Molecular Genetics and Metabolism 100 (2010) 46–50

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Diagnoses of newborns and mothers with carnitine uptake defects through newborn screening

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ARTICLE INFO

Article history:
Received 23 December 2009
Accepted 23 December 2009
Available online 28 December 2009

Keywords:
Carnitine uptake defect
Primary carnitine deficiency
Newborn screening
Maternal effect
SLC22A5
OCTN2

ABSTRACT

Carnitine uptake defect (CUD) is an autosomal recessive fatty acid oxidation defect caused by a deficiency of the high-affinity carnitine transporter OCTN2. CUD patients may present with hypoketotic hypoglycemia, hepatic encephalopathy or dilated cardiomyopathy. Tandem mass spectrometry screening of newborns can detect CUD, although transplacental transport of free carnitine from the mother may cause a higher free carnitine level and cause false negatives during newborn screening. From Jan 2001 to July 2009, newborns were screened for low free carnitine levels at the National Taiwan University Hospital screening center. Confirmation tests included dried blood spot free acylcarnitine levels and mutation analyses for both babies and their mothers. Sixteen newborns had confirmation tests for persistent low free carnitine levels; four had CUD, six had mothers with CUD, and six cases were false positives. All babies born to mothers with CUD had transient carnitine deficiency. The six mothers with CUD were put on carnitine supplementation (50–100 mg/kg/day). One mother had dilated cardiomyopathy at diagnosis and her cardiac function improved after treatment. Analysis of the *SLC22A5* gene revealed that p.S467C was the most common mutation in mothers with CUD, while p.R254X was the most common mutation in newborns and children with CUD. Newborn screening allows for the detection of CUD both in newborns and mothers, with an incidence in newborns of one in 67,000 (95% CI: one in 31,600–512,000) and a prevalence in mothers of one in 33,000 (95% CI: one in 18,700–169,000). Detection of CUD in mothers may prevent them from developing dilated cardiomyopathy.

Introduction

Carnitine uptake defect (MIM #212140; CUD), also known as primary carnitine deficiency, is an autosomal recessive disorder of fatty acid oxidation caused by mutations of the *SLC22A5* gene [1,2]. *SLC22A5* encodes the high-affinity carnitine transporter OCTN2 in the plasma membrane [1,3–5]. Carnitine is responsible for transporting fatty acids into mitochondria. Defective carnitine uptake results in urinary carnitine wasting and systemic and intracellular carnitine deficiencies [3]. Carnitine deficiency then leads to defects in beta-oxidation of fatty acids [2].

CUD is a potentially lethal disease. Patients with CUD usually have early-onset cardiomyopathy, muscle weakness, recurrent hypoketotic hypoglycemic coma or Reye-like syndrome [3,6–9]. Some patients may present later with isolated cardiomyopathy, including poor contractility, thickened ventricular walls or increased T waves on EKG [6,9]. Laboratory evaluations reveal extremely low blood and tissue carnitine concentrations (<5% of normal) [6,7]. With early treatment, the outcome is good, as most symptoms are reversible [9].

The recent implementation of tandem mass (MS/MS) spectrometry screening of newborns has been critical for diagnosing CUD [10–12]. By measuring dry blood spot (DBS) free carnitine levels, newborns with low DBS free carnitine levels can be evaluated for CUD. However, carnitine can be transported through the placenta [6,14,15]. Thus, a fetus with CUD can have a carnitine supply from the mother. In addition, a normal fetus can have a low free carnitine level when the mother has carnitine deficiency [3,6,13]. MS/MS screening was begun in Taiwan in 2001 [16,17]. Because

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doi:10.1016/j.ymgme.2009.12.015

of the prevalence of the *SLC22A5* gene founder mutation p.R254X in our population [18], special attention has been paid to low DBS free carnitine levels in the screening program. In this article, we report the diagnoses for four newborn cases and six cases of maternal CUD through newborn screening.

