In vitro probe acylcarnitine (IVP) assay using MS/MS

An IVP assay was performed, as described, with some modifications [13, 15, 17], and principle of IVP assay was shown Fig. 2. Briefly, 3×10^6 cells were seeded in triplicate onto a six-well microplate (35 mm i.d.; Iwaki) and cultured until confluent. After washing twice with Dulbecco's phosphate buffered saline (DPBS; Invitrogen, Carlsbad, CA, USA), the cells were subsequently cultured for 96 h in 1 ml of a special experimental minimal essential medium (MEM) containing bovine serum albumin (0.4 % essential fatty acid-free BSA; Sigma), two different concentrations of C0 (Sigma)—10 μmol/L (reduced level, lower compared with physiological level) and 400 μmol/L (excess level)—and unlabelled palmitic acid (0.2 mmol/L; Nacalai Tesque). C0 and AC levels in the culture medium (extracellular fraction) and in the intracellular extract were analyzed after a 96-h incubation period using MS/MS (API 3000; Applied Biosystems, Foster City, CA, USA), as described [18].

Intracellular acylcarnitine extraction

Intracellular C0 and ACs were extracted using the Folch method, with some modification [16]. Briefly, harvested cells were washed twice with DPBS buffer. The cell pellet was resuspended in 100 μl volume of DPBS buffer and immediately frozen in liquid N_2. In order to separate phospholipids and cell debris, 250 μl of Folch reagent (chloroform/methanol, 2:1) was added to the resuspended cell pellet. After vigorous mixing using a vortex mixer, the solution was centrifuged for 10 min at 15,000 rpm at 4 °C. The debris layer around the interface between the aqueous and lipid phases was removed, and the extracted aqueous and lipid phases were mixed and thereafter dried under a nitrogen stream at 50 °C. ACs in culture medium supernatants and extracted intracellular ACs lysate were analyzed

using MS/MS (API 3000; Applied Biosystems, Foster City, CA, USA). Briefly, methanol (200 μl) including an isotopically labeled internal standard (Cambridge Isotope Laboratories, Kit NSK-A/B, Cambridge, UK) was added to 10 μL of supernatant from culture medium and extracted intracellular ACs, for 30 min. Portions were centrifuged at 1,000×g for 10 min, and then 150 μL of supernatant was dried under a nitrogen stream and butylated with 50 μL of 3 N n-butanol-HCl at 65 °C for 15 min. The dried butylated sample was dissolved in 100 μL of 80 % acetonitrile/water (4:1 v/v), and then the ACs in 10 μL of the aliquots were determined using MS/MS [18] and quantified using Chemo-View™ software (Applied Biosystems/MDS SCIEX, Toronto, Canada).

Protein concentration and cell viability

Protein concentrations were measured by a modification of the Bradford method using the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA) [19]. The percentage of viable cells was determined at 24, 48, 72, and 96 h of incubation using the modified 3-(4,5-dimethyl-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay [20].

Data and statistical analysis

The results are expressed as mean±SD from at least three independent experiments for IVP assay in each cultured cell and three intra-assays and three inter-assays for recovery of standard AC solutions, and statistical significance was evaluated using Student's t test in Microsoft Excel. The AC concentrations were expressed as nanomoles per milligram protein.

Results

Recovery of ACs during Folch extraction

The AC standards in the aqueous or lipid fraction were analyzed separately using MS/MS, after extraction by the Folch procedure, and compared with direct analysis of the total mixed standard solutions using three inter-assays and three intra-assays of analysis of standard AC solution. As shown in Fig. 3, most of the C6 and C8-carnitines fractionated to the aqueous phase, while almost all C16-carnitine was exclusively retained in the lipid phase. The amount of C10-carnitine was comparable in both aqueous and lipid phases.

