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ORIGINAL ARTICLE

Mitochondrial acetoacetyl-CoA thiolase deficiency: basal ganglia impairment may occur independently of ketoacidosis

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Abstract

Background Mitochondrial acetoacetyl-CoA thiolase (T2) deficiency affects ketone body and isoleucine catabolism. Neurological impairment may occur secondary to ketoacidotic episodes. However, we observed neuromotor abnormalities without ketoacidotic events in two T2-deficient families. We hypothesized that the neurological signs were related to the genetic defect and may occur independently of ketoacidotic episodes. We therefore conducted a retrospective review on a French T2-deficient patient series searching for neuromotor impairment.

Methods In total, 26 cases were retrospectively analysed for clinical, biological and neuroimaging data.

Results Neurological findings were observed for 6/26 (23%) patients. Among these, two had never experienced ketoacidotic episodes, though they developed extrapyramidal signs with putamen involvement. Two of the other four patients developed neurological abnormalities before the first ketoacidotic crisis, with putamen involvement in one case. The third patient developed extrapyramidal symptoms more than 10 years after the initial decompensation with globus pallidus involvement. The last patient developed extrapyramidal signs immediately after a severe ketoacidotic crisis with putaminal lesions.

Conclusions Most T2-deficient patients achieved normal neurodevelopment. However, on account of the role of T2 in isoleucine catabolism, these patients are potentially exposed to accumulation of toxic isoleucine-derived metabolites, which may contribute to neurological impairment. Our findings confirm previous observations that

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neurological symptoms in T2 deficiency may occur unrelated to ketoacidosis. The role of protein restriction as a preventive measure against neurological symptoms could not be established in this study and deserves further evaluation. Long-term follow-up data on children diagnosed by newborn screening may clarify the pathogenesis of this neurometabolic association.

Introduction

Mitochondrial acetoacetyl-CoA thiolase (β-ketothiolase, 3-oxothiolase, abbreviated as T2, E.C. 2.3.1.9) deficiency (OMIM 203750) is a disorder of both ketone body and isoleucine catabolism caused by mutations in *ACAT1* (OMIM 607809). T2 catalyzes the cleavage of 2-methylacetocetyl-CoA into acetyl-CoA and propionyl-CoA in isoleucine catabolism, as well as the interconversion of acetyl-CoA and acetoacetyl-CoA in ketone body metabolism. The condition typically manifests as intermittent ketoacidotic episodes, triggered by infections or fasting in an otherwise healthy child. The biochemical diagnosis may be suspected following urinary excretion of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate and tiglylglycine. Blood acylcarnitine analysis can be useful if it shows elevated levels of 2-methyl-3-hydroxybutyrylcarnitine (C5OH) and tiglylcarnitine (C5:1) (Korman 2006). Enzyme assay in fibroblasts or leucocytes as well as mutation analysis of *ACAT1* can be performed in order to confirm the diagnosis (Fukao et al 1995). Patients with a detectable residual enzymatic activity develop metabolic crises as severe as do those with complete enzymatic deficiency. Hence, despite the existence of a correlation between genotype and biochemical data, there appears to be no clear genotype/phenotype correlation in T2 deficiency (Fukao et al 1995, 2001, 2014).

T2-deficient patients usually have a favourable outcome and are generally asymptomatic between episodes. However, neurological sequelae from severe and prolonged ketoacidotic crises have been described (Ozand et al 1994). Furthermore, neurodevelopmental abnormalities and especially motor delays prior to acidotic attacks were reported long ago, suggesting the presence of a T2-related encephalopathy (Ozand et al 1994). Neurological impairment, including extrapyramidal signs have been observed in our hospital in the absence of ketoacidotic events in two T2-deficient children, diagnosed via presymptomatic screening in affected families. This observation stimulated us to initiate a retrospective analysis of 26 T2-deficient French patients. The aim of the present study was to evaluate the frequency of such neurological involvement in T2 deficiency, to better characterize its clinical findings, and to understand the physiopathological mechanisms underlying this neurometabolic association.