Materials and methods

Patients and CUD screening

Beginning in January 2001, newborns were screened for CUD at the Newborn Screening Center of the National Taiwan University Hospital by measuring dry blood spot (DBS) free carnitine levels. Blood spots were obtained from infants 48 h after birth and 24 h of feeding. One 1/8-inch punch was extracted by methanol and butylated acylcarnitines were analyzed using either API 2000 or API 3000 tandem mass spectrometry (Applied Biosystems, Foster City, CA, USA) [16].

The cut-offs for free carnitine were 2.6 μM from 2001 to 2005 and 2.86 μM in 2006, but only one case of CUD was detected. Because of the low detection rate, we increased the cut-offs to 6.44–10.95 μM beginning in 2007 (Table 1). For calculations of incidence and prevalence, we only included the period after 2007 (n = 202,076, Table 1). Two cases (MAT7 and NBS5) that were screened by other screening centers, but treated in our hospital, were also included for case analysis. Eight clinically diagnosed CUD cases were used for comparisons; some of these were described previously [19–21]. These 10 cases were not included in the calculations of incidence and prevalence.

For newborns who had DBS free carnitine levels lower than the cut-off, a recall DBS was requested. Babies who were premature, given a special formula or had poor oral intake were also recalled (rescreen). Babies who were positive for the recall or rescreen DBS were subjected to confirmation tests, including DBS free carnitine, liver enzymes, blood glucose, ammonium, urine ketone, electrocardiography, urinary organic acid analysis and molecular analysis. DBS free carnitine measurements and molecular analyses were also done for their mothers after receiving informed consent. For confirmed cases, carnitine supplementation (50–100 mg/kg/day) was given.

Molecular analysis

The molecular analysis study was approved by the Institutional Review Board (No. 2002.401). For molecular analysis of the *SLC22A5* gene, genomic DNA was isolated from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (Qiagen®, Hilden, Germany). The coding regions and their flanking intronic sequences were amplified by polymerase chain reaction (PCR) using primers as previously reported [18]. Sequencing of the PCR products was done using the BigDye method (Applied Biosystems, USA) [18].

Data analysis

Results are given as means ± SDs or ranges. Statistical analyses used SPSS statistical package version 11.5. Mann–Whitney Rank Test was used to compare different groups. A p value <0.05 was considered statistically significant.

Table 1
Free carnitine cut-off values and numbers of cases detected during the study period.

Year	Screened number	Cut-off (μM)	CUD case number	Maternal CUD case number
2001–2005	304,536	<2.6	0	0
2006	88,200	<2.86	1	0
2007/1–2007/5	31,329	<10.95	0	0
2007/6–2007/12	59,785	<6.44	1	1
2008/1–2009/7	110,962	<8	2	5

Results

From 2001 to 2006, 392,736 newborns were screened. Among them, only one newborn was recalled and then confirmed as a case of CUD (NBS1). Since 2007, the cut-off value was much higher (Table 1). Among 202,076 newborns screened during this period, 101 newborns were recalled due to low DBS free carnitine levels (Fig. 1); subsequently, nine entered the confirmation process. Among these nine newborns, three had CUD (NBS 2–4) and six had mothers affected by CUD (maternal CUD) (MAT1–6). Among 202,076 newborns, 12 were rescreened due to prematurity, use of special formula or poor oral intake; six required a confirmation, but none was positive. Therefore, the incidence of newborns with CUD after 2007 was around one in 67,000 (95% CI: one in 31,600–512,000), while the prevalence of maternal CUD was around one in 33,000 (95% CI: one in 18,700–169,000). We did not detect any missed CUD patients in our screening system during the study period.