To determine the loss of C0 and ACs during Folch extraction, the standard AC solution was analyzed directly after routine sample preparation for MS/MS and compared with that after Folch extraction. The recovery of ACs in the
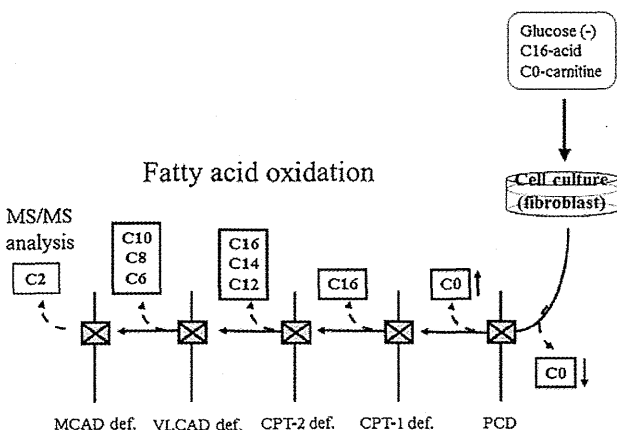

Fig. 2 Principle of in vitro probe acylcarnitine assay. *C2, C4, C6, C8, C10, C12, C14,* and *C16* represent acylcarnitines
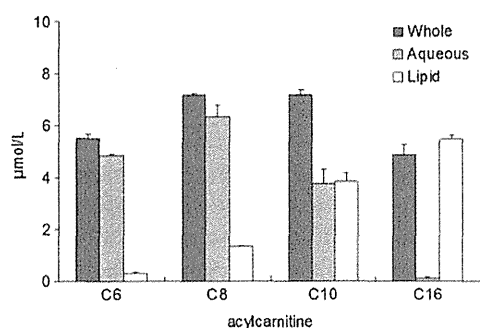
Fig. 3 Recovery of ACs during extraction using the Folch method. Standard solutions of 10 μmol/L each of C6-, C8-, C10-, and C16-carnitine were used to determine the recovery of ACs in the aqueous and lipid fractions during extraction using the Folch method. *Grey column*: ACs in the whole extract after Folch method; *striped column*: ACs in the aqueous fraction of Folch extraction; *open column*: ACs in lipid fraction of Folch extraction. Data are expressed as mean±SD (micromoles per liter) from three intra-assays and three inter-assays, and statistical significance was evaluated using Student's *t*test in Microsoft Excel

standard solutions after direct analysis and Folch extraction procedure was analyzed three times by inter-assay. The inter-assay CV of acylcarnitines ranged from 3.21 to 8.33 %. No statistical difference was seen between direct analysis and after Folch extraction.

Acylcarnitine profile in extracellular medium of cultured fibroblasts with excess and reduced concentrations of free carnitine

Using fibroblasts from various carnitine cycle disorders, AC profiles were determined in the extracellular medium with reduced or excess concentration of C0. Reported conventional IVP assay used excess levels of C0 (400 μmol/L) [14,

15, 17, 21]. With excess amount of C0 (Table 1, "Medium (C0-excess, 400 μM)"), a selective increase in C16 and a decrease in acetylcarnitine (C2) was observed in cases of CPT2-deficient fibroblasts. AC profiles in media from PCD- and CPT1-deficient fibroblasts were similar to that of healthy controls. In PCD fibroblasts, C2 was 53.1 % of the normal control while C2 in CPT1-deficient fibroblasts was 140 % of the normal control. No statistical difference in C0 level was observed among CPT2-, PCD-, and CPT1-deficient fibroblasts and a healthy control.

In the extracellular medium containing reduced C0, C16 remains higher in cells with CPT2 deficiency, while AC profiles were similar to those observed in C0-excess for PCD- and CPT1-deficient cells and the healthy controls (Table 1, "Medium (C0-reduced, 10 μM)").

Acylcarnitine profile in intracellular lysate with various concentrations of free carnitine

The intracellular C0 and ACs were measured after AC extraction using the Folch method. C16 in the intracellular lysate from CPT2-deficient fibroblasts was significantly elevated in both reduced and excess C0 conditions similar to those in extracellular medium, and diagnostic significant was kept. In the excess C0 condition, CPT1- and PCD-deficient fibroblasts could not be distinguished clearly; based on the C0 levels, even C16 level was relatively low (Fig. 4a). On the other hand, the intracellular C0 under conditions with reduced C0 was 41.78±1.47 and 6.31± 2.88 nmol/mg protein/96 h in the normal controls (*n*=6) and patients with PCD (*n*=3), respectively, and the C0 levels of PCD cells were significantly lower (*p*<0.001) as shown in Fig. 4b. This indicated that the C0 uptake was significantly decreased in PCD compared with control in

