3%) with ASL deficiency.

A precipitating factor was found in 23 cases (77%), and among them 7 patients (23%) had two precipitating factors (Table I). The most frequent factor was increased protein intake (7 patients, 23%). Other factors were sodium valproate (4 patients, 14%), pregnancy and/or delivery, surgery, infections, fasting, high-protein diet (exceeding normal dietary recommendations) to lose weight, intense exercice, psychological stress and immunotherapy for hepatocarcinoma.

In this group, chronic symptoms, prior to diagnosis, were identified in 24 patients (80%) – including 12 OTC deficient female: spontaneous low-protein or vegetarian diet (13 patients, 43%), psychiatric symptoms (11 patients, 37%), chronic headaches (10 patients, 33%), recurrent episodes of nausea or vomiting (8 patients, 27%), episodes of confusion (7 patients, 23%), learning disabilities (4 patients, 14%), seizure or epilepsy (4 patients, 14%) and anorexia (2 patients, 7%). Five patients did not have any chronic symptom before their first hyperammonemic crisis and data were missing for 1 patient.

Hyperammonemia was found in all patients, with a mean initial level of 275 µmol/L (median 218, range 45-1254), and a mean peak of 414 µmol/L (median 268, range 75-2500). Mean glutamine level was 924 µmol/L (median 862, range 250-1941). For patients with OTC deficiency, plasma ammonia was high at 262 µmol/L (median 175, range 45-1254), with a peak of 418 µmol/L (median 267, range 75-2500), as well as glutamine at 955 µmol/L (median 862, range 250-1941) and urinary orotic acid at 204 µmol/mmol of creatinine (median 122, range 1,6-579). All patients had a genetic analysis with available results for 28 patients (93%). Pathogenic variants were identified in 25 patients (89%).

Eighty-six percent of patients (25/29) required an admission in intensive care units (data not available for one patient). Hemodialysis was performed in 50% (15 patients). Noticeably, one patient presented with central pontine myelinolysis following hemodialysis, possibly related to a rapid change in serum tonicty with prompt correction of ammonia[10]. Nitrogen scavenger drugs were used in 72% (21 patients) – benzoate in 18 patients (64%), phenylbutyrate in 14 patients (50%) and both in 11 patients (39%). Carglumic acid was used in 4 patients (14%), including two CPS1 deficient patients, and 3 patients (12%) received antiepileptic drugs. Long-term treatment of the 26 remaining patients included low-protein diet (22 patients, 85%) with a

median protein restriction of 0.7g/kg/day (range 0.4 to 1 g/kg/day), nitrogen scavengers (21 patients, 81%), citrulline and/or arginine supplementation (17 patients, 65%). The median dose required for stability was 6g/day (range 3-15, 11 patients) for phenylbutyrate and 5g/day of benzoate (range 2-12; 12 patients). Glycerol phenylbutyrate was prescribed for two patients (9.9 and 17.6 g/day, respectively).

Long-term follow-up for these patients – except for 3 patients lost during follow-up – were: return to baseline for 11 patients (including 3 patients with previous intellectual disability and/or epilepsy), neurological sequelea for 7 patients, psychiatric symptoms for 2 patients, subsequent decompensations for 6 patients including 2 patients who died.

Patients diagnosed following chronic symptoms (table III)

Two men were diagnosed because of chronic symptoms. One patient (16 years old) was diagnosed with OTC deficiency because of abdominal pain along with headaches, drowsiness, and dizziness, with episodes of agitation and aggressive behavior that required a psychiatric follow-up, without other comorbidities. After diagnosis, he was treated with a low-protein diet, sodium benzoate and arginine supplementation. The other patient (26 years old) was diagnosed with HHH syndrome after recurent episodes of drowsiness, lethargy and aphasia. He also had intellectual deficiency. He was treated with a low-protein diet, sodium phenylbutyrate, citrulline supplementation and antiepileptic drugs. Both patients had hyperammonemia at diagnosis.