Referred from other screening centers, we had NBS5 who had CUD and MAT7 who had maternal CUD. We also evaluated eight CUD patients found by clinical presentation (CLN1–8). In our hospital, a total of 20 cases were diagnosed; some of them have been previously described [19–21]. In contrast to clinically diagnosed cases who presented with cardiomyopathy, hyperammonemia or Reye-like syndrome, all newborns with CUD or babies born to mothers with CUD were asymptomatic at diagnosis. NBS1, two and five received carnitine supplementations immediately after diagnosis. A transient drug withdrawal at three months of age was followed by a rapid drop in free carnitine levels; thus, we immediately resumed the treatment. NBS3 did not take carnitine, and at age one year of age his parents reported that he was well. However, NBS4 had irregular medication before 18 months of age, and she had several episodes of Reye-like syndrome during that period. Her elder brother was also affected (CLN6). One mother with CUD (MAT3) reported herself as asymptomatic, but was found by us to have dilated cardiomyopathy and frequent ventricular premature beats (VPCs). Her heart size decreased after three months' carnitine supplementation. One mother (MAT6) felt more energetic after carnitine supplementation.

When we compared DBS free carnitine levels between different patient groups, we found that the clinically diagnosed CUD patients (lowest available DBS free carnitine level from 0.19 to 4.51 μM) and mothers with CUD (first available DBS free carnitine

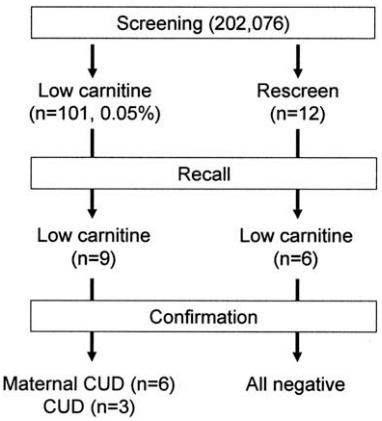

Fig. 1. Newborn screen, recall, and confirmation flow-chart from January 2007 to July 2009. CUD, carnitine uptake defect.

level from 0.89 to 2.17 μM) had lower free carnitine levels than either newborns with CUD (2.57–6.08 μM) or babies born to mothers with CUD (2.85–5.87 μM) (Fig. 2). Babies born to mothers with CUD had a gradual increase in free carnitine level during follow-up (Fig. 3). All of the six false-positive cases were confirmed based on a positive rescreen; five of them had normal first DBS free carnitine levels. However, their secondary carnitine deficiencies were quite prolonged, which may have led to a false diagnosis of CUD.

Ten different *SLC22A5* gene mutations were identified in this study; five of the mutations were novel (p.F17L, p.P143L, p.G234R, p.S362L and p.R471C; Table 2). The most common mutation in clinically diagnosed cases and newborns with CUD was p.R254X (50% and 30%, respectively), while the most common mutation in mothers with CUD was p.S467C (40%).

Discussion

Prevalence of CUD

In this paper, we report the results of newborn screening for CUD at a single screening center. During a 2.5-years period with an adequate cut-off, the prevalence of CUD in newborns was one in 67,000. The prevalence found in the current study is higher than results from most other studies [22]. Screening programs in North Carolina (n = 239,415; cut-off = 13 μM), California (n = 755,698), and Massachusetts and New England (n = 200,000) did not identify any cases of CUD [23–25]. A German program (n = 250,000) [10] and an Australian (New South Wales) program (n = 149,000, cut-off = 10 μM) found one case each [12]. The free carnitine cut-off values employed in each program differed, but their results were comparable [3,12].

The high incidence of CUD in the current study is due to a founder mutation (p.R254X) of the *SLC22A5* gene among Southern Chinese [18]. Tang et al. studied 250 control samples in which two heterozygote carriers of p.R254X were identified; thus, the population carrier rate for p.R254X would be about one in 125 [18]. Haplotypes of p.R254X alleles were examined and patients homozygous for p.R254X were also homozygous for the same haplotype of intragenic and microsatellites markers [18]. However, given the estimate that p.R254X accounts for 50% of all mutated alleles in clinical cases, the total mutation carrier rate will be ~ one in 62.5, and the incidence of CUD will be ~ one in 15,625, which is even higher than the incidence from the current study. It is interesting that, although one screening program in Japan found no cases of CUD among 102,200 newborns [26], an earlier study employing molecular methods gave different results [27]. In the latter report, from among 973 unrelated individuals in Akita, they identified 36 with low serum free carnitine levels. *SLC22A5* gene mutations were identified in nine of the 36, making an estimated incidence of CUD as one in 40,000. They also found recurrent mutations of p.W132X (n = 3) and p.S467C (n = 4) [27].