Table 1 Acylcarnitine profiles of in vitro probe acylcarnitine assay

	Acylcarnitines, nmol/mg protein/96 h						
	C0	C2	C6	C8	C12	C14	C16
Medium (C0 excess, 400 μM)							
Control (*n*=6)	411.74±23.08	11.80±1.54	2.60±0.09	1.70±0.47	0.79±0.22	0.34±0.19	2.06±0.77
PCD (*n*=3)	432.18±18.76	6.25±0.96	2.09±0.40	0.94±0.54	0.41±0.33	0.20±0.10	1.72±0.57
CPT-1 (*n*=1)	357.69±34.16	16.52±5.60	1.73±0.87	0.54±0.94	0.18±0.14	0.17±0.16	1.36±0.98
CPT-2 (*n*=3)	376.56±42.71	6.88±0.72	0.94±0.65	0.41±0.22	1.70±0.35	0.80±0.05	18.73±1.07
Medium (C0 reduced, 10 μM)							
Control (*n*=6)	9.85±0.30	1.70±0.74	0.78±0.30	0.18±0.09	0.10±0.08	0.03±0.01	0.51±0.11
PCD (*n*=3)	10.03±0.71	0.74 ±0.33	0.75±0.31	0.06±0.04	0.03±0.01	0.01±0.01	0.20±0.08
CPT-1 (*n*=1)	11.06±0.75	7.56±3.10	0.98±0.30	0.55±0.62	0.09±0.09	0.08 ±0.07	0.01 ±0.02
CPT-2 (*n*=3)	9.73±1.94	0.64±0.23	0.54±0.20	0.11±0.03	0.22±0.06	0.04±0.01	2.79±0.38

The results are expressed as mean±SD from three independent experiments with triplication in each cell line. The AC concentration was expressed as nanomoles per milligram protein. *C0* free carnitine, *C2* acetylcarnitine, *C6* hexanoylcarnitine, *C8* octanoylcarnitine, *C12* dodecanoylcarnitine, *C14* myristoylcarnitine, *C16* palmitoylcarnitine

C0-reduced condition. Concentration of C16 was also significantly low in PCD in C0-reduced condition. Under the C0-reduced condition, intracellular C0 was much higher, but C16 was much lower in CPT1-deficient fibroblasts, compared with the levels in controls (Fig. 4b).

The ratio of intracellular C0 to extracellular C0 in PCD was significantly lower than that of the controls ($p<0.001$) in the C0-reduced condition, while that in C0-excessive condition was not significantly different (Fig. 5). Cell viability was measured using the MTT assay under reduced or excess concentrations of C0. The percentage of viable cells cultured in C0-reduced medium was equivalent to that in C0-excess media (data not shown).

Discussion

The present study developed a novel IVP assay for the accurate diagnosis of PCD and CPT1 deficiency. Although previous studies reported that IVP assay was a powerful method for the diagnosis of most FAO disorders [13, 14, 21], this assay turned out to be unable to identify PCD and CPT1 deficiencies. At first, we used a C0-excess experimental medium, which contained 400 μmol/L of C0, according to previous reports [13, 14, 21]. Extracellular

a

C0-EXCESS

o Control
□ CPT1
▲ PCD
◇ CPT2

C16
(nmol/mg protein/96h)

b

C0-REDUCED

o Control
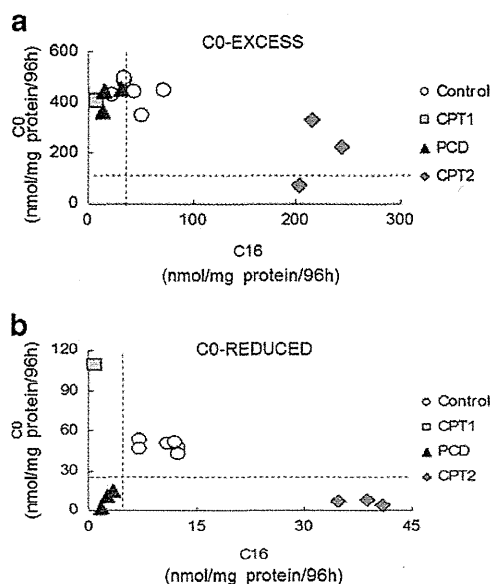□ CPT1
▲ PCD
◇ CPT2

C16
(nmol/mg protein/96h)