Patients diagnosed from family screening (table IV)

Thirty-nine patients were screened because of a familial proband: 32 women (82%) and 7 men (18%). All of them were diagnosed with OTC deficiency and we observed clear differences in

clinical presentations within members of the same family. Mean age at diagnosis was 37 years (median 32 years, range 17-86). For 7 patients, clinical or biological data were not available. Seven patients did not have any comorbidity, whereas 11 patients had surgical comorbidities, 3 patients had migraines or chronic haedaches and 2 were depressive. All patients had a genetic test. Forty-four percent of patients (14/32) had symptoms that could be related to chronic or recurrent hyperammonemia: 11 patients (34%) were spontaneously avoiding proteins, 7 patients (22%) had chronic episodes of nausea or vomiting, 6 patients (19%) reported chronic headaches, 3 patients had previous episodes of drowsiness, 2 patients had a psychiatric disorder, and one patient had an episode of confusion. Fourteen patients (44%) had never experienced symptoms before diagnosis.

After diagnosis, only 4 patients (3 men and 1 woman) presented with metabolic decompensations, including one severe hyperammonemic crisis leading to coma and hospitalisation in the ICU. Fourteen patients initiated a restricted protein diet, 5 patients were put on nitrogen scavenger drugs and 7 patients on arginine, citrulline and/or carnitine. Two patients died from causes unrelated to OTC deficiency.

Discussion

Our study describes the largest cohort of 71 patients with adult-onset UCD. First, we confirmed the clinical spectrum previously described for late-onset UCDs with 3 major symptom categories: neurological, gastrointestinal and psychiatric. Second, we observed that a large proportion of OTC deficient patients with acute decompensations in adulthood were women, which underlines that heterozygous females can have life-threatening onset in adulthood. This suggests that DNA testing should be considered for females at risk for OTC deficiency as they

can appear asymptomatic for decades before presenting in hyperammonemic crisis during adulthood. Third, we emphasized that UCDs are life-threatening conditions, with mortality or neurologic sequelae if not rapidly treated and/or prevented.

Unlike most previous cohorts of UCD patients where late-onset was defined as onset after the first month of life [4–6,13–21], we specifically included patients with adult-onset (after 16 years of age). Not surprisingly, the most frequent diagnosis in our cohort was OTC deficiency (89%), as previously reported[5,6,13–16,18,21]. Our cohort also comprised 2 patients with HHH syndrome, which is in line with a literature review of all cases of HHH syndrome, in which Martinelli *et al.*, showed that more than 30% of patients are diagnosed during adulthood[22]. A triggering factor of decompensation was found in 80% of patients, which is comparable to children's cohorts, but some factors were specific to adults such as increased protein intake (7 patients) or high-protein diet (exceeding normal dietary recommendations) (patient 10) and pregnancy or post-partum for 5 women. Infection was present in only 4 patients although this is the most common triggering factor during childhood.

Three studies[4-6] have reported adult patients with UCDs. In one study, 3 patients were diagnosed during adulthood[6]. In another study, 26 patients were older than 16 years at diagnosis – including 14 females with OTC deficiency and one patient with arginase deficiency – but without clinical and biochemical data[4]. In the third study[5], 105 patients (50%) were older than 16 years at the time of inclusion (74 with OTC deficiency, 17 with ASL deficiency, 8 with ASS deficiency, 4 with arginase deficiency, one patient with CPS1 deficiency and one patient with NAGS deficiency), but no information was provided about their age at diagnosis. Nevertheless, some of their results were comparable to our patient's cohort: (1) 52% of patients presented one episode of symptomatic hyperammonemia, vs 51% in our cohort, (2) 58% of patients followed

a restricted protein diet, vs 53% in our cohort, (3) 69% of patients with OTC deficiency were female, vs 63% in our cohort. Brassier *et al*. also described 11 OTC deficient patients (5 males, 6 females) with a median age at diagnosis of 28 years of age, which is younger than our OTC deficient patients (mean age 37 years)[7], but similar findings in terms of symptoms during acute decompensations, preexisting history in some patients, triggering factors and death rate. Noticeably, 3 pathogenic variants of *OTC* were frequent in our cohort: c.622G>A (p.Ala208Thr)[23] in 12 patients (4 male; 8 female) from 4 families, c.119G>A (p.Arg40His)[24] in 8 patients (5 male, 3 female) from 6 families and c.350A>G (p.His117Arg) in 5 women from 3 families. These variants are known to be associated with late-onset presentation[7,25].