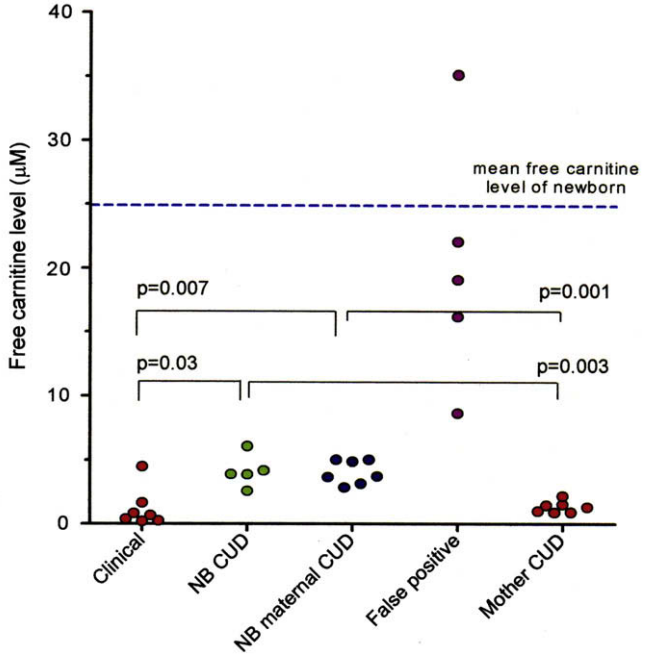

Fig. 2. First available dry blood spot free carnitine levels in different groups. Clinical, clinically diagnosed CUD patients; NB CUD, newborns with CUD; NB maternal CUD, babies born to mothers with CUD; Mother CUD, mothers with CUD.

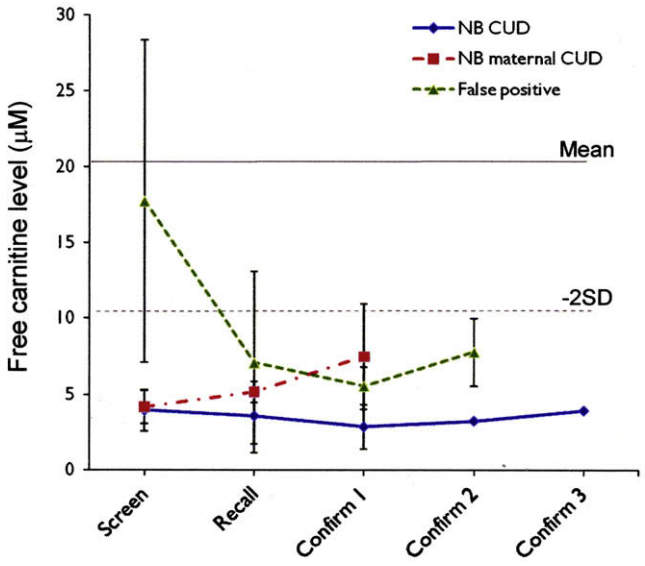

Fig. 3. Changes in free carnitine level during serial follow-up before carnitine supplementation. The solid line indicates the normal mean of newborns, and the dashed line indicates -2SD. NB CUD, newborns with CUD; NB maternal CUD, babies born to mothers with CUD.