Methods

Data were collected from the 26 French T2-deficient patients registered by the Biochemical Diagnostic Laboratory in Lyon between 1986 and 2014. T2 deficiency was confirmed by enzymatic assay except for five patients, four of whom were detected presymptomatically by urinary organic acid analysis after the diagnosis was made in siblings. The last patient was diagnosed by means of a typical organic acid profile of T2 deficiency during the first ketoacidotic episode. Enzymatic assay was performed in fibroblasts, as already reported (Gibson et al 1992, 2000; Middleton and Bartlett 1983). Sequencing of *ACAT1* was performed based on DNA extracted from patients' fibroblasts and was available for 20/26 patients (primers available on request). For 4/26 patients, molecular analysis was not conducted, due to it being already available for the index case of the family. Physicians involved in the clinical care and follow-up of individual patients were contacted, and their consent to share the retrospective data set in anonymized form was obtained. Data were collected, including age at diagnosis and follow-up, origin of the family, presence of parental consanguinity, psychomotor development, neuroradiological findings, as well as clinical and biochemical data during the first metabolic crisis at the time of its occurrence, including the following information: intensive care requirement, blood pH, bicarbonate, anion gap, blood lactate, ketoacidemia/ketoaciduria, blood glucose, plasma ammonia, urinary organic acid chromatography, blood acylcarnitine analysis, enzymatic assay and molecular analysis, if available.

Patients were divided into two groups based on the presence or absence of neurological symptoms. Among patients with neurological abnormalities, a distinction was made between children with or without a history of acute ketoacidotic episodes. Clinical and neuroradiological findings were characterized, with particular attention to patients who did not have metabolic crises accounting for their neurological symptoms.

Results

In total, 26 patients, 15 males and 11 females from 19 families, were included in the study. Median age at diagnosis was 15 months. Parental consanguinity was present in 4/14 families for which the information was available. The diagnosis was confirmed following the first acute ketoacidotic episode for 21/26 (80%) patients. T2 deficiency was documented for 5/26 asymptomatic siblings. The first ketoacidotic attack typically occurred during an infectious episode in association with fasting and catabolic conditions. Overall, 13/21 (61%) patients required hospitalization in an intensive care unit.

Laboratory parameters and clinical data in the 26 T2-deficient patients have been summarized in Table 1.

Neurological abnormalities were noted for 6/26 (23%) patients. Among these, 2/26 children developed extrapyramidal signs but never exhibited ketoacidotic episodes owing to presymptomatic testing. Among the four others, two children developed neurological abnormalities prior to the first ketoacidotic episode. For the last two, the first patient developed neurological sequelae with extrapyramidal signs more than 10 years after the initial acute episode, whereas the second patient developed extrapyramidal signs immediately after a severe acute ketoacidosis crisis.

Clinical and neuroradiological features displayed by the six patients with neurological involvement have been summarized in Table 2.

Patients with neurological abnormalities without a history of metabolic crisis

Patient #11 was the fourth child of a consanguineous Tunisian couple. The elder sister had been diagnosed as T2-deficient at the age of 18 months secondary to a severe ketoacidotic coma requiring supportive care and hemofiltration. Despite the severity of the initial crisis and further mild ketoacidotic episodes, her psychomotor development remained normal. Patient #11 was diagnosed in the neonatal period by presymptomatic screening in view of the family history. Urine organic acid profile revealed typical metabolites of T2 deficiency. So far, the boy never experienced ketoacidotic events. Psychomotor development was normal until the age of 5 months. From this age, truncal hypotonia was noted, along

