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ORIGINAL PAPER

Intracellular in vitro probe acylcarnitine assay for identifying deficiencies of carnitine transporter and carnitine palmitoyltransferase-1

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Abstract Mitochondrial fatty acid oxidation (FAO) disorders are caused by defects in one of the FAO enzymes that regulates cellular uptake of fatty acids and free carnitine. An in vitro probe acylcarnitine (IVP) assay using cultured cells and tandem mass spectrometry is a tool to diagnose enzyme defects linked to most FAO disorders. Extracellular acylcarnitine (AC) profiling detects carnitine palmitoyltransferase-2, carnitine acylcarnitine translocase, and other FAO deficiencies. However, the diagnosis of primary carnitine deficiency (PCD) or carnitine palmitoyltransferase-1 (CPT1) deficiency using the conventional IVP assay has been hampered by the presence of a large amount of free carnitine (C0), a key molecule deregulated by these deficiencies. In the present study, we developed a novel IVP assay for the diagnosis of PCD and CPT1 deficiency by analyzing intracellular ACs. When exogenous C0 was reduced, intracellular C0 and total AC in these deficiencies showed specific profiles clearly distinguishable from other FAO disorders and control cells. Also, the ratio of intracellular to extracellular C0 levels showed a significant difference in cells with these deficiencies compared with control. Hence, intracellular AC profiling using the IVP assay under reduced C0 conditions is a useful method for diagnosing PCD or CPT1 deficiency.

Keywords Fatty acid oxidation · Carnitine cycle disorder · Acylcarnitine profile · ESI-MS/MS

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Introduction

L-Carnitine plays an essential role in the transfer and activation of long-chain fatty acids across the outer and inner mitochondrial membranes during which it is acted upon by enzymes including carnitine transporter (OCTN2), carnitine palmitoyltransferase-1 (CPT1), carnitine palmitoyltransferase-2 (CPT2), and carnitine acylcarnitine translocase (CACT) (Fig. 1) [1, 2]. Carnitine penetrates into cells across the plasma membrane against a high concentration gradient of free carnitine with the aid of the plasma membrane OCTN2 protein encoded by the SLC22A5 gene [3]. Deficiency of OCTN2 causes primary carnitine deficiency (PCD, OMIM 212140), which is characterized by systemic carnitine deficiency in tissues and blood but in concord with increased excretion of free L-carnitine in the urine [4–6]. Clinical symptoms in patients with PCD such as cardiomyopathy,

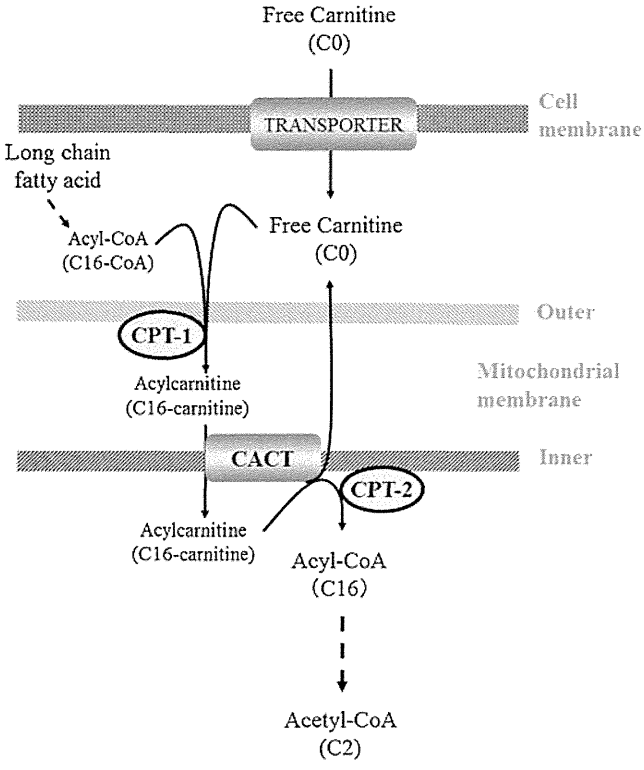

Fig. 1 Pathway for mitochondrial fatty acid beta-oxidation. Transporter: carnitine uptake transporter; *CPT-1*: carnitine palmitoyltransferase-1, *CACT*: carnitine acylcarnitine translocase, *CPT-2*: carnitine palmitoyltransferase-2. *Solid arrows* indicate single reactions; *dashed arrows* indicate multiple reactions or steps

encephalopathy, hepatomegaly, myopathy, hypoglycemia, and hyperammonemia, mainly result from low carnitine concentration in the tissues. On the other hand, secondary carnitine deficiency occurs in some conditions such as organic acidemias, renal dialysis, long-term medication (antiepileptic drugs or some antibiotics), and alimentary deficiency of L-carnitine [7–9].

It is necessary to make a differential diagnosis of PCD from the secondary carnitine deficiency or other false-positive cases, and diagnosis is confirmed by demonstrating reduced transport in skin fibroblasts from the patients. Until now, cluster-tray method using radioisotope-labeled substrate was used for the diagnosis of PCD [4, 10–12]. However, such a diagnostic method requires handling of radioactive substrates and focused only on diagnosis of PCD. Gene sequencing in SLC22A5 is one diagnostic method for PCD. However, it is molecularly heterogenous, and around 50 different mutations have been identified [6]. After acylcarnitine analysis using tandem MS analysis became available in the worldwide, blood acylcarnitine analysis was used as an initial method for diagnosis of FAO disorders and a detection of FAO disorders has been increased. However, it is necessary to confirm the diagnosis of the diseases with detailed analysis. The in vitro probe acylcarnitine (IVP) assay using cultured fibroblasts and tandem mass spectrometry (MS/MS) has been used to evaluate FAO capacity in the cultured cells and make a diagnosis of FAO disorders [13–15]. However, conventional IVP assay is not feasible to diagnose PCD or CPT1 deficiency, because excess amount of free carnitine is added to the experimental medium at the beginning. Estimation of free carnitine, which is the key marker for the above diseases, in experimental medium was nonsense for diagnosis of these disorders. We developed a novel functional assay for PCD and CPT1 deficiency using the IVP assay, with some modifications. This method uses different concentrations of exogenous free carnitine and measures intracellular as well as extracellular acylcarnitine (AC) levels, which overcomes the disadvantage of the conventional IVP assay in the diagnosis of carnitine cycle disorders.