Fig. 4 Intracellular C0 and C16 correlation in patients with carnitine cycle disorders. **a** C0-excessive condition (−E); **b** C0-reduced condition (−R). open circle: healthy control (n=6); closed triangle: PCD (n=3); closed square: CPT1 deficiency (n=1); closed diamond: CPT2 deficiency (n=3). Cells were incubated in experimental medium with 400 or 10 μmol/L of free carnitine and 200 μmol/L of palmitic acid. After 96-h incubation, cells were harvested, and intracellular free carnitine (C0) and palmitoylcarnitine (C16) were extracted using Folch method and measured using MS/MS. Data of mean values of triplicates are presented (nanomoles per milligram protein per 96 h)
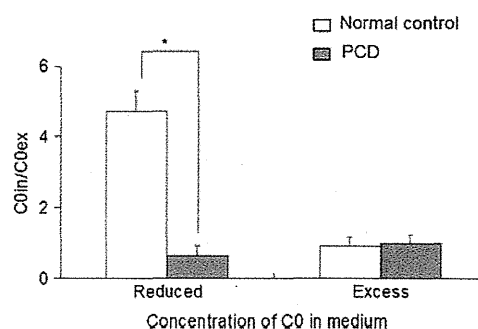
□ Normal control
▩ PCD

C0in/C0ex

Reduced   Excess
Concentration of C0 in medium

Fig. 5 Ratio of intracellular C0 to extracellular C0. Open square: normal control (n=6); closed square: PCD (n=3). Extra- and intracellular C0 of cells with normal control and PCD were measured in C0-reduced (10 μmol/L) and C0-excess (400 μmol/L) conditions using MS/MS. Data are expressed as mean±SD of six normal controls and three patients with PCD. Experiment in each cell line was repeated twice with triplications. Significant differences between normal control and PCD are shown as *$p<0.001$

AC profiles of patients with PCD and CPT1 deficiency showed a pattern similar to that of normal controls by the conventional assay that contains excessive C0 (400 μmol/L) in the culture medium, since C0 moves across the cell membrane down its concentration gradient by passive diffusion. Long-chain fatty acids are transferred across the inner mitochondrial membrane with the assistance of carnitine and carnitine cycle enzymes. The subsequent FAO functions normally even in PCD, and AC profile in PCD is similar to that in normal FAO. Next, we used 50 μmol/L of C0 because the normal range of free carnitine in human plasma was approximately 25 to 50 μmol/L [6]. However, there was no diagnostic difference compare with C0-excess condition, and data are not shown. We analyzed IVP assay in C0-deficient condition (10 μmol/L of C0).

It is known that fibroblasts and muscle and cardiac cells have a high-affinity, low-capacity transporter system [22], and carnitine concentrations in the tissues are much higher than those in serum [23]. Analysis of intracellular C0 and ACs is more relevant for the diagnosis of PCD and CPT1 deficiency because it was shown that C0 was decreased in PCD and increased in CPT1 deficiency in those tissues. When we analyzed cell lysates with MS/MS after direct sonication, artificial peaks of ACs were detected, and the background peaks of mass spectrum were high and hampered the subsequent analyses (data not shown). Hence, we extracted intracellular ACs using a modified Folch method and analyzed both the intracellular lysate and the extracellular medium. This allowed visualization of clear peaks of C0 and ACs in the intracellular lysate, validating that the Folch extraction can be used for simultaneous quantitation of intracellular C0 and a wide range of ACs (short- to long-chain AC).