In our study, hemo(dia)filtration was used in 50% of symptomatic patients, whereas no clear data are reported in other cohorts. If we consider the 2012 UCD guidelines[26] and their revision[27] as a reference, hemo(dia)filtration should be started immediatly in an undiagnosed patient if ammonia level is above 500 µmol/L. Between 250 and 500 µmol/L, it should be used only if there is no improvement in the ammonia levels with nitrogen scavenging drugs. These recommandations were established for all patients including neonates. In our cohort, the mean peak of ammonia level was 414 µmol/L, and 50% of our patients (all of them receiving nitrogen scavenging drugs) were under hemofiltration. This is in agreement with the only recommandations for adults that suggest treating all patients with ammonia levels higher than 200 µmol/L with extra-renal filtration[28]. Management of patients was decided following local protocol, in district or university hospital, and one center followed international guidelines. There were no differences regarding availability of nitrogen scavenger drugs or hemofiltration in the various centers, except for one patient who died after being treated with only hemofiltration as nitrogen scavengers were not available (ammonia level around 600 µmol/L).

In our cohort, patients who died exhibited the highest levels of peak ammonia (mean 753 µmol/L), which could explain their poor prognosis. Peak ammonia above 500 µmol/L has been associated with a poor neurological outcome[29]. Similarly, two other patients (patients 17, 26) with high peak ammonia at initial presentation (around 800 µmol/L) survived but manifested poor neurological outcomes. Overall, a third of our patients presented with neurologic and/or psychiatric manifestations following their initial metabolic crisis. It appeared that patients who died despite being diagnosed with UCD had very low treatment and diet compliance related to the complex psychosocial issues of their social and/or cognitive disabilities. Notably, 3 patients presented stroke-like episodes during their inaugural decompensation. Brain MRI was available for one patient and showed slight hypersignal of the white matter (internal capsule and semiovale center) initially, with persistent signal abnormality at 4 months follow-up but without neurologic sequelae.

Based on our patient cohort and data from the literature, we recommend searching for UCDs in all adult patients presenting with unexplained encephalopathy, especially when they are associated with gastrointestinal and/or psychiatric symptoms. The diagnosis of UCD may be further supported by the identification of triggering factors – e.g., infection, medication or recent increase of protein intakes –, and/or evocative chronic symptoms – e.g., vomiting, headaches, protein-restricted diet, episodes of unexplained neurological and/or psychiatric symptoms. Ammonia should be measured immediately in these patients. Hemo(dia)filtration in association with nitrogen scavenger drugs should be performed as soon as hyperammonemia is confirmed in patients with neurologic signs and/or if level of ammonia is greater than 200 µmol/L[28]. In our study, adult patients diagnosed with OTC deficiency during an acute metabolic decompensation were mostly female. Hence, we stress the importance of extended family

screening that includes OTC women. All situations that put adult UCD patients at risk of metabolic decompensation should be anticipated. All patients, even asymptomatic individuals should be issued with emergency certificates or cards. These patients should be made aware of factors that could trigger metabolic decompensations (prolonged fasting, anesthesia, infection), with specific triggers for women such as pregnancy and post-partum.

Our study has some limitations: this is a retrospective analysis, and patients from this cohort were ascertained thanks to a collaboration, which might not be exhaustive for all French patients. However, this is the first study focusing on adult patients that highlights the need to educate clinicians working in adult care about UCD decompensation first occurring in adulthood (with neurologic, gastro-intestinal and sometimes psychiatric symptoms), as well the importance of prompt management of acute metabolic crisis to avoid neurologic damage. We also insist on the importance of family screening, especially for OTC women who may be symptomatic.