Maternal effects

Free carnitine can be transported through the placenta. Fetuses with CUD have a supply of free carnitine through the placenta, although their mothers could be heterozygotes and have a significant chance of being carnitine deficient themselves. Babies with CUD have decreasing free carnitine levels after birth, but at the time of newborn screening (usually 2–3 days old) they may not have a level low enough to be detected by screening. This may be one of the reasons for false-negative cases in newborn screening for CUD, although certainly an inappropriate cut-off like that used during the first period of the current study causes false negatives. Free carnitine levels in the acylcarnitines profile analysis using the butylation method can be overestimated because of the breakdown of acylcarnitines. However, this method is reliable for detecting CUD, as sample acylcarnitines levels are also low.

By comparison, babies born to mothers with CUD do not have an adequate supply of carnitine and are carnitine deficient before birth. The prevalence of mothers with CUD was very high in the current study (one in 33,000). There have been other reports describing the detection of mothers with CUD through newborn screening programs [3,6,28]. Schimmenti et al. reported six mothers with CUD in 2007 [3]. Through cases that occurred in Minnesota, they estimated an incidence of CUD as one in 40,000 (four out of approximately 161,420 births), which was quite close to

Table 2
Clinical features and mutations in patients with carnitine uptake defect (CUD).

Case	Age at diagnosis	Gender	C0 level (μM)	SLC22A5 mutations allele1/allele2	Symptoms
MAT1	26 y	F	1.00 (3.15)[c]	S467C/Y396X	No
MAT2	32 y	F	1.52 (5.87)[c]	R254X/S467C	No
MAT3	34 y	F	2.17 (3.67)[c]	R282Q/S467C/S467C	CM
MAT4	31 y	F	1.30 (5.03)[c]	F17L*/F17L*	No
MAT5	24 y	F	0.90 (2.85)[c]	F17L*/S467C	No
MAT6	31 y	F	0.89 (3.73)[c]	R254X/?	LE
MAT7[a]	33 y	F	1.44 (4.90)[c]	R254X/S467C	No
NBS1	78 d	F	3.88	R254X/S362L*	No
NBS2	21 d	F	4.19	R254X/P143L*	No
NBS3	19 d	M	6.08	R254X/?	No
NBS4	18 d	F	2.57	?/?	No
NBS5[a]	30 d	F	3.00	?/?	No
CLN1[b]	20 y	M	0.38[d]	R254X /IVS3 + 1G > A	CM, HA, RL
CLN2	6 m	F	4.51[d]	R254X/Y387X	RL
CLN3	2y5 m	M	1.64[d]	R254X/R254X	CM, HA, RL, MA
CLN4	1y2 m	M	0.25[d]	R254X/R254X	CM, HA, HG, RL, MA
CLN5[b]	1y7 m	F	NA	R254X/R254X	RL
CLN6	6 m	M	0.82[d]	?/?	CM, HA, HG, RL, MA
CLN7	5y3 m	F	0.19[d]	F17L*/R471C*	HA, HG, RL
CLN8	8y7 m	F	0.66[d]	G234R*/R254X	HA, HG, RL

[a] From other screening center.
[b] Expired.
[c] Her baby's first dried blood spot data.
[d] Lowest available data.
* Mutation not reported before; CLN, clinically diagnosed cases; NBS, newborns with CUD; MAT, mothers with CUD; C0, free carnitine; CM, cardiomyopathy; HA, hyperammonemia; HG, hypoglycemia; LE, less energetic; MA, metabolic acidosis; No, no symptoms; NA, not available; RL, Reye-like syndrome; ?, mutation not found; CLN4 and CLN5 are sibs; CLN6 and NBS4 are sibs. The first C0 concentration in CLN6 at 3-day-old was 6.52 μM by other screening center.

the incidence discovered by our study. If the prevalence of mothers with CUD (one in 33,000) represents a true incidence for CUD, the false-negative rate for newborn screening will be 50% based on our current performance (an incidence of one in 67,000).