Materials and methods

Materials

Hexanoylcarnitine (C6), octanoylcarnitine (C8), decanoylcarnitine (C10), and palmitoylcarnitine (C16) were purchased from Sigma–Aldrich (St Louis, MO, USA). Methanol, acetonitrile, and formic acid were purchased from Wako (Osaka, Japan). As an internal standard, a labeled carnitine standard kit (NSK-B), which contains $^{2}[H]_{9}$-carnitine, $^{2}[H]_{3}$-acetylcarnitine, $^{2}[H]_{3}$-propionylcarnitine, $^{2}[H]_{3}$-butyrylcarnitine, $^{2}[H]_{9}$-isovalerylcarnitine, $^{2}[H]_{3}$-octanoylcarnitine, $^{2}[H]_{9}$-myristoylcarnitine, and $^{2}[H]_{3}$-palmitoylcarnitine, was purchased from Cambridge Isotope Laboratories (Andover, MA, USA).

Preparation of standard solutions of ACs

Standard solutions containing 1, 10, 25, and 50 μmol/L each of C6, C8, C10, and C16 were used to validate the recovery and determine linear concentration range of ACs after extraction by the Folch method [16]. The ACs were dissolved in methanol (99.8 %), and the prepared standard solution was analyzed directly and after extraction by the Folch method.

Subjects

Human skin fibroblasts from six healthy controls (volunteers) and seven patients with various carnitine cycle disorders—three each with PCD and CPT2 deficiency and one with CPT1 deficiency—were analyzed. In all cases, diagnoses were confirmed by mass spectrometric analyses (gas chromatography-mass spectrometry and MS/MS), enzyme assay, and protein or mutational analyses. Informed consent was obtained from the patients or their families. This study was approved by the Ethical Committee of the Shimane University School of Medicine.

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 ChemoView™ software (Applied Biosystems/MDS SCIEX, Toronto, Canada).

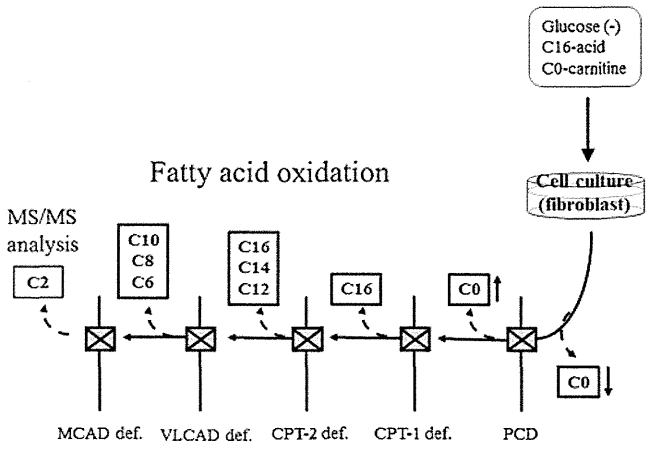

Fig. 2 Principle of in vitro probe acylcarnitine assay. *C2, C4, C6, C8, C10, C12, C14*, and *C16* represent acylcarnitines

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

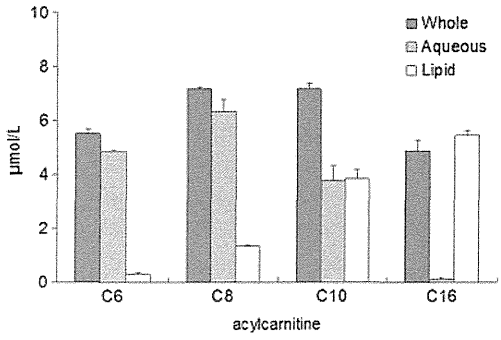

Fig. 3 Recovery of ACs during extraction using the Folch method. Standard solutions of 10 µmol/L each of C6-, C8-, C10-, and C16-carnitine were used to determine the recovery of ACs in the aqueous and lipid fractions during extraction using the Folch method. *Grey column*: ACs in the whole extract after 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

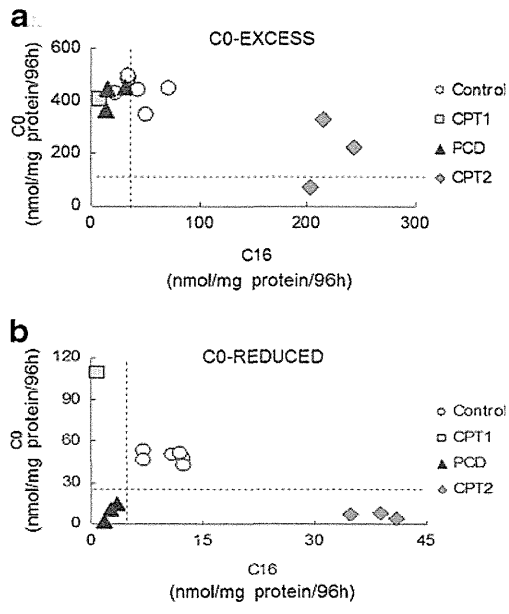

Fig. 4 Intracellular C0 and C16 correlation in patients with carnitine cycle disorders. **a** C0-excessive condition (−*E*); **b** C0-reduced condition (−*R*). *open circle*: healthy control ($n=6$); *closed triangle*: PCD ($n=3$); *closed square*: CPT1 deficiency ($n=1$); *closed diamond*: CPT2 deficiency ($n=3$). 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)