Table 1 Laboratory parameters and clinical data in 26 T2-deficient patients

Patients ($n = 26$)	Ethnic origin	Age at onset/ diagnosis	Current age	Enzyme assay	*ACAT1* mutations	Neurological impairment
1	Caucasian	2 days	13 years	Yes[a] results NA	c.826 + 1G>T/c.826 + 1G>T exon8/exon8	No
2	Caucasian	30 m	11 years	NA	NA	No
3	Caucasian	10 m	29 years	Yes[b]	NA	No
4	Caucasian	12 m	8 years	Yes[a], +KCl/-KCl 0.6 vs 2.7	c.79A>T/c.1223_1226dup exon2/exon12	Yes
5	Caucasian	15 m	17 years	Yes[c] 6% of normal activity	c.1189c>G/c.1189C>G exon12/exon12	No
6	Caucasian	15 m	6 years	Yes[a], +KCl/-KCl 1.1 vs 2.6	c.446del/c.1163G>A exon6/exon11	No
7	Caucasian	8 years	21 years	Yes[a], +KCl/-KCl 1 vs 2.8	c.455G>C/c.1016_1018dup exon6/exon11	No
8	Caucasian	18 m	17 years	Yes[c] 1% of normal activity	c.455G>C/c.52dupC exon6/exon2	No
9	Caucasian	31 m	5 years	Yes[a], +KCl/-KCl 1.2 vs 2.5	NI/NI	No
10	Tunisian	18 m	14 years	Yes[a], +KCl/-KCl 1 vs 2	c.83_84del/c.83_84del exon2/exon2	No
11 (sib of 10)	Tunisian	Neonatal FS	3 years	NA	c.83_84del/c.83_84del exon2/exon2#	Yes
12	Moroccan	24 m	8 years	Yes[a], +KCl/-KCl 0.9 vs 2.2	c.1033_1034del/c.1033_1034del exon11/exon11	No
13 (sib of 12)	Moroccan	10 m	7 years	NA	c.1033_1034del/c.1033_1034del exon11/exon11	No
14	Caucasian	19 m	19 years	Yes[c] 6% of normal activity	c.534G>T/c.1059T>G exon6/exon11	No
15	Caucasian	24 m	18 years	Yes[c] 5.5% of normal activity	c.760G>A/c.760G>A exon8/exon8	Yes
16	Caucasian	22 m	11 years	Yes[a], +KCl/-KCl 0.7 vs 2.1	c.414_415del/c.414_415del exon5/exon5	No
17 (sib of 16)	Caucasian	FS at 5 m	9 years	NA	c.414_415del/c.414_415del exon5/exon5#	Yes
18	Caucasian	11 m	6 years	Yes[a], +KCl/-KCl 1.1 vs 2.3	c.83_84del/c.83_84del exon2/exon2	No
19 (sib of 18)	Caucasian	FS at 2 m	4 years	NA	c.83_84del/c.83_84del exon2/exon2#	Yes
20 (sib of 18/19)	Caucasian	FS at 7 m	2 years	NA	c.83_84del/c.83_84del exon2/exon2#	No
21	Caucasian	11 m	27 years	Yes[b]	c.946_948dup/c.1167G>A exon10/exon12	Yes
22 (sib of 21)	Caucasian	FS at 7 m	26 years	Yes[c] 9% of normal activity	c.946_948dup/c.1167G>A exon10/exon12	No
23	Caucasian	22 m	12 years	Yes[a], +KCl/-KCl 0.8 vs 1.6	c.765A>T/c.814C>T exon8/exon8	No
24	Caucasian	11 m	15 years	Yes[a], +KCl/-KCl 1.3 vs 2.3	c.760G>A/NI exon8/NI	No
25	Caucasian	24 m	23 years	Yes[c] 2% of normal activity	c.578T>C/c.826+5G>T exon6/exon8	No
26	Caucasian	72 m	15 years	Yes[a], +KCl/-KCl 1.5 vs 3.2	c.578T>C/c.826+5G>T exon6/exon8	No

FS familial screening, *m* months, *NA* not available, *NI* not identified, # performed in the index case

[a] enzyme assay, Gibson et al 2000; the T2 enzyme activity is expressed as the ratio of the activity in the presence of K+ to the activity in the absence of K+. In Table 1, the first value of the ratio refers to the patient while the second one after vs refers to the control

[b] enzyme assay, Middleton and Bartlett 1983

[c] enzyme assay, Gibson et al 1992

Table 2 Clinical and neuroradiological features in the six patients with neurological involvement