Phenotypes of CUD

Detection of CUD has lead to the discovery of undiagnosed, asymptomatic siblings [29]. This raised a concern that biochemically affected individuals may not have any symptoms. The first four mothers reported as diagnosed through their babies were asymptomatic [6]. However, in the report by Schimmenti et al., among the six mothers, one had fasting intolerance and fatigue, one had decreased stamina, and another had ventricular arrhythmia and prolonged QT intervals [3]. In our study, one mother had dilated cardiomyopathy and arrhythmia and another complained of fatigue.

It is likely that adult females with CUD may have a disease phenotype different from classic CUD. This may be explained by the mild mutations found in the mothers, such as p.S467C, p.F17L and p.R282Q. *In vitro* carnitine uptake assays showed that the function of p.S467C was around 11% of control [27]. A homozygous p.R282Q mutation was described in a 7-week-old girl presenting with failure to thrive, gastroesophageal reflux and hypotonia, and this mutation had about 10% residual transport activity compared with other mutations reported in patients with classic presentations (0–5%) [30]. Newborns with mild mutations may be more likely to be missed by screening, which explains the differences in mutations between newborns and mothers with CUD.

In our cohort, most clinically diagnosed cases carried the p.R254X mutation, which indicates that this mutation may cause significant clinical presentations, such as dilated cardiomyopathy, hyperammonemia and others. The mother with dilated cardiomyopathy in the current study may have had three mutations: one chromosome with p.S467C and another chromosome with both p.S467C and p.R282Q. Because of the relatively low enzyme activity of p.S467C *in vitro*, it is likely that p.S467C plus another severe mutation would be clinically significant. For example, MAT2 and MAT7 (both had p.R254X/p.S467C) may be symptomatic at a later time because p.R254X is a well-known mutation in clinically diagnosed CUD cases. Nonetheless, MAT6 who had two severe mutations (p.R254X/p.R254X) should also be carefully monitored for her clinical presentations in the future. Because acute death is one of the major presentations in adults with CUD, carnitine supplementation is the safest and most acceptable management for any asymptomatic individual with "profound" carnitine deficiency.

In conclusion, this study demonstrated the high incidence and importance of CUD in the Southern Chinese population. What cannot be covered at this time are possible undiagnosed male adult patients who are at risk for cardiomyopathy and acute death [29,31]. Long term follow-up of patients detected by screening and searching for modifying genes will be necessary in the future [29,32].

Acknowledgments

We thank all doctors and nurses who helped with the collection of samples for newborn screening, and the Bureau of Health Promotion, Department of Health in Taiwan for supporting newborn screening.

References

[1] J. Nezu, I. Tamai, A. Oku, R. Ohashi, H. Yabuuchi, N. Hashimoto, H. Nikaido, Y. Sai, A. Koizumi, Y. Shoji, G. Takada, T. Matsuishi, M. Yoshino, H. Kato, T. Ohura, G. Tsujimoto, J. Hayakawa, M. Shimane, A. Tsuji, Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter, Nat. Genet. 21 (1999) 91–94.
[2] Online Mendelian Inheritance in Man, OMIM (TM). Johns Hopkins University, Baltimore, MD. MIM Number: 212140: 8/3/2009. Available from http://www.ncbi.nlm.nih.gov/omim/.
[3] L.A. Schimmenti, E.A. Crombez, B.C. Schwahn, B.A. Heese, T.C. Wood, R.J. Schroer, K. Bentler, S. Cederbaum, K. Sarafoglou, M. McCann, P. Rinaldo, D. Matern, C.A. di San Filippo, M. Pasquali, S.A. Berry, N. Longo, Expanded newborn screening identifies maternal primary carnitine deficiency, Mol. Genet. Metab. 90 (2007) 441–445.