Uptake of C0 and abnormalities in ACs were associated with the concentration of C0 in culture medium. In the C0-excess condition, it was hard to differentiate PCD from control

cells. Levels of C0 and C16 were overlapped with those of normal control. On the other hand, in the C0-reduced condition, intracellular C0 was significantly decreased in PCD while being increased in CPT1 deficiency, compared with that in normal control. C0-reduced medium was changed after fibroblasts equilibrated in MEM, and normal control could force to uptake free carnitine in C0-deficient condition while cells with PCD could not uptake sufficiently in that condition. Furthermore, the following fatty acid oxidation cycle interrupted, and C16 also decreased in PCD. This correlation of C0 and C16 in the C0-reduced condition is more informative for the diagnosis of carnitine cycle disorders (Fig. 4b). Since cells with PCD cannot uptake C0 via the cell membrane, the finding of reduction of both C0 and C16 is specific for PCD. In case of CPT1 deficiency, C0 uptake is normal, but it cannot bind acyl-CoA ester, resulting in reduced long-chain acylcarnitine production, and FAO is disturbed. Therefore, the stored intracellular ACs were consumed by FAO, and intracellular C16 as well as total ACs were decreased, and C0 was accumulated in intracellular lysate. In contrast, the AC profile of low level of C0 and high level of C16 is diagnostic for CPT2 deficiency. In this disease, normally transferred long-chain AC cannot be converted back from ACs to acyl-CoA esters and C0, the substrate for FAO. Additionally, the ratio of intracellular and extracellular C0s can sensitively distinguish PCD from control in the C0-reduced medium because carnitine transporter of normal cells was forced to uptake C0 up to physiological level in C0-reduced condition while cells with PCD failed for it. In excessive C0 condition, ratio of intracellular and extracellular C0 was similar to that in normal control and PCD since C0 transfer by passive diffusion across the cell membrane.

In conclusion, the simultaneous analysis of intracellular and extracellular C0 and ACs under the various concentrations of free carnitine in the culture medium is useful for diagnosis of FAO, especially carnitine cycle disorders. This study confirms that the newly modified IVP assay is an easy and safe method to diagnose PCD and CPT1 deficiency.

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Coagulopathy in Patients With Late-Onset Ornithine Transcarbamylase Deficiency in Remission State: A Previously Unrecognized Complication
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Coagulopathy in Patients With Late-Onset Ornithine Transcarbamylase Deficiency in Remission State: A Previously Unrecognized Complication

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abstract

The late-onset type of ornithine transcarbamylase (OTC) deficiency is almost asymptomatic before an abrupt onset of metabolic crisis in adolescence. This study focused on coagulopathy in OTC deficiency. We collected laboratory data regarding coagulation from OTC-deficient patients in Kyushu University Hospital in Japan or from cases reported from previous articles. Five patients with late-onset OTC deficiency, admitted to Kyushu University Hospital at the first metabolic attack or who presented at the outpatient clinic in the hospital, were analyzed, and 3 additional cases of OTC deficiency with coagulopathy in previous articles were included. As a result, the blood ammonia levels in these patients were remarkably high at the time of the metabolic attack, and prothrombin times were far below the normal level. The prothrombin times remained significantly abnormal on remission, despite almost normal levels of blood ammonia, serum aspartate aminotransferase, and alanine aminotransferase. Coagulation abnormality is a previously unidentified complication of OTC deficiency in remission state. This information will aid in the identification of patients with OTC deficiency before a lethal metabolic crisis occurs during adolescence. *Pediatrics* 2013;131:e1–e4

Ornithine transcarbamylase (OTC) deficiency, an X-linked disorder, is the most frequent urea cycle disorder characterized by an acute clinical manifestation of hyperammonemia, accounting for >60% of all urea cycle disorders.[1] The classic type of OTC deficiency demonstrates a severe hyperammonemia during infancy and sometimes proves fatal, whereas the late-onset type is almost asymptomatic before an abrupt onset of metabolic crisis in adolescence.[2–4] The early diagnosis of the late-onset type is sometimes difficult because the first crisis usually appears in either adolescent or adult patients who have exhibited normal growth and development. Specific findings of abnormal metabolites in urine such as orotic acid or uracil are critical for making an accurate diagnosis of OTC deficiency.[5] In contrast, the biochemical markers in routine examinations are usually within the normal ranges. Consequently, the identification of specific markers in a routine biochemical analysis would be clinically beneficial for patients with OTC deficiency. Coagulopathy in OTC deficiency is usually accompanied by acute liver failure, and slight changes in the coagulation data in a remission state have been overlooked as nonspecific findings. Hence, coagulopathy is not recognized as a consequence of OTC deficiency by itself.