Patients (n = 6)	Consanguinity	Age at diagnosis	Acute ketoacidosis	Age at metabolic crisis	Neurological evaluation		Evolution	Brain MRI
					Before metabolic crisis	After metabolic crisis		
Patient #11	Yes	Neonatal FS	No				Motor delay, axial hypotonia and dystonia from the age of 5 m	Bilateral T2 hyperintensities in putamen at 6 m
Patient #17	No	FS at 5 m	No				Motor delay, ataxia, dyskinesia, dystonia from the age of 19 m	Bilateral T2/FLAIR hyperintensities in putamen at 6 years
Patient #19	Yes	FS at 2 m	Yes	5 m	Motor delay, axial hypotonia	Unchanged	Axial hypotonia, dystonia at 2.5 years	Bilateral T2 hyperintensities in putamen at 11 m
Patient #4	No	1 years	Yes	1 years	Motor delay, axial hypotonia, dyskinesia	Unchanged	Improvement of dyskinesia, dyspraxia	Normal
Patient #15	UD	2 years	Yes	2 years	Normal	Hypotonia, dyskinesia, dystonia, ataxia	Progressive improvement with physical support, attentional disturbance, learning disabilities	Increased T2 intensities in both putamen and dentate nuclei, 4 m after the acute episode
Patient #21	No	11 m	Yes	11 m	Normal	Normal	Choreoathetosis and dystonia from the age of 15 years	Increased T2 intensities in posterior part of internal capsules at 15 years

FS familial screening, *UD* undefined, *m* months

with involuntary movements and dystonia in the upper limbs. Weight and growth failure occurred since the age of 4 months. Metabolic work-up including thyroid function, creatine phosphokinase, lactate and amino acid chromatography in plasma, urine and cerebrospinal fluid (CSF) proved normal. CSF neurotransmitter profile and blood uric acid were unremarkable. Brain magnetic resonance imaging (MRI) at the age of 6 months showed bilateral increased signal intensity and mild putamen atrophy in T2-weighted sequences (Fig. 1). Magnetic resonance spectroscopy (MRS) produced nonspecific findings, with a mild decrease in N-acetylaspartate (NAA) peak.

Patient #17, a 9-year-old boy, was the second child of non-consanguineous parents. His older sister had been diagnosed as T2-deficient following a severe ketoacidotic crisis with coma at the age of 22 months. Her psychomotor development was subsequently normal. Patient #17 was presymptomatically diagnosed at the age of 5 months based on a typical urinary organic acid profile. He never experienced an acute ketoacidotic episode until now. However, his motor development was delayed. He sat at 12 months of age and walked at 2.5 years with unsteady gait. Clinical examination at the age of 19 months revealed truncal ataxia and dystonic postures in his upper limbs. Follow-up was interrupted until the age of 6 years. At this age, gait disturbance persisted with ataxia, mild dyskinesia, fine motor difficulties and dystonic postures. Neuropsychological evaluation at the age of 5 years showed normal intellectual abilities with a total intellectual quotient of 126. Brain MRI at 6 years revealed bilateral T2/FLAIR hyperintensities in the putamen (Fig. 2 a-b). MRS showed a mild decrease in NAA level. Further metabolic investigations, including congenital disorders of glycosylation, blood α-foetoprotein, uric acid, copper, ceruloplasmin, lactate

Fig. 1 MR imaging of patient #11, aged 6 months at the time of imaging. Axial T2-weighted sequence through basal ganglia: bilateral hypersignal of the putamen (*arrows*)