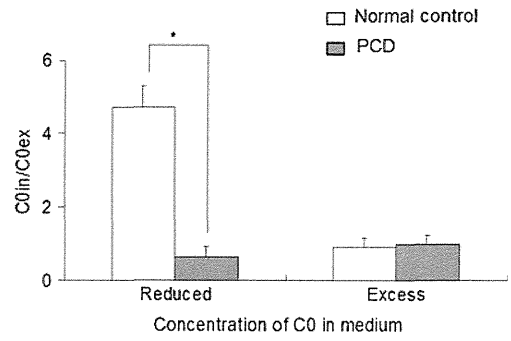

Fig. 5 Ratio of intracellular C0 to extracellular C0. *Open square*: normal control ($n=6$); *closed square*: PCD ($n=3$). Extra- and intracellular C0 of cells with normal control and PCD were measured in C0-reduced (10 μmol/L) and C0-excess (400 μmol/L) conditions using MS/MS. Data are expressed as 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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Expanded newborn mass screening with MS/MS and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in Japan

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Abstract

Expanded newborn mass screening (NBS) with tandem mass spectrometry (MS/MS) and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in Japan are described. Prognosis of patients with inborn metabolic disease was compared between groups detected in the symptomatic and pre-symptomatic stages. Furthermore, clinical, biochemical and genetic findings of Japanese children with MCAD deficiency, which is a most important target of MS/MS screening, was investigated. Our study concluded as follows: 1) the detection incidence in MS/MS screening in Japan is totally about 1 in 9,000, which might be smaller than that of the other countries; 2) Outcomes of patients detected by NBS (pre-symptomatic stage) is more favorable than that of cases detected after symptomatic onset; 3) the incidence of MCAD deficiency in the Japanese population is 1 in 110 thousands, which is approximately 10 times smaller than that in Caucasian; 4) Japanese patients with MCAD deficiency have a common mutation, c.449-452delCTGA, which covers about 45% of alleles of MCAD gene, but not have 985A>G, which is a common mutation of Caucasians patients; 5) genotype/phenotype correlation was not observed in MCAD deficiency; 6) prognosis of the non-symptomatic group is much more favorable than that of the symptomatic group. In conclusion, it is indicated that detection of inborn metabolic disease in the pre-symptomatic stage by NBS is essential to prevent children affected with target diseases of NBS including MCAD deficiency from neurological impairments or infant death.

Key words

expanded newborn mass screening, MS/MS, MCAD deficiency, genotype/phenotype correlation, prevention of neurological impairment

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1. Introduction

The expanded newborn screening (NBS) using tandem mass spectrometry (MS/MS) is becoming popular worldwide. In Japan, a pilot screening was initiated at Fukui University in 1997 (1), and the national

project of MS/MS screening (Principle Investigator, Dr. Seiji Yamaguchi, Shimane University) funded by Grant-in-Aid for Scientific Research from the Ministry of Health, Labour, and Welfare was started in 2004. For the pilot screening, about 10 laboratories in Japan joined up to 2012. The Japanese government issued notice urging implementation of MS/MS to NBS in 2011. Eventually, the MS/MS screening is becoming spread from 2012 to 2013 (2), and will initiate officially nationwide in next year (2014).

In the expanded NBS, it is considered that medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is a most important screening target, particularly in Caucasian people, because the incidence is high (1 in 10,000), and MCAD deficiency is a causative disease of sudden infant death, and preventable by detection in NBS (3). In this paper, clinical and genetic aspects of MCAD deficiency as well as the results of the pilot MS/MS screening in Japan are described.

2. Pilot MS/MS Screening In Japan

a) Results of pilot MS/MS screening in Japan

A total number of babies screened by MS/MS during the period between 1997 and 2012 was 1,949,987 (about 1.95 million), and 217 cases affected with disease were found as shown in Table 1. The detection incidence of Japanese babies was calculated to be 1 in 8,986, while that in USA was estimated to be 1 in 4,353 (4). The prevalence of Japanese is likely smaller than that of the other countries.

b) The detection prevalence of each disease group

The incidence of amino acidemias was totally 1 in 27 thousands; that of organic acid disorders, 1 in 22 thousands; and that of fatty acid disorders, 1 in 34 thousands. The most common disease in Japan was propionic acidemia, which was found in 1 in 45,349 (about 45 thousands); and phenylketonuria, 1 in 52,702 (53 thousands); followed by methylmalonic acidemia and MCAD deficiency, each of which in 1 in 108,333 (110 thousands). A considerable number of Japanese patients with propionic acidemia show a mild phenotype with a common mutation, 1304T>C (Y435C) in *PCCB* gene (5).

c) Comparison of outcomes between cases detected by NBS and by tests after symptomatic onset

Outcomes of cases with organic and fatty acid disorders detected in NBS were compared with those detected after symptomatic onset by MS/MS, GC/MS and/or molecular investigation in Shimane University. As shown in Table 2, in organic acid disorder, normal development was achieved in 58 of 70 cases (83%) of the NBS group, as of at least 1 year of age. In contrast, normal development was gotten in only 28 of 114 cases (19%) in the "symptomatic" group.

In fatty acid disorder, normal development was achieved in 40 of 45 cases (89%) in the NBS group, although that was in 25 of 52 cases (48%) in the symptomatic group. Hence, beneficial effect of NBS using MS/MS was indicated. Through the results of the pilot study, we proposed 16 kinds of disease which should be screened as primary targets as marked with black circle in Table 1, in consideration of the false negative rate, complexity of diagnostic approaches, or the benefit of detection by NBS.