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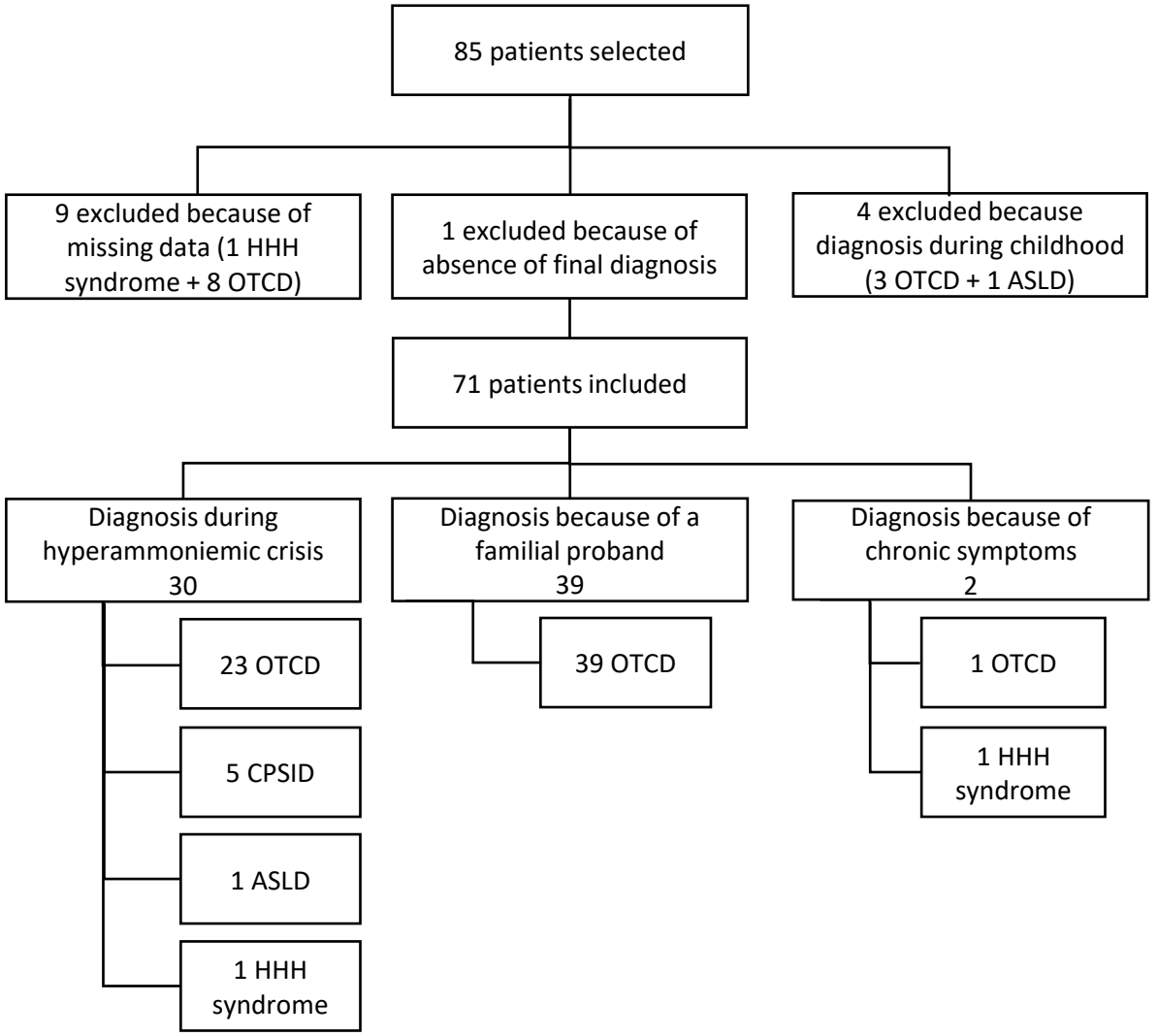

Figure 1: Flow chart. Figure explaining the inclusion of all patients in this study, the reason of the diagnosis and the final diagnosis with the number of patients by diagnoses.

OTCD ornithine transcarbamylase deficiency, CPSID carbamoylphosphate syntethase deficiency, ASLD Argininosuccinate lyase deficiency, HHH syndrome Hyperornithinemia-Hyperammonemia-Homocitrullinuria syndrome

Table I: Characteristics of patients diagnosed during their first hypermmoniemic decompensatio