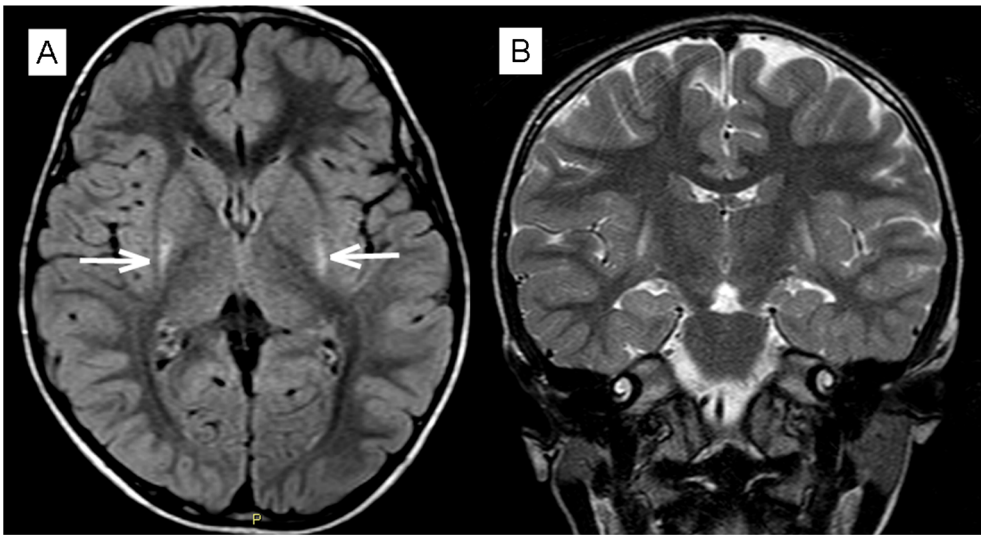

Fig. 2 **a-b** MR imaging of patient #17, aged 6 years at the time of imaging. Axial FLAIR (**a**), coronal T2-weighted-(**b**) sequences: bilateral hypersignal of posterior part of the putamen (*arrows*); note that myelination allows a good delineation of the lesions

and amino acid chromatography in plasma, urine and cerebrospinal fluid, were normal.

Patients with neurological abnormalities before the first ketoacidotic episode

Patient #19 was the fourth child born of French consanguineous parents. The elder brother had experienced a severe ketoacidotic episode at 11 months of age, but the clinical evolution was favourable, and his psychomotor development remained normal. Patient #19 was presymptomatically diagnosed using systematic screening at 2 months of age in view of the family history. Axial hypotonia was noted from the first months of life. At age 5 months, he presented with a bronchiolitis and simultaneously the first ketoacidotic episode, but the latter rapidly resolved with a glucose infusion. There was no alteration of consciousness. Laboratory data included blood pH of 7.2 (normal range: 7.30-7.44), bicarbonate 8.7 mmol/L (normal range: 21-27) and glucose 3.6 mmol/L. Neurological examination after this episode was unchanged. At age 10 months, axial hypotonia persisted without regression. He was able to remain in a sitting position at the age of 12 months and could walk at 22 months of age. Brain MRI was performed at 11 months of age and revealed bilateral increased signal intensity in the putamen on T2-weighted sequences (Fig. 3 a-b).

Neurological examination at 2.5 years of age showed dystonic postures of the right upper limb. Diagnostic work-up was normal, including uric acid and creatine metabolites, lactate and amino acids in blood and urine. His current development is marked by mild learning and speech disabilities requiring educational and physical support.

Patient #4 is a 7-year-old girl and the third child of non-consanguineous parents. She was evaluated at 11 months for