Table 1. Results of the pilot screening using MS/MS in Japan (1997 to 2012)

Disease		No. of Cases	Incidence (Japan)	Estimated in USA*
AMINO ACIDEMIA		72	(1:27 K)	(1:15 K)
• Phenylketonuria		37	1: 53 K	1: 19 K
• MSUD		1	1: 1,950 K	1: 159 K
• Homocystinuria		3	1: 650 K	1: 38 K
• Citrulinemia type I		6	1: 330 K	1: 173 K
• Argininosuccinic A		2	1: 980 K	1: 591 K
Citrin deficiency		23	1: 85 K	n.a.
ORGANIC ACIDEMIA		87	(1: 22 K)	(1:16 K)
• Methylmalonic acidemia		18	1: 110 K	1: 67 K
• Propionic acidemia		43	1: 45 K	1: 276 K
• Isovaleric acidemia		3	1: 650 K	1: 129 K
• MCD		3	1: 650 K	1: 1,380K
• Methylcrotonylglycinuria		13	1: 150 K	1: 44 K
• HMGL deficiency		—	—	1: 1,380K
• Glutaric acidemia type1		7	1: 280 K	1: 109 K
β-ketothiolase deficiency		—	—	1: 591 K
FATTY ACID DISORDER		58	(1: 34 K)	(1:10 K)
• CPT1 deficiency		5	1: 390 K	n.a.
• VLCAD deficiency		12	1: 160 K	1: 69 K
• MCAD deficiency		18	1: 110 K	1: 17 K
• TFP/LCHAD deficiency		2	1: 980 K	1: 276 K
CPT2 deficiency		7	1: 280 K	n.a.
CACT deficiency		—	—	n.a.
Glutaric acidemia type 2		6	1: 330 K	n.a.
Carnitine uptake defect		7	1: 280 K	1: 49 K
SCHAD deficiency		1	1: 1,950 K	n.a.
TOTAL	Affected cases	217	1: 8,986	1: 4,353
	Screened babies	1,949,987		

•, primary target disease in the MS/MS screening in Japan. "K" means thousands. * Estimated incidence in USA, based on live birth for 2006 (n=4,138,349) (4). Abbreviation: −, not detected; n.a., not applicable; MCD, multiple carboxylase deficiency; HMGL, 3-hydroxy-3- methyl-glutaryl-CoA lyase; CPT1 and CTP2, carnitine palmitoyl transferase-I and -II, respectively; VLCAD and MCAD, very-long-, and medium-chain acyl-CoA dehydrogenase, respectively; TFP, mitochondrial trifunctional protein; CACT, carnitine acylcarnitine translocase; LCHAD and SCHAD, long- and short-chain 3-hydroxyacyl-CoA dehydrogenase, respectively.

3. Clinical, Biochemical and Genetic Investigation of Japanese MCAD Deficiency

a) Outline of MCAD deficiency

MCAD deficiency was first discovered in children with sudden infant death syndrome-like illness in 1982, and has been found at an incidence of 1 in 10,000. A common mutation, 985A>G, covering about 90% of the alleles in this disease was seen among Caucasian population. Acute symptoms of MCAD deficiency include acute

Table 2. Comparison of outcomes between presymptomatic and symptomatic detection cases in Japanese children with organic academia and fatty acid disorder.

Disease	NBS (MS/MS screening)	Symptomatic (Shimane)
No. of cases	115	196
ORGANIC ACID DISORDER	70	144
Normal development	58 (83%)	28 (19%)
Handicaps or death	12 (17%)	116 (81%)
FATTY ACID DISORDER	45	52
Normal devlopment	40 (89%)	25 (48%)
Handicaps or death	5 (11%)	27 (52%)

NBS, newborn mass screening, MS/MS screening, cases detected by the pilot study between 2004 and 2011. Symptomatic, cases after symptomatic onset and detected by MS/MS, GC/MS or molecular tests in Shimane University from 2001 to 2011.

encephalopathy-like symptoms, or even sudden death (6), following after long fasting or infection, although the patients have no symptoms in the stable condition. Concerning the prognosis, it has been claimed that as many as 35% of MCAD deficiency patients are asymptomatic lifelong, but that over 25% of the symptomatic cases die suddenly during the first episodic attack (3).

Abnormal laboratory tests in the acute condition include hypoglycemia or hyperammonemia. Biochemical markers in MS/MS analysis of blood acylcarnitines are elevation of C8, C6 or C10, or C8/C10. Elevation of hexanoylglycine (HG) and suberylglycine (SG) as well as dicarboxylic acids is often observed by GC/MS analysis of urinary organic acids.

b) Prevalence of MCAD deficiency in Japan

According to the results of pilot screening using MS/MS, MCAD deficiency was found at the incidence of 1 in 110 thousands, and was most common among fatty acid disorders in the pilot study of Japan, as shown in Table 1. However, the incidence is about 10 times smaller than that of Caucasian whose incidence is 1 in 10 thousands (3).

c) Subjects of MCAD deficiency

Ages at onset, and diagnosis, clinical, biochemical and genetic findings of a total 20 Japanese cases whose blood C8 acylcarnitine, a diagnostic marker of MCAD deficiency, was over the cut off value (0.3 nmol/mL) in MS/MS analysis were investigated (Table 3). Cases 1 through 9 were identified after symptomatic onset (symptomatic group), while cases 10 through 18 were detected in the non-symptomatic or pre-symptomatic stage (non-symptomatic group). Cases 10 through 17 were detected by NBS while Case 18 was by the sibling screening using MS/MS. Acylcarnitine analysis was performed at Shimane University or the other laboratories. GC/MS analysis (7) and gene analysis (8) were performed at Shimane University. The final diagnosis was confirmed by gene analysis, which revealed that cases 19 and 20 were heterozygotes (carrier group). Clinical information was surveyed using questionnaire.