[4] Y. Wang, J. Ye, V. Ganapathy, N. Longo, Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency, Proc. Natl. Acad. Sci. USA 96 (1999) 2356–2360.
[5] N.L. Tang, V. Ganapathy, X. Wu, J. Hui, P. Seth, P.M. Yuen, R.J. Wanders, T.F. Fok, N.M. Hjelm, Mutations of OCTN2, an organic cation/carnitine transporter, lead to deficient cellular carnitine uptake in primary carnitine deficiency, Hum. Mol. Genet. 8 (1999) 655–660.
[6] S. Vijay, A. Patterson, S. Olpin, M.J. Henderson, S. Clark, C. Day, G. Savill, J.H. Walter, Carnitine transporter defect: diagnosis in asymptomatic adult women following analysis of acylcarnitines in their newborn infants, J. Inherit. Metab. Dis. 29 (2006) 627–630.
[7] I. Tein, D.C. De Vivo, F. Bierman, P. Pulver, L.J. De Meirleir, L. Cvitanovic-Sojat, R.A. Pagon, E. Bertini, C. Dionisi-Vici, S. Servidei, et al., Impaired skin fibroblast carnitine uptake in primary systemic carnitine deficiency manifested by childhood carnitine-responsive cardiomyopathy, Pediatr. Res. 28 (1990) 247–255.
[8] C.A. Stanley, S. DeLeeuw, P.M. Coates, C. Vianey-Liaud, P. Divry, J.P. Bonnefont, J.M. Saudubray, M. Haymond, F.K. Trefz, G.N. Breningstall, et al., Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake, Ann. Neurol. 30 (1991) 709–716.
[9] C.A. Stanley, M.J. Bennett, E. Mayatepek, Disorders of mitochondrial fatty acid oxidation and related metabolic pathways, in: J. Fernandes, J.M. Saudubray, G. van den Berghe, J.H. Walter (Eds.), Inborn Metabolic Diseases, Springer, Heidelberg, 2006, pp. 177–188.
[10] A. Schulze, M. Lindner, D. Kohlmuller, K. Olgemoller, E. Mayatepek, G.F. Hoffmann, Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: results, outcome, and implications,, Pediatrics 111 (2003) 1399–1406.
[11] B. Wilcken, V. Wiley, J. Hammond, K. Carpenter, Screening newborns for inborn errors of metabolism by tandem mass spectrometry, N. Engl. J. Med. 348 (2003) 2304–2312.
[12] B. Wilcken, V. Wiley, K.G. Sim, K. Carpenter, Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry, J. Pediatr. 138 (2001) 581–584.
[13] M. Schoderbeck, B. Auer, E. Legenstein, H. Genger, P. Sevelda, H. Salzer, R. Marz, A. Lohninger, Pregnancy-related changes of carnitine and acylcarnitine concentrations of plasma and erythrocytes, J. Perinat. Med. 23 (1995) 477–485.
[14] F. Scaglia, N. Longo, Primary and secondary alterations of neonatal carnitine metabolism, Semin. Perinatol. 23 (1999) 152–161.
[15] J. Christodoulou, S.H. Teo, J. Hammond, K.G. Sim, B.Y. Hsu, C.A. Stanley, B. Watson, K.C. Lau, B. Wilcken, First prenatal diagnosis of the carnitine transporter defect, Am. J. Med. Genet. 66 (1996) 21–24.
[16] H.P. Huang, K.L. Chu, Y.H. Chien, M.L. Wei, S.T. Wu, S.F. Wang, W.L. Hwu, Tandem mass neonatal screening in Taiwan–report from one center, J. Formos. Med. Assoc. 105 (2006) 882–886.
[17] W.L. Hwu, A.C. Huang, J.S. Chen, K.J. Hsiao, W.Y. Tsai, Neonatal screening and monitoring system in Taiwan, Southeast Asian J. Trop. Med. Public Health 34 (Suppl. 3) (2003) 91–93.
[18] N.L. Tang, W.L. Hwu, R.T. Chan, L.K. Law, L.M. Fung, W.M. Zhang, A founder mutation (R254X) of SLC22A5 (OCTN2) in Chinese primary carnitine deficiency patients, Hum. Mutat. 20 (2002) 232.