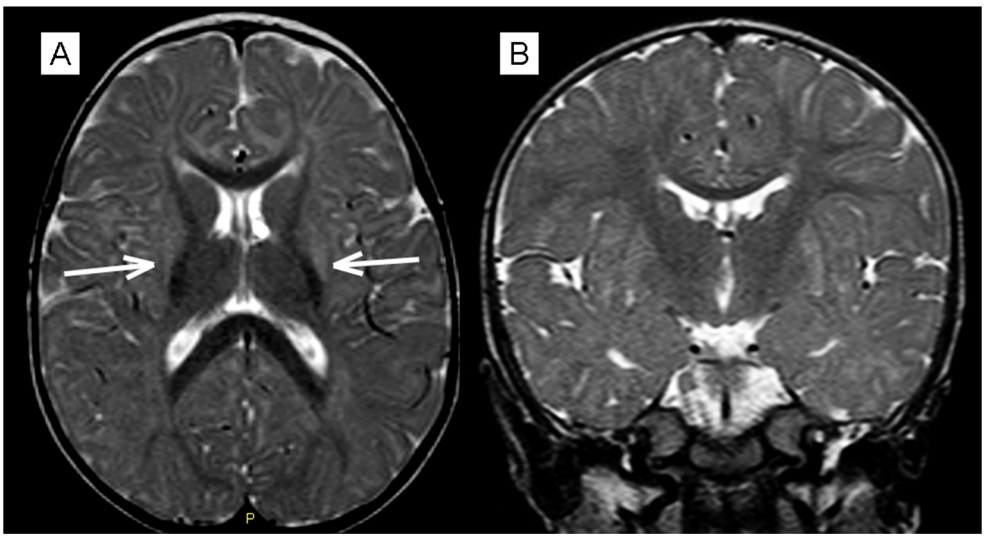

Fig. 3 **a-b** MR imaging of patient #19, aged 11 months at the time of imaging. Axial (**a**) and coronal (**b**) T2-weighted sequences: bilateral hypersignal of the putamen (*arrows*)

psychomotor delay. Sitting position was unsteady, and continuous involuntary movements of the trunk and limbs were noted. The girl failed to gain weight after the 4th month of life. The first ketoacidotic episode occurred at 12 months during a febrile episode. Blood pH was 7.26, bicarbonate 8 mmol/L, anion gap 28 mmol/L (normal range: 8-16) and glucose was severely decreased (0.9 mmol/L). She recovered quickly after glucose and fluid infusions. Brain MRI performed after the decompensation proved normal. Nocturnal enteral feeding was used 1 month after the metabolic crisis, until the age of 4 years. Enteral feeding enabled restoration of weight parameters. The girl walked at 26 months. Clinical follow-up showed global hypotonia, coordination disorders, dyskinesia and speech delay, all of which progressively resolved during childhood. However, her dyspraxia still persisted at the time of writing.

Patients with neurological abnormalities after the first ketoacidotic episode

Patient #15 was the second child of non-consanguineous parents. Psychomotor development was normal until the age of 2 years, when the boy experienced his first metabolic crisis during an intercurrent infection, resulting in ketoacidotic coma with profound hypoglycaemia (1.27 mmol/L). Blood pH was 6.92, bicarbonate 6 mmol/L. Invasive ventilation support was needed over 48 h. On day 2 post-admission, the boy suffered from seizures. Clinical examination revealed truncal hypotonia, as well as dyskinesia and dystonic postures of the limbs. Brain MRI performed 4 months after the initial decompensation showed signal abnormalities in the putamen and dentate nuclei, with improvement on brain MRI at the age of 4 years. Motor and visuospatial disabilities persisted with learning difficulties, along with attentional and behaviour disturbances.

Patient #21 displayed her first severe ketoacidotic episode at the age of 11 months. Blood pH was 6.95, with bicarbonate levels at 3 mmol/L. While intensive care support was required, there was no detailed information available on this first episode. Immediate outcome was favourable without neurological sequelae. Annual neurological examination was normal until the age of 15 years, when she developed choreoathetoid movements of the left lower limb and postural tremor in the left upper limb. Dystonic postures of the left foot appeared during walking. Brain MRI at the age of 15 years revealed increased signal intensity within the posterior arms of internal capsule and within the globus pallidi.

Discussion

Most T2-deficient patients have normal psychomotor development and a favourable neurological outcome if acute ketoacidotic episodes are promptly managed. Acute ketoacidosis may, however, be a life-threatening condition. Profound metabolic acidosis, osmotic diuresis, dehydration and glucose disturbances are metabolic derangements that may potentially lead to cerebral lesions, impaired consciousness and ultimately death.

Neurological impairment in T2 deficiency is usually attributed to the sequelae of severe metabolic crises (Yalçinkaya et al 2001), as was the case for patient #15. This patient recovered from a severe ketoacidotic decompensation with residual movement and cognitive disorders along with basal ganglia damage. In our series, however, 5/26 patients (19%) suffered from neurological involvement, including delayed motor development and extrapyramidal signs, without any clear relationship to a ketoacidotic event.