Table 3. Clinical, biochemical and genetic profiles of Japanese cases of MCAD deficiency

No.	Age at onset	Age at diag.	NBS	MS/MS	GC/MS		Genotype		Outcome
				C8 (<0.3)	HG	SG	Allele 1	Allele 2	
Symtpomatic group									
1	8m	8m	−	5.97	11.1	44.5	c.449-452del	c.157C>T	impair
2	1y0m	1y0m	−	4.52	n.a	n.a	IVS4+1G>A	c.422 A>T	SID
3[a]	1y0m	8y10m	−	1.57	45.4	29.6	c.449-452del	c.449-452del	impair
4	1y1m	1y1m	−	7.00	14.7	112.2	del. ex 11-12	del. ex 11-12	impair
5	1y3m	1y3m	−	high*	n.a	n.a	del. ex 11-12	del. ex 11-12	impair
6[b]	1y4m	1y4m	−	3.33	9.9	15.3	c.449-452del	c.449-452del	impair
7	1y7m	1y7m	−	4.12	6.1	6.4	c.275C>T	c.157C>T	impair
8[a]	1y8m	1y8m	−	4.75	69.3	1.2	c.449-452del	c.449-452del	SID
9	2y2m	2y2m	−	1.71	n.a	n.a	c.449-452del	c.449-452del	normal
Non-symptomatic group									
10	−	5d	+	5.92	12.9	14.8	c.1085G>A	c.843A>T	normal
11	−	5d	+	5.37	6.3	39.9	c.449-452del	c.154A>G	normal
12	−	5d	+	4.82	15.3	3.8	IVS3+2T>C	c.843 A>T	normal
13	−	5d	+	4.04	n.a	n.a	c.449-452del	c.212 G>A	normal
14	−	5d	+	2.78	11.5	5.9	c.449-452del	c.134 A>G	normal
15	−	5d	+	2.59	3.1	(-)	c.1085G>A	c.1184A>G	normal
16	−	5d	+	2.58	(-)	3.2	c.449-452del	IVS3+5G>A	normal
17	−	5d	+	0.49	9.7	1.5	c.449-452del	c.820 A>C	normal
18[b]	−	5y5m	−	1.37	n.a	n.a	c.449-452del	c.449-452del	normal
Carrier group									
19	−	5d	+	0.44	(-)	(-)	c.845C>T	(-)	normal
20	−	4m	−	0.51	(-)	(-)	c.843A>T	(-)	normal

Cases 3 and 8 (a-a), and cases 6 and 18 (b-b) are sibling cases, respectively. Abbreviation: diag, diagnosis; NBS, newborn mass screening; −, none; +, NBS received; MS/MS, blood acylcarnitine analysis; GC/MS, urinary organic acid analysis; HG and SG, hexanoylglycine and suberylglycine, respectively; high*, elevated but detailed value not available. n.a, data not available in Shimane University; (-), not detected; SID, sudden infant death; c.449-452del, 4 base deletion of CTGA. impair, neurological impairments as sequallae. Unit: C8, nmol/mL (cut off, <0.3); HG and SG, peak area ratio to IS (%) (7) on GC/MS (normal, both undetectable).

d) Comparison between Symptomatic and non-symptomatic groups of MCAD deficiency

1) Ages at onset and diagnosis: In the symptomatic group, the ages at onset was 8 mo to 2 yr 2 mo. Cases 3 and 8, and cases 6 and 18, were siblings. In 9 cases of the non-symptomatic group, 8 cases were detected on 5 day after birth by NBS, and the other one (case 18) was identified at the age of 5 yr 5 mo by sibling screening.

2) Clinical findings of symptomatic case: In the symptomatic group, all 9 cases had acute encephalopathy- or sudden death-like illness in the acute stage. Hypoglycemia was observed in all 7 cases tested, while hyperammonemia was seen in 4 of the 9 cases.

3) Biochemical findings: As shown in Table 2, C8 (cut off, <0.3 nmol/mL) ranged between 1.57 and 7.00 in the symptomatic group, while C8 did between 0.49 and 5.92

in the non-symptomatic group. No significant difference between these two groups was seen in the level of C8. The C8 value of the carrier group (cases 19 and 20) was 0.44 and 0.51, respectively, which was lower compared to those of the 18 affected cases. No significant difference was seen in the urinary excretion amounts of HG or SG between these two groups (Table 3).

4) Gene mutation: c.449-452delCTGA (c.449del4) was identified in 16 of 36 alleles (44%) in 18 Japanese patients with MCAD deficiency. The homozygote of c.449del4 mutants were observed in 4 and 1 cases in the symptomatic and non-symptomatic groups, respectively. A common mutation, 985A>G, found in Caucasian population was never identified in Japanese cases (8). On the other hand, it was reported that the c.449del4 mutation was in 3 of 5 alleles of 3 Korean MCAD deficiency cases (9). It would be interesting to investigate and compare the genotypes of Japanese MCAD deficiency with those of the other Asian countries and the other ethnic groups.

5) Outcomes: With respect to the outcomes, 8 of 9 cases of the symptomatic group resulted in severe handicaps or sudden death, whereas all 9 cases of the non-symptomatic group showed normal development and growth (Table 3). It was likely that there were no genotype/phenotype correlation, although existence of the correlation is not clear enough in the present point (10). These findings indicate that pre-symptomatic detection is important for the favorable outcome in MCAD deficiency. Namely, NBS is essential (11, 12). Furthermore, our data suggests no clear genotype/phenotype in MCAD deficiency.

4. Conclusion

Our study indicated that: 1) the detection incidence in MS/MS screening is totally about 1 in 9,000 in Japan, which might be 2 times smaller than that of other countries; 2) the outcomes of patients detected by NBS is more favorable compared with that of cases detected after symptomatic onset; 3) the incidence of MCAD deficiency is 1 in 110 thousands in Japanese population. This is approximately 10 times smaller than that in Caucasian population; 4) 45% of alleles of *MCAD* gene in Japanese patients have a common mutation, c.449-452delCTGA. The genetic background of Japanese cases is likely the same with Korean patients, but different from those in Caucasians with MCAD; 5) clinical severity of MCAD deficiency may be similar despite the different genetic mutations, suggesting that genotype does not necessarily predict phenotype in MCAD deficiency; 6) prognosis of the symptomatic cases with MCAD deficiency was poor, whereas that of the non-symptomatic group was excellent, indicating "pre-symptomatic detection" is essential to prevent children affected with MCAD deficiency from impairments or sudden death.