This report focused on the presence of any underlying coagulation abnormality in OTC deficiency during a metabolically compensated state. We retrospectively collected laboratory data on coagulation from the clinical records of OTC-deficient patients of Kyushu University Hospital in Japan. A bibliographic search was also conducted to determine whether any coagulopathy had previously been noticed before the abrupt onset of metabolic attack in cases of OTC deficiency.

METHODS

Patients with OTC deficiency, admitted to Kyushu University Hospital at the first metabolic attack or who presented at the outpatient clinic in the hospital from January 1993 to December 2010, were enrolled in this study. A systematic search of the PubMed database was conducted for all articles from 1990 through 2012 with the terms "OTC deficiency," "coagulopathy," "coagulation," or "prothrombin time" in the title, abstract, or key words to determine whether any articles described coagulation abnormality before and after the onset of metabolic attack in OTC-deficient patients.

RESULTS

Five patients were identified at our hospital as follows: patient 1 was a 10-year-old boy with OTC deficiency underlying asymptomatic coagulopathy of unknown etiology before the onset of metabolic crisis. Two patients (patients 2 and 4) were diagnosed during the asymptomatic period because the brother of patient 2 and the maternal uncle of patient 4 died of OTC deficiency at the first metabolic attack. Patients 3 and 5 experienced the first attack during infancy (10 months) and childhood (10 years), respectively. The database search identified 3 publications, and 3 cases of OTC deficiency from these articles with the description of the coagulation at remission state are shown in Table 1 (patients 6–8).[6–8]

Six of these 8 patients were male. Five patients were successfully treated and maintained normal growth and development for years. One patient died a sudden death at 19 years of age. The 2 patients described in the literature developed a metabolic attack at 24 years of age and died at the first attack. The levels of blood ammonia of these patients were remarkably high during the metabolic attack, whereas other laboratory data such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were indistinctive.

In contrast, prothrombin times (PTs) were below the normal level at the metabolic attack and were also considerably abnormal during remission. The blood ammonia, serum AST, ALT, or albumin levels were close to normal ranges while the patients were being managed by using either mild restriction of protein intake or oral arginine supplementation (Table 1).

DISCUSSION

Coagulation abnormalities have rarely been recognized in association with OTC deficiency. Coagulopathy may occur if the metabolic attacks cause severe liver damage or disseminated intravascular coagulation, but such critical conditions were not found in any of these patients. These cases also demonstrated the presence of abnormalities in coagulation even during remission. The findings suggest that coagulopathy may be a useful sign for detecting an underlying OTC deficiency, especially in boys with nonspecific clinical symptoms such as cyclic vomiting or psychological problems. To the best of our knowledge, no common genetic coagulopathy has yet been identified in the Japanese population; therefore, it is likely that this coagulation abnormality may be specific to OTC deficiency.

The pathogenesis of such a coagulation abnormality is unclear. This coagulopathy does not seem similar to that in fulminant hepatic failure with the collapse of protein synthesis in the liver. The coagulopathy in OTC deficiency might be under the same mechanism that occurs with vitamin K (VK) deficiency because elevation of serum des-γ-carboxyprothrombin (PIVKA-II) levels was sometimes detected, and VK administration seemed effective for these patients. The coagulation factors II, VII, IX, and X are activated in a VK-dependent manner during carboxylation at the γ-terminus-glutamate in