Four T2-deficient children with encephalopathic presentation prior to the first ketoacidotic episode were reported as long ago as 1994 (Ozand et al 1994). All four had delayed development and axial hypotonia. For three of them, neuroradiologic imaging showed abnormal signal in the putamen bilaterally. More recently, a 17-year-old male was described (Buhas et al 2013) who experienced delayed development and continuous choreoathetoid movements from the age of 10 months onwards, despite his first ketoacidotic episode only occurring at the age of 5 years. Brain MRI at the age of 4 years revealed bilateral hyperintensities in both the putamen and cerebral peduncles. Diagnosis of T2 deficiency was confirmed at the age of 17 years.

The pathomechanisms underlying the neurological involvement and basal ganglia lesions in T2 deficiency remain unclear, especially when these occur independently of an identifiable ketoacidotic episode.

Interestingly, similar clinical and neuroradiological findings have not yet been observed in the two other genetic causes of recurrent ketoacidosis, namely succinyl-CoA: 3-oxoacid CoA transferase (SCOT, EC 2.8.3.5) deficiency (OMIM 245050) (Mitchell and Fukao 2001; Fukao et al 2014) and the recently described monocarboxylate transporter 1 (MCT1) deficiency (OMIM 616095) (van Hasselt et al 2014; Balasubramaniam et al 2016).

In contrast to SCOT deficiency and MCT1 disease, T2 deficiency involves the impaired catabolism of isoleucine, a branched-chain amino acid (BCAA) and exposes affected patients to the accumulation of 2-methylacetoacetate, 2-methyl-3-hydroxybutyrate and tiglylglycine. It has not yet been identified which of these metabolites is responsible for neurotoxicity, and the underlying pathomechanisms have only been hypothesized.

The *in vitro* impact of 2-methylacetoacetate and 2-methyl-3-hydroxybutyrate on energy metabolism was investigated in the cerebral cortex of young rats (Rosa et al 2005; Leipnitz et al 2010). These metabolites were found to inhibit aerobic energy metabolism and induce oxidative stress. In brief, 2-methyl-acetoacetate was shown to induce lipid peroxidation and protein oxidative damage, and to reduce glutathione levels

(GSH), pathomechanisms which may be involved in the brain damage of T2-deficient patients (Leipnitz et al 2010).

Pathological metabolites that accumulate in T2 deficiency are monocarboxylic acids that can cross plasma membranes through monocarboxylate transporters (Murín et al 2009; Yudkoff 2016), limiting the intracerebral trapping of organic acids (Kölker et al 2006, 2008). However, these intermediates are metabolized as CoA esters through the catabolic pathway of isoleucine. Acyl-CoA esters do not cross biological membranes, thus facilitating the toxic intramitochondrial accumulation of acyl-CoA esters (Mitchell et al 2008). Furthermore, despite the fact that brain metabolism of isoleucine has not been fully elucidated to date, much evidence points to its major contribution to healthy neurologic function (Suryawan et al 1998; Bixel et al 2001; Murín et al 2009; Hull et al 2012; Yudkoff 2016).

In classical organic aciduria, basal ganglia lesions usually occur during metabolic decompensations or in the early recovery period. However, prominent neurological disease, including extrapyramidal signs without acute episodes of ketoacidosis, has also been described (Nyhan et al 1999; Sholl-Bürgi et al 2009). In these patients, increased brain lactate levels on magnetic resonance spectroscopy (MRS) can even occur under stable metabolic conditions, supporting the hypothesis of a chronic mitochondrial dysfunction (Chemelli et al 2000; Baker et al 2015), possibly due to the toxicity of accumulated organic acids and their CoA esters.

Due to the underlying organic aciduria in T2 deficiency, L-carnitine supplements are often given, and all patients in our series were treated with the standard oral dose of 50-100 mg/kg/day. The role of a protein-restricted diet in preventing cerebral toxicity in T2-deficient patients remains controversial. There may be a vulnerable period for acute metabolic crises in the first years of life, as well as a risk of chronic neurological impairment without an acute episode, as in glutaric aciduria type I. Dietary treatment in T2 deficiency could be effective in preventing both metabolic crises in the first years of life and chronic neurological intoxication in older children.