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MS解析による代謝障害の診断

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SUMMARY

質量分析（MS）によって生体内の微量成分を一斉分析し，その代謝プロフィールから代謝障害部位が特定（生化学診断）される．保険収載されているMSは，タンデム型質量分析計（タンデムマス）とガスクロマトグラフィー質量分析計（GC/MS）である．タンデムマスで血中アミノ酸とアシルカルニチンを同時測定し，GC/MSによって尿中有機酸分析がおこなわれ，アミノ酸代謝異常症，有機酸・脂肪酸代謝異常症が診断される．チアミン（ビタミンB1）欠乏症（脚気），ビオチン欠乏症，カルニチン欠乏症，あるいは肝硬変によるチロシン代謝障害などの後天性代謝障害も診断される．最近カルニチン欠乏症が注目されているが，その評価にはタンデムマスが不可欠である．

はじめに

代謝異常症の診断に質量分析（mass spectrometry：MS）が普及しつつある．先天代謝異常診断のためのメタボロミクス解析，プロテオミクス解析にMSが応用されている．なかでも「ガスクロマトグラフィー質量分析計（gas chromatograph MS：GC/MS）による先天代謝異常症の診断」と「タンデム型質量分析計（タンデムマス）によるアシルカルニチン分析」は条件つきではあるものの最近保険収載された．さらに新生児マススクリーニングにタンデムマスが導入され，MSの臨床応用が広がりつつある[1,2]．MSでは，微量の検体で高感度・高精度な網羅的分析が可能であり，先天的な代謝異常のみならず，後天的な代謝障害の評価にも応用される．また最近注目されているカルニチン欠乏の診断にも，タンデムマスが不可欠である．そこで，上記のタンデムマスとGC/MSによる代謝障害の評価法について述べたい．

KEY WORDS

- タンデムマス
- ガスクロマトグラフィー質量分析計（GC/MS）
- アシルカルニチン
- 有機酸
- カルニチン欠乏症

代謝障害の診断に使われるMS

1）タンデムマス

タンデムマスは，MSが2つ直列に並んだ構造（MS1とMS2）で，高感度分析が可能である[3]．MS1とMS2で測定された粒子の質量数を比較して測定したい代謝産物の分子量が推定される．そしてそれぞれの分子のイオン強度によって定量される．検体の量は，新生児マススクリーニングでは，血液ろ紙の3 mm大のディスクでよく，血清ならば10 μLでも分析可能である[4,5]．

タンデムマスでは，アミノ酸とアシルカルニチンが同時分析される．アミノ酸に関しては，アミノ酸分析計にくらべると精度は低く，分析項目も少ない．アシルカルニチン分析では，遊離カルニチン（C0）とアシルカルニチンが測定される．

2）GC/MS

検体を誘導体化して揮発性の物質に変えてGC分析する．GCから出てきた粒子は電子衝撃イオン化法で断片化されMSに導入される[6]．マススペクトルは，分子構

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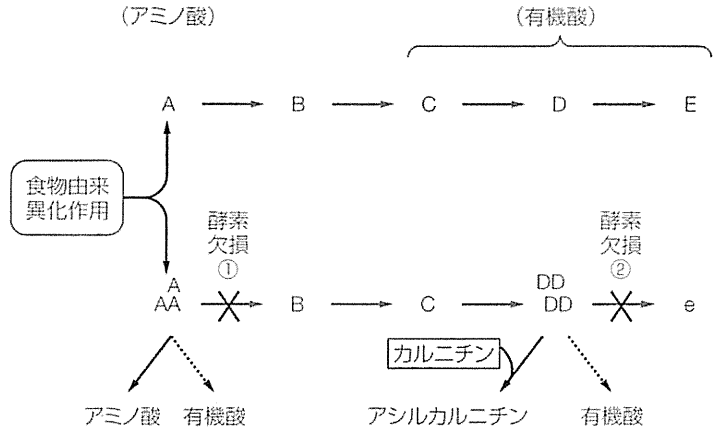


図❶ 先天代謝異常と生化学診断の原理
酵素欠損①はアミノ酸代謝異常，酵素欠損②は有機酸代謝障害の例である．アミノ酸代謝異常では，上流アミノ酸とそれから由来する特徴的な有機酸が検出される．有機酸代謝異常では，上流の中間体にあたる有機酸とそれに由来する代謝産物が増加する．

造の情報となる断片イオンが比較的多いため，異性体の同定も可能である．分析項目は尿中有機酸分析である．有機酸は弱酸であり体内に増加するとただちに尿中に排出されるため，尿の分析が用いられる．MSで一斉分析し，有機酸全体のプロフィールから代謝障害部位が特定される．

MSによる代謝異常の生化学診断

体内で代謝障害があると，図❶に示すように障害部位の上流物質とそれに由来する異常代謝産物がMSで一斉分析される．タンデムマスとGC/MSの分析プロフィールから，代謝障害部位が推定される[7]．

1）生化学診断の原理

食物あるいは体内タンパクの異化に由来するアミノ酸の第1段階（または第2段階）の代謝障害のために，アミノ酸が増加した状態をアミノ酸代謝異常症という（図❶のA→B）．タンデムマス（またはアミノ酸分析計）でアミノ酸を測定し，さらにGC/MSによる有機酸分析によってアミノ酸由来の代謝産物を検出して代謝障害部位を特定する．たとえば，表❶に示すようにフェニルケトン尿症では，アミノ酸分析やタンデムマス分析で血中フェニルアラニン（Phe）の増加，GC/MS分析（尿中有機酸）でフェニルピルビン酸などのような有機酸の増加がみられる．高チロシン血症では，血中チロシン（Tyr）の増加と，有機酸分析で4-OH-フェニル乳酸，サクシニルアセトンなどの増加がみられる．