Number of patients	Sex	Age at diagnosis (years)	Triggering factors	During first hyperammoniemic c: Gastro intestinal symptoms	During first hyperammoniemic c: Neurologic symptoms
Patients diagnosed with OTCD					
1	F	18	NA	x	x
2	M	18	diabetic ketoacidosis	x	x
3 [9]	F	21	valproate		x
4	F	21	increased protein intake		x
5	F	22	increased protein intake	x	x
6	F	23	increased protein intake + valproate	x	x
7	M	27	immunotherapy		x
8	M	27	NA		x
9 [10]	F	29	pregnancy (9 WA) + fasting	x	x
10	F	31	infection + high-protein diet (exceeding normal dietary recommendations)	x	
11	F	33	pregnancy (20 WA)		x
12 [11]	M	35	intense physical effort	x	x
13	M	36	infection	x	x
14	F	37	psychological stress	x	x
15	F	43	increased protein intake		x
16	F	44	increased protein intake + valproate	x	x
17	M	49		x	x
18	F	52	surgery		x
19	M	54	psychological stress	x	x
20	M	55	increased protein intake + valproate	x	x
21	F	57			x
22	F	59	surgery		
23	M	64	increased protein intake	x	x
Patients diagnosed with CPS1D					
24	F	21	caesarean section		x
25 [12]	F	35	infection + postpartum		x
26	M	46	surgery	x	x
27	M	52	infection		x
28	M	68			x
Patient diagnosed with ASLD					
29	M	22		x	x
Patient diagnosed with HHH syndrome					
30	M	69			x

Abbreviations: F female, M male, OTCD ornithine transcarbamylase deficiency, ASLD argininosucci
hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, NA not available, WA weeks of ame
[9; 10; 11; 12] references of primary publications where these patients were previously published

ın. Epidemiological, clinical and biological data as well as initial treatment.

ɛrisis		Ammonia level		Treatment of first decompensation		
Psychiatric symptoms	**Death**	**Initial level (µmol/L)**	**Peak (µmol/L)**	**Intensive care units**	**Hemo(dia) filtration**	**Nitrogen scavengers**
		high	NA	x	NA	NA
		113	272	x	x	x
x		510	510	x	x	x
		high	NA			x
		267	267	x	x	x
x		106	161	x		x
		144	687	x	x	x
	x	1254	2500	x	x	x
x		150	281	x	x	x
		45	75	x		
x		173	173	x		x
		256	256	x		
x		190	190	x		x
x	x	600	NA	x	x	x
		78	78	x		
x		290	290	x	NA	NA
		176	794	x	x	x
		high	NA	NA	NA	NA
		120	120	x		x
x	x	270	700	x	x	
		327	327	x	x	x
NA		65	105			
		114	155			x
		268	268	x	x	x
x		high	224	x		x
		800	800	x	x	x
x	x	218	715	x		x
		329	329	x	x	x
		101	237	x	x	x
		235	235			

ıate lyase deficiency, CPS1D carbamoylphosphate synthetase deficiency, HHH syndrome
ɛnorrhea

Table II: Characteristics of patients diagnosed during their first hype decompensation).

	Chronic symptoms before diagnosis			
Number of patients	**Gastro intestinal**	**Neurologic**	**Psychiatric**	**Spontaneous restricted protein diet**
Patients diagnosed with OTCD				
1	x	x		
2				
3 [9]	x	x		x
4		x		
5				x
6	x	x		x
7	x	x		
8	NA		NA	NA
9 [10]				
10	x	x		x
11		x		x
12 [11]				
13	x		x	x
14	NA	NA	NA	NA
15		x		
16	x	x	x	x
17				
18	x	x		
19		x		
20			x	x
21		x		
22				x
23				
Patients diagnosed with CPS1D				
24		x		x

25 [12]		x	x	x
26		x		x
27		x	NA	
28				
Patient diagnosed with ASLD				
29		x		x
Patient diagnosed with HHH syndrome				
30		x		

Abbreviations: OTCD ornithine transcarbamylase deficiency, ASLD argi
hyperammonemia-homocitrullinuria syndrome, NA not available, $ homoz
[9; 10; 11; 12] references of primary publications where these patients we

Genetic results (verified with mutalyzer 2.0.32 and varsome)		Long-term treatm	
Pathogenic variants	**Type of variants**	**Restricted protein diet**	**Arginine and/or citrulline**
***OTC* , transcript NM_000531.6**			
exon 2 c.119G>A, p.Arg40His	missense	x	x
exon 8 c.817_819del, p.Glu273del	deletion	x	x
exon 6 c.583G>A p.Gly195Arg	missense	x	x
exon 2 c.119G>A, p.Arg40His	missense	x	
exon 10 c.1052del, p.Lys351Serfs*44	frameshift	x	x
exon 1 c.3G>A, p.?	misense (start-loss)		
not found		x	x
exon 2 c.119G>A, p.Arg40His	missense		
exon 9 c.919A>G, p.Lys307Glu	missense		x
exon 3 c.275G>A, p. Arg92Gln	missense		
exon 6 c.626C>A, p. Ala209Glu	missense	x	x
exon 9 c.903A>T, p.Leu301Phe	missense	x	x
exon 2 c.119G>A, p.Arg40His	missense	x	x
exon 6 c.622G>A, p.Ala208Thr	missense		
exon 2 c.119G>A, p. Arg40His	missense	x	x
exon 6 c.638T>A, p.Met213Lys	missense	x	x
exon 6 c.622G>A, p.Ala208Thr	missense	x	x
not found		x	x
exon 6 c.653C>T, p.Ala218Val	missense		x
exon 2 c.214G>A, p.Glu72Lys	missense		
NA		x	
exon 8 c.740C>G, p.Thr247Arg	missense	x	
exon 2 c.206A>T, p.Gln69Leu	missense		
***CPS1* , transcript NM_001875.5**			
exon 38 c.4451A>T, p.Asp1484Val [$]	missense	x	x