TABLE 1 Clinical, Molecular, and Laboratory Data of the Patients

Variable	RR	Patient No. 1	2	3	4	5	6 (Thurlow et al,[7] 2010)	7 (Schimanski et al,[6] 1996)	8 (Zammarchi et al,[8] 1996)
Gender		M	M	M	M	M	M	F	F
Proband		Himself	Brother[a]	Himself	Maternal uncle[a]	Himself	Himself	Herself	Herself
Transmission type		ND	Maternal	Maternal	Maternal	ND	Maternal	ND	ND
OTC gene mutation		R40H	R40H	IVS2-1G>A	R40H	R40H	R40C	Deletion	ND
Diagnosis									
Age		10 y	PP	8 mo	1 mo	10 y	24 y	24 y	11 mo
Method		BDx	GDx	BDx	BDx	BDx	BDx	BDx	BDx
Age at first attack		10 y	12 y	7 d	None	10 y	24 y	24 y[c]	None
Representative data at attack									
AST/ALT, U/L	13–33/6–30	31/23	16/14	86/126[d]	—	67/154[d]	114 (AST)[d]	52/47[d]	—
PT, % [INR]	≥70% [0.90–1.10]	44 [1.59][d]	56 [1.37][d]	11[d]	—	17.5%[d,e]	ND [2.7][d]	ND [3.0][d]	—
APTT, s	26.0–41.0	38.3	38.4	64.0[d]	—	60.7[d]	ND	ND	—
Ammonia, μmol/L	7–39	175[d]	399[d]	112[d]	—	214[d]	348[d]	380[d]	—
Representative data at remission, age		15 y	17 y	23 y	16 y	12 y	24 y	24 y	11 m
AST/ALT, U/L	13–33/6–30	21/21	12/11	25/34[d]	16/8	29/18	Normal	32/32[d]	223/103[d]
PT, % [INR]	≥70 [0.90–1.10]	58 [1.40][d]	68 [1.20][d]	69 [1.22][d]	82 [1.11][d]	64.1[d,e]	ND [2.2][d]	ND [3.0][d]	50[d]
APTT, s	26.0–41.0	42.1[d]	40.6	44.0[d]	32.0	30.3	47.0[d] (RR: 29–37)	Normal	42 (RR: 30–45)
Ammonia, μmol/L	7–39	19	27	41	9	19	Normal	23.5	74[d]
PIVKA-II, mAU/mL	<40	32	27	37	56[d]	ND	ND	ND	ND
Serum albumin, g/dL	4.0–5.0	4.1	4.2	3.9	5.0	4.0	ND	ND	ND
Frequency of severe attack		Once	3 times	3 times	None	3 times	Once	Once	None
Long-term treatment		A, P	A	A, P	A	A, B	—	—	B, P, C
Outcome (age)		Healthy adolescent (16 y)	Healthy adolescent (17 y)	Healthy adult (24 y)	Healthy adolescent (17 y)	Dead (19 y)	Dead (24 y)	Dead (24 y)	Healthy (ND)

A, oral arginine supplement; APTT, activated partial thromboplastin time; B, oral sodium benzoate treatment; BDx, biochemical diagnosis based on the hypersecretion of orotic acid and uracil in urine without elevation of any specific amino acid in blood; C, oral citrulline treatment; GDx, genetic diagnosis by the analysis of the *OTC* gene; INR, international normalized ratio; ND, not described or not available; P, intake protein restriction; PP, during the prenatal period; RR, reference range.

[a] Dead.
[b] Deletion, *T892del,G893del in exon 9.*
[c] In pregnancy.
[d] The laboratory data out of RRs.
[e] Hepaplastin test (normal range: 67%–138%).

their N-terminal domains, whereas in the absence of VK or in the presence of VK antagonists, hepatic VK-dependent carboxylase activity is inhibited, and PIVKA-II is released into the blood.[9] It is possible that abnormal metabolites in association with OTC deficiency, such as orotic acid, might inhibit the hepatic VK-dependent carboxylase. In fact, hyperornithinemia-hyperammonemia-homocitrullinuria syndrome, which is a congenital error in the metabolism of ornithine accompanied with substantial elevations of orotic acid, is also associated with coagulopathy.[10,11] In addition, a large amount of polyunsaturated fatty acids reduced the expression of γ-glutamyl carboxylase in apolipoprotein E knock-out mice.[12] Taken together, orotic acid or other unknown abnormal products in common with hyperornithinemia-hyperammonemia-homocitrullinuria syndrome may affect the lipid metabolism and reduce the γ-glutamyl carboxylase activity and, consequently, cause coagulopathy. Further investigation is needed to understand the pathophysiology of coagulation in OTC deficiency. Moreover, the discovery of novel factor-specific inhibitors may provide valuable information for the design of new anticoagulation drugs with mechanisms of action distinct from warfarin or novel oral anticoagulants.[13]

CONCLUSIONS

The current results suggest that coagulation abnormality is a previously unidentified complication of late-onset OTC deficiency in a metabolically compensated state. This information would be beneficial for undiagnosed patients to avoid lethal metabolic crises during adolescence.

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