有機酸代謝異常症は，アミノ酸の中間代謝過程に障害によって有機酸が増加し臓器障害を起こす疾患である（図❶のD→E）．脂肪酸代謝異常症は，ミトコンドリアβ酸化障害のためにエネルギー産生不全に陥る疾患である[8]．有機酸・脂肪酸代謝異常症では，特異的なアシルカルニチン，および有機酸プロフィールから生化学診断される．

2）先天代謝異常症

アミノ酸の上昇があればそれに対応するアミノ酸代謝異常症が推定される．一方，有機酸・脂肪酸代謝異常症では，増加した有機酸や脂肪酸がアシルカルニチンとして細胞の外に排出される．血液中に排出されたアシルカルニチンをタンデムマスで測定する．そのアシルカルニチンプロフィールとGC/MS分析による有機酸プロフィールによって，障害部位が特定される．

たとえば，表❶に示すように，プロピオン酸血症とメチルマロン酸血症は，アシルカルニチン分析（タンデム

表❶ 代謝障害と診断マーカーの例

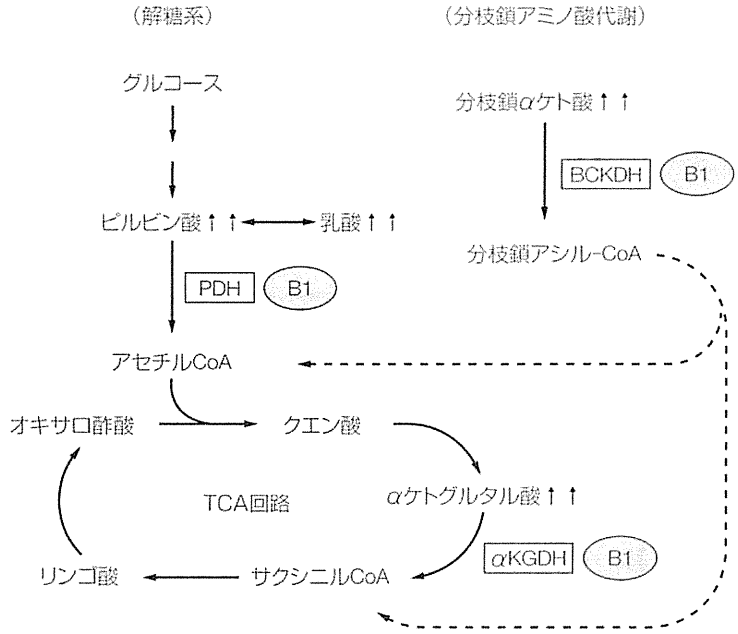
分類	疾患	タンデムマス所見	有機酸所見（GC/MS）
アミノ酸代謝異常	フェニルケトン尿症	Phe	フェニルピルビン酸 フェニル乳酸
	高チロシン血症	Tyr	4-OH-フェニル乳酸 サクシニルアセトン
有機酸代謝異常	プロピオン酸血症	C3	メチルクエン酸 プロピオニルグリシン
	メチルマロン酸血症		メチルクエン酸 プロピオニルグリシン メチルマロン酸
	イソ吉草酸血症	C5	イソバレリルグリシン
	ピバロイル基結合抗菌薬の長期投与によるカルニチン欠乏症		ジカルボン酸
脂肪酸代謝異常	MCAD 欠損症	C8	ヘキサノイルグリシン スベリルグリシン ジカルボン酸
	CPT2 欠損症	C16	ジカルボン酸
	原発性カルニチン欠乏症	C0 低下	ジカルボン酸
その他	B1 欠乏症（脚気）	—	乳酸・ピルビン酸 分枝鎖 α ケト酸 α ケトグルタル酸

—：異常認めず．
C3：プロピオニルカルニチン，C5：ペンタノイルカルニチン（イソバレリル-，またはピバロイルカルニチン），C8：オクタノイルカルニチン，C16：パルミトイルカルニチン．

マス）ではC3が上昇するが両者の区別はできない．有機酸分析（GC/MS）によって鑑別される．脂肪酸代謝異常症である中鎖アシル-CoA 脱水素酵素（medium-chain acyl-coenzyme A dehydrogenase：MCAD）欠損症ではC8の増加，長鎖脂肪酸代謝障害のカルニチンパルミトイルトランスフェラーゼ-2（carnitine palmitoyltransferase 2：CPT2）欠損症ではC16などの増加が決め手となる．

3）後天性代謝異常症

後天的代謝異常として脚気を例示する（図❷）．清涼飲料水の過剰摂取，アルコール中毒などのような栄養の偏りによって，現在でもチアミン（ビタミンB1）欠乏が起こることが知られている．B1を補酵素とする酵素として，分枝鎖 α ケト酸脱水素酵素（branched chain α-keto acid dehydrogenase：BCKDH）複合体，ピルビン酸脱水素酵素（pyruvate dehydrogenase：PDH）複合体，あるいは α ケトグルタル酸脱水素酵素（α-ketoglutarate dehydrogenase：αKGDH）複合体などがある．B1が欠乏するとこれら3ヵ所が同時に障害され，それに対応する有機酸の異常がみられる[9]．このほかにも後天的要因によるビオチン欠乏や，カルニチン欠乏，あるいは重篤な肝障害によるチロシン代謝産物の増加なども，MSによる生化学診断が可能である．



図❷ ビタミンB1欠乏症の代謝障害
B1欠乏症では，B1を補酵素とする3つの酵素が同時に障害されて，乳酸，ピルビン酸，分枝鎖αケト酸，αケトグルタル酸が増加する．