exon 3 c.259C>T, p.Pro87Ser; exon 20 c.240C>T, p.Tyr80-	missense; silent	x	x
c.1201G>C, p.Gly401Arg; c.2810T>A, p.Ile937Asn; c.3097G>T, p.Glu1033*	missense; missense; nonsense	x	x
NA			
exon 28 c.3464C>A, p.Ala1155Glu [$]	missense	x	x
***ASL*, transcript NM_000048.4**			
intron 6 c.446+1G>A; exon 8 c.566A>G, p.Glu189Gly	splicing; missense	x	x
not found		x	x

ininosuccinate lyase deficiency, CPS1D carbamoylphosphate synthetase deficiency, HHH syndrome hyperorni

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ıent

Nitrogen scavengers drugs	Current status at last follow up	
x	alive	headaches and decompensations
x	alive	stable
x	alive	neurological and psychiatric sequelae and decompensations
	alive	stable
x	alive	stable
x	NA	NA
x	deceased	
	deceased	
	alive	neurological sequelae
	alive	other decompensation
x	alive	neurological sequelae
	NA	NA
x	alive	neurological sequelae
	deceased	
	alive	stable
x	alive	stable
x	deceased	
x	alive	neurological and psychiatric sequelae and decompensations
x	alive	stable
	deceased	
x	alive	neurological sequelae
x	alive	stable
	alive	stable
x	alive	stable

x	alive	stable
x	NA	NA
	deceased	
x	deceased	
x	alive	stable
x	deceased	

thinemia-

Table III: Characteristics of patients diagnosed because of chronic symptoms. Epidemiological data, c

Number of patients	Sex	Age at diagnosis (years)	Chronic symptoms before diagnosis				Ammon
			Gastro intestinal	Neurologic	Psychiatric	Spontaneous restricted protein diet	Initial level (µmol/L)
Patient diagnosed with OTCD							
31	M	16		x	x		167
Patient diagnosed with HHH syndrome							
32	M	26		x			100

Abbreviations: F female, M male, OTCD ornithine transcarbamylase deficiency, HHH syndrome hyperorni

clinical description, biological data and treatment.

nia level	Genetic results (verified with mutalyzer 2.0.32 and		Long-term treatm	
Peak (µmol/L)	**Pathogenic variants**	**Type of variants**	**Restricted protein diet**	**Arginine and/or citrulline**
	***OTC*, transcript NM_000531.6**			
276	exon 6 c.622G>A, p.Ala208Thr	missense	x	x
100	NA		x	x

ithinemia-hyperammonemia-homocitrullinuria syndrome, NA not available

ıent	
Nitrogen scavengers drugs	**Current status at last follow up**
x	alive
x	alive

Table IV : Description of OTC deficient patients diagnosed because of a familial proband. Epid