In recent guidelines for the treatment of ketone body utilization disorders (Hori et al 2015), mild protein restriction (1.5-2 g/kg/day) is proposed. In our retrospective study, a rigorous evaluation of individual protein intake could not be performed for all patients, limiting our ability to analyse the relationship between protein intake and clinical course. There was, however, no obvious correlation. For example, patient #17 developed neurological symptoms whereas his older sister who had experienced a ketoacidotic episode did not, though both had a protein intake of approximately 2.5-3 g/kg/day, despite recommendations to reduce this. Patient #11 presented with truncal hypotonia and dystonia and his older sister remained healthy, though both had a protein intake of approximately 1-1.5 g/kg/day. There was no significant difference in terms of clinical monitoring, dietary management and biochemical findings during follow-up between patient #11 and #17 and their respective older siblings.

In line with this, patients with neurological symptoms were compared to the 20 others with metabolic crisis but without neurological manifestations, and no clear difference could be observed in terms of biochemical and diet patterns, nor in terms of frequency of metabolic episodes. Dietary management varied across the different metabolic centres. Both groups included some patients with no dietary restriction while others experienced protein and fat restriction. However, among the five patients who developed neurological symptoms independent of ketoacidosis, only patient #11 followed a low-protein diet before neurological symptoms appeared. Moreover, this patient had severe failure to thrive from the age of 4 months, which may have caused a chronic catabolic state. Nevertheless, most T2-deficient patients are healthy, even without any dietary restriction and despite initial decompensation, which is related to catabolic conditions. Consequently, effective methods to evaluate the impact of protein intake on clinical outcome may prove challenging. While correlations between protein intake and body fluid excretion of T2 metabolites exists, the neurological outcome cannot be predicted solely based on quantifying body fluid metabolites (Chemelli et al 2000; Kölker et al 2013). Furthermore, as previously described (Fukao et al 2001, 2014), there was no genotype-phenotype relationship established, with great phenotypic variability observed within the same family. Thus, it remains unclear why some patients develop neurological signs without ketoacidosis while their siblings who developed metabolic crises remained healthy, despite having a similar diet and carrying the same mutations (and therefore having similar residual enzyme activity and biochemical phenotype). Other genetic factors could be involved, which might modulate an individual critical threshold of cellular dysfunction.

Finally, early detection of T2-deficient patients can influence clinical management through the implementation of preventive measures to avoid ketoacidotic crises. However, preventing ketoacidotic episodes does not appear to prevent neurological symptoms. Newborn screening could allow us to better define the neurological outcome of T2-deficient patients, independent of ketoacidosis. Further data are needed to assess the potential impact of dietary management on neurological outcome. Therefore, it would be useful to collect clinical, dietary, neuroimaging, biochemical and genetic data using registries, which could provide descriptions of the wider natural history outside metabolic crises, compare the treatments between different centres and countries, and allow the identification of potential prognostic indicators. With that in mind, this study invites clinicians to actively evaluate the

neurological findings and to record the metabolite levels and details of dietary management during follow-up.

Conclusion

In our series of T2-deficient patients, 5/26 (19%) developed neurological impairment with extrapyramidal signs without a clear relationship to a ketoacidotic decompensation. Four of them exhibited basal ganglia lesions on brain imaging with the putamen being preferentially involved. No genotype-phenotype relationship could be established since siblings carrying similar mutations exhibited highly variable phenotypes. These results emphasize that neurological involvement may occur in T2-deficient patients, independently of acute episodes. The impact of a low-protein diet as a preventive measure designed to avoid neurological impairment deserves further evaluation. Multicentre and long-term follow-up of newborns diagnosed by means of newborn screening may help to clarify the pathogenesis of this neurometabolic association.

Compliance with ethical standards

Conflict of interest None declared.

Informed consent 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 2000.

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