カルニチンと代謝障害

1）生体におけるカルニチンの役割

カルニチンは，体内では99%以上が組織内（大半は骨格筋，心筋）に貯蔵されている．カルニチンのおもな役割は以下の2つである（**図❸**）．

①β酸化の活性化：長鎖脂肪酸をミトコンドリアに転送してβ酸化の基質を供給する．

②蓄積物質の解毒：代謝障害のためにミトコンドリア内に増加した有機酸や脂肪酸を細胞外に排出する．

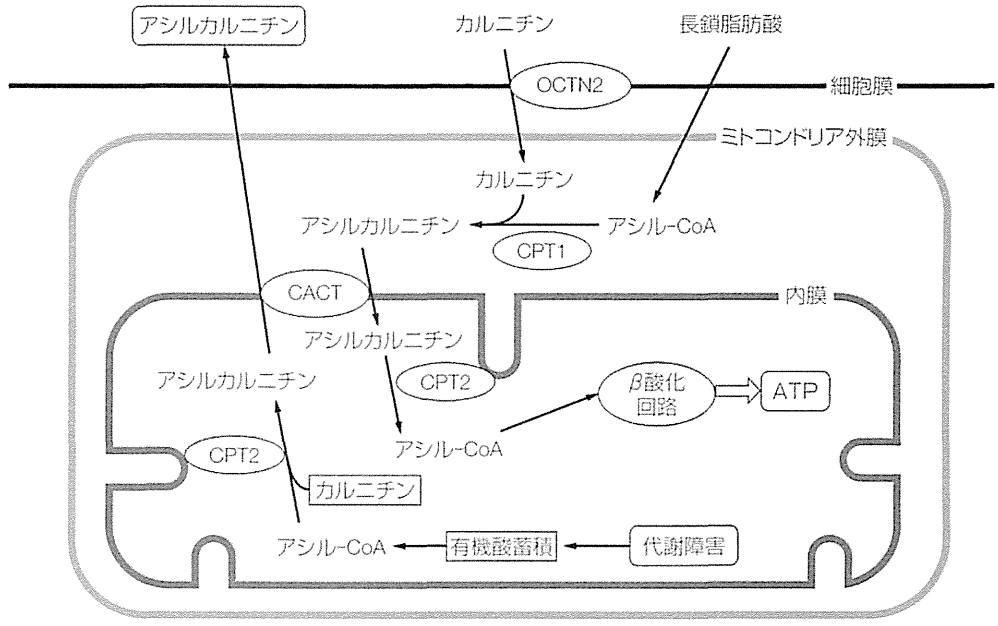
2）カルニチン欠乏をきたす疾患

カルニチン欠乏をきたす疾患を**表❷**にあげている．

①原発性カルニチン欠乏症（primary carnitine deficiency：PCD）[10]：カルニチントランスポーター（organic cation transporter novel type 2：OCTN2）の先天的欠損によって起こる．とくに尿細管におけるカルニチン再吸収障害のために，尿中にカルニチンが失われ，一方，体内ではカルニチン欠乏をきたす．したがって，カルニチンクリアランスは高値を示す．タンデムマス分析でC0低値，GC/MS分析ではβ酸化障害を反映して非ケトン性ジカルボン酸尿症を呈する．大量のレボカルチニン塩化物投与が奏効する．

②有機酸代謝異常症：蓄積する有機酸によってカルニチンが過剰に消費されるために低カルニチン血症をきたす．C0低下のほかに，疾患特異的なアシルカルニチンがみられる．脂肪酸β酸化異常症の一部〔CPT2欠損症やカルニチン・アシルカルニチントランスロカーゼ（carnitine acylcarnitine translocase：CACT）欠損症〕でもカルニチン転送の障害のためにカルニチン低下をきたす．これらは原則としてレボカルチニン塩化物の投与が必要である．

③カルニチン摂取障害：カルニチンの75%は食物から摂取され，25%が体内で生合成される．偏った食事などによる食事性欠乏や，慢性腸疾患などによるカルニチン吸収障害があると，カルニチン欠乏が起こることがある．タンデムマス分析ではPCDと類似した所見がみられるが，尿中C0排泄の増加はみられない点が異なる．



図❸　カルニチンの役割
①β酸化の活性化：長鎖脂肪酸をアシルカルニチンとしてミトコンドリアに運搬しβ酸化の基質を提供する．
②異常代謝産物の解毒：蓄積した有機酸とアシルカルニチンとして細胞外に排出．

表❷　カルニチン欠乏の起こる原因

1．原発性カルニチン欠乏症	OCTN2 異常，カルニチン転送障害
2．過剰消費による 2 次性欠乏	有機酸代謝異常症
3．カルニチン摂取障害	食事性欠乏，慢性腸疾患（吸収障害）
4．薬剤性	ピバロイル基結合抗菌薬の長期投与，バルプロ酸投与
5．過剰喪失	透析（腎不全），重症下痢
6．その他	肝硬変，新生児などにおける合成能低下

④薬剤性：ピバロイル基をもつ抗菌薬（セフェム系など）の長期投与，バルプロ酸投与などによってカルニチンの消費が亢進して低カルニチンをきたすことがある[11]．とくにピバロイルカルニチンは炭素鎖 5 の脂肪酸であるため，タンデムマス分析では C0 の低下と C5 の増加がみられる．この所見はイソ吉草酸血症のそれに類似しているが，鑑別できない．有機酸分析をおこなうと図❹に示すような 2 つのパターンがある．イソ吉草酸血症ならばイソバレリルグリシンが増加し，ピバリン酸によるカルニチン欠乏ならばβ酸化障害を反映してジカルボン酸尿がみられる．GC/MS 分析によって先天性疾患か，後天性代謝異常かが鑑別される（表❶参照）．
⑤カルニチンの過剰喪失：腎不全の透析患者[12]，重篤な下痢などである．
⑥その他：肝硬変患者，新生児（とくに未熟児）のようなカルニチン合成能の低下などである．

3）カルニチン欠乏の臨床所見

低カルニチン血症とは，遊離カルニチンの血中濃度が 20 μM 以下，アシルカルニチン/遊離カルニチンのモル比 0.4 以上をいう．カルニチンが欠乏するとβ酸化が阻害され，表❸に示すようなβ酸化異常症に類似した所見を呈する．すなわち，全身倦怠，筋力低下，不整脈，心筋症状，急性脳症（痙攣，意識障害）などである．検査

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