Number of patient	Sex	Age at diagnosis (years)	Index case (age at diagnosis)	Number of family	Chroni	
					Gastro intestinal	Psychiatric
33	M	26	brother	1		
34	M	32	brother	1	NA	NA
35	M	36	brother	1	x	
36	F	38	son (3d)	2		
37	F	61	grandson (3d)	2	x	
38	F	23	son (15d)	3		
39	F	NA	grandson (15d)	3		
40	F	24	son (birth)	4	NA	NA
41	F	NA	brother	4		
42	F	21	father (49y)	5	NA	
43	M	21	uncle (49y)	5		NA
44	M	27	uncle (49y)	5		
45	F	83	son (49y)	5		
46	F	NA	brother (49y)	5	Deceased before investigatio	
47	F	33	nephew	6		NA
48	F	NA	cousin	6		NA
49	F	33	son (10m)	7	NA	NA
50	F	43	nephew (10m)	7		
51	F	NA	son (18m)	8	NA	NA
52	F	NA	brother (18m)	8	NA	NA
53	F	32	cousin	9		x
54	F	42	sister	9		
55	F	60	daughter	9	NA	NA
56 [9]	F	28	sister (21y)	10		
57 [9]	F	52	daughter (21y)	10	x	
58	F	17	mother			
59	F	23	son (8d)		x	
60	F	25	son (1m)		x	
61	F	27	son			
62	F	29	daughter (9m)			
63	F	29	nephew (2y)		x	
64	F	30	son (18m)			
65	F	30	son			
66	F	32	son (13m)			
67	F	33	son			x
68	F	46	grandson (5d)		x	
69	M	64	nephew			
70	M	86	niece			
71	F	NA	brother			NA

Abbreviations: OTC ornithine transcarbamylase, M male, F female, NA not available, d days, y years
[9] reference of primary publication where these patients were previously published

lemiological, clinical and genetic data.

ic symptoms		Genetic results (verified with mutalyzer 2.0.32 a
Neurological	**Spontaneous restricted protein diet**	**Pathogenic variants of *OTC*, variant NM_000531.6**
x	x	exon 2 c.119G>A, p.Arg40His
NA		exon 2 c.119G>A, p.Arg40His
x	x	exon 2 c.119G>A, p.Arg40His
	NA	exon 6 c.608C>T, p.Ser203Phe
x	x	exon 6 c.608C>T, p.Ser203Phe
		exon 5 c.422G>A, p.Arg141Gln
		exon 5 c.422G>A, p.Arg141Gln
NA	NA	exon 4 c.386G>A, p.Arg129His
		exon 4 c.386G>A, p.Arg129His
		exon 6 c.622G>A, p.Ala208Thr
NA	NA	exon 6 c.622G>A, p.Ala208Thr
		exon 6 c.622G>A, p.Ala208Thr
		exon 6 c.622G>A, p.Ala208Thr
ns		exon 6 c.622G>A, p.Ala208Thr
		exon 4 c.386G>A, p.Arg129His
		exon 4 c.386G>A, p.Arg129His
NA	NA	exon 4 c.350A>G, p.His117Arg
		exon 4 c.350A>G, p.His117Arg
NA	NA	exon 4 c.350A>G, p.His117Arg
NA	NA	exon 4 c.350A>G, p.His117Arg
		exon 6 c.622G>A, p.Ala208Thr
		exon 6 c.622G>A, p.Ala208Thr
NA	NA	exon 6 c.622G>A, p.Ala208Thr
		exon 6 c.583G>A, p.Gly195Arg
x	x	exon 6 c.583G>A, p.Gly195Arg
x	x	exon 6 c.638T>A, p.Met213Lys
x	x	Not found
x		exon 6 c.584G>C, p.Gly195Ala [$]
		exon 3 c.269G>A, p.Ser90Asn
	x	exon 9 c.996G>A, p.Trp332*
	x	exon 2 c.158T>C, p.Ile53Thr
x	x	NA
		exon 5 c.533C>T, p.Thr178Met- polymorphism exon 2 c.137A>G, p.Lys46Arg
		exon 4 c.350A>G, p.His117Arg
		exon 6 c.622G>A, p.Ala208Thr
	x	NA
		exon 8 c.817_819del, p.Glu273del
x	x	not done
		NA

s, m months, [$] homozygote mutation

nd varsome) Type of variants	Current status at last follow up
missense	alive
missense	NA
missense	alive
missense	alive
missense	alive
missense	alive
missense	alive
missense	NA
missense	alive
missense	alive
missense	alive
missense	alive
missense	alive
missense	deceased
missense	alive
missense	alive
missense	NA
missense	NA
missense	alive
missense	alive
missense	NA
missense	NA
missense	NA
missense	NA
missense	alive
missense	NA
	alive
missense	alive
missense	NA
nonsense	alive
missense	alive
	alive
missense; missense	NA
missense	alive
missense	NA
	alive
deletion	NA
	deceased
	alive