[19] N.L. Tang, J. Hui, L.K. Law, K.F. To, K.L. Cheung, H.N. Magnus, P.M. Yuen, T.F. Fok, Primary carnitine deficiency in the Chinese, Chin. Med. J (Engl.) 113 (2000) 376–380.
[20] W.L. Hwu, S.C. Chiang, M.H. Chang, T.R. Wang, Carnitine transport defect presenting with hyperammonemia: report of one case, Acta Paediatr. Taiwan 41 (2000) 36–38.
[21] W.L. Hwu, Y.H. Chien, N.L. Tang, L.K. Law, C.Y. Lin, N.C. Lee, Deficiency of the carnitine transporter (OCTN2) with partial *N*-acetylglutamate synthase (NAGS) deficiency, J. Inherit. Metab. Dis. 30 (2007) 816.
[22] D.J. Dietzen, P. Rinaldo, R.J. Whitley, W.J. Rhead, W.H. Hannon, U.C. Garg, S.F. Lo, M.J. Bennett, National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary, Clin. Chem. 55 (2009) 1615–1626.
[23] L. Feuchtbaum, F. Lorey, L. Faulkner, J. Sherwin, R. Currier, A. Bhandal, G. Cunningham, California's experience implementing a pilot newborn supplemental screening program using tandem mass spectrometry, Pediatrics 117 (2006) S261–269.
[24] D.M. Frazier, D.S. Millington, S.E. McCandless, D.D. Koeberl, S.D. Weavil, S.H. Chaing, J. Muenzer, The tandem mass spectrometry newborn screening experience in North Carolina: 1997–2005, J. Inherit. Metab. Dis. 29 (2006) 76–85.
[25] D. Marsden, Expanded newborn screening by tandem mass spectrometry: the Massachusetts and New England experience, Southeast Asian J. Trop. Med. Public Health 3 (2003) 111–114.
[26] Y. Shigematsu, S. Hirano, I. Hata, Y. Tanaka, M. Sudo, N. Sakura, T. Tajima, S. Yamaguchi, Newborn mass screening and selective screening using electrospray tandem mass spectrometry in Japan, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 776 (2002) 39–48.
[27] A. Koizumi, J. Nozaki, T. Ohura, T. Kayo, Y. Wada, J. Nezu, R. Ohashi, I. Tamai, Y. Shoji, G. Takada, S. Kibira, T. Matsuishi, A. Tsuji, Genetic epidemiology of the carnitine transporter OCTN2 gene in a Japanese population and phenotypic characterization in Japanese pedigrees with primary systemic carnitine deficiency, Hum. Mol. Genet. 8 (1999) 2247–2254.
[28] G. la Marca, S. Malvagia, B. Casetta, E. Pasquini, M.A. Donati, E. Zammarchi, Progress in expanded newborn screening for metabolic conditions by LC-MS/MS in Tuscany: Update on methods to reduce false tests, J. Inherit. Metab. Dis. (2008), Short Report #127 Online, doi:10.1007/s10545-008-0965-z.
[29] U. Spiekerkoetter, G. Huener, T. Baykal, M. Demirkol, M. Duran, R. Wanders, J. Nezu, E. Mayatepek, Silent and symptomatic primary carnitine deficiency within the same family due to identical mutations in the organic cation/carnitine transporter OCTN2, J. Inherit. Metab. Dis. 26 (2003) 613–615.
[30] C. Amat di San Filippo, M. Pasquali, N. Longo, Pharmacological rescue of carnitine transport in primary carnitine deficiency, Hum. Mutat. 27 (2006) 513–523.
[31] K. Karmaniolas, P. Ioannidis, S. Liatis, M. Dalamanga, T. Papalambros, I. Migdalis, Primary carnitine deficiency in a male adult, J. Med. 33 (2002) 105–110.
[32] Y. Wang, F. Taroni, B. Garavaglia, N. Longo, Functional analysis of mutations in the OCTN2 transporter causing primary carnitine deficiency: lack of genotype–phenotype correlation, Hum. Mutat. 16 (2000) 401–407.