Carnitine transporter defect diagnosed by newborn screening with electrospray tandem mass spectrometry

1651

Bridget Wilcken, MBChB, FRACP, Veronica Wiley, PhD, Keow Giak Sim, BSc, and Kevin Carpenter, PhD

The carnitine transporter defect is a potentially fatal but treatable disorder. We used electrospray tandem mass spectrometry in the New South Wales (Australia) Newborn Screening Programme to measure free carnitine and acylcarnitine species in the newborn population. Free carnitine levels in dried blood samples from 149,000 neonates did not vary markedly between 2 and 8 days of age. Two of 4 babies subsequently diagnosed clinically with the carnitine transporter defect had a free carnitine level in the neonatal blood sample low enough to be detected by screening. (J Pediatr 2001;138: 581-4)

Carnitine is necessary for the transport of long-chain fatty acids, as acyl coenzyme A esters, across the mitochondrial membrane and is thus an essential component of normal fatty acid oxidation. Carnitine is synthesized from lysine, but the greater part is derived from diet.[1] There is an active transport system across membranes in small intestine, renal tubule, and skeletal muscle, which is also expressed in skin fibroblasts.[2-4] Defective transport of carnitine across cell membranes causes a variety of potentially fatal symptom complexes in infancy and childhood, including neonatal death, hepatic encephalopathy, skeletal myopathy, and more commonly, dilated cardiomyopathy.[5] More than 40 cases have been recorded, variously described as primary carnitine deficiency, carnitine uptake defect, and plasma membrane carnitine transporter defect. The genetic defect is in a sodium ion–dependent carnitine transporter that has been mapped at 5q31.1.[6] Mutations in the gene, OCTN2, have been shown to impair carnitine transport in patients with the carnitine transporter defect.[7]

The carnitine transporter defect is treatable with oral carnitine therapy.[8] Clinical detection is only possible when there are significant symptoms, and affected patients have died of hepatic encephalopathy or cardiac failure before the diagnosis was made.[5] This makes the defect an ideal candidate for newborn screening. The recent introduction of electrospray tandem mass-spectrometry for newborn screening makes possible the diagnosis of a number of inborn errors of metabolism, including fatty acid oxidation defects, but the sensitivity of this technique for the various defects is so far unknown. We introduced this technology to the New South Wales Newborn Screening Programme in 1998.

We recently identified a neonate with carnitine transporter defect by routine newborn screening and retrospectively analyzed newborn screening samples from patients previously identified clinically to investigate the sensitivity of MS/MS for detecting this disorder in neonates.

MS/MS Tandem mass spectrometry

METHODS

Patients

The patient identified by newborn screening (patient 1) was a term baby with a birth weight of 3980 g. The newborn screening sample taken on day 4 had a low level of free carnitine, confirmed in a repeat sample on day 23 (Table I). He was breast fed and was well at all times. A baseline echocardiogram showed no abnormalities at 6 weeks, when carnitine therapy was started.

Five patients from 3 families have been identified clinically in New South

From New South Wales Newborn Screening and Biochemical Genetics Services, The New Children's Hospital, Westmead, Sydney, Australia.

Submitted for publication Mar 17, 2000; revisions received July 6, 2000, and Sept 11, 2000; accepted Sept 20, 2000.

Reprint requests: Bridget Wilcken, MBChB, FRACP, New Children's Hospital, PO Box 3515, Parramatta, New South Wales 2124, Australia.

0022-3476/2001/$35.00 + 0 9/22/111813
doi:10.1067/mpd.2001.111813

Table I. Babies with the carnitine transporter defect: Clinical features and free and acetyl carnitine species measured by electrospray MS/MS in routine newborn screening dried blood spots assayed either prospectively or retrospectively

Patient No.	Day of sample	Screened/retrospective	Clinical presentation	Free carnitine (μmol/L)	Acetyl carnitine (μmol/L)
1	4	Prospective	Clinically well, identified by screening	4	5
1	23	Prospective test, repeat sample	Clinically well	1	4
2	3	Pilot study	Cardiac failure at 18 mo	10	45
2	3	Sample re-assayed after storage for 2 y		11	10
3	4	Retrospective.	Brother had cardiac failure at 17 mo and died at 18 mo; patient 3 well at 9 mo on diagnosis	7	†
4°	3	Retrospective	Sister had cardiac failure at 6 y; patient 4 diagnosed prenatally°	24*	†

Timing of samples in relation to feeding is unknown.
°Patient 4 was diagnosed prenatally. The mother received carnitine, 1.25 g/d, during pregnancy, and the baby was breast fed.
†Stored card: acetyl carnitine measurement was inaccurate.

Table II. Free carnitine levels (μmol/L whole blood) in the neonatal population, measured by tandem mass spectrometry

Neonate class	Number	Free carnitine median	Free carnitine 5th and 95th centiles	Number (%) <10 μmol/L (0.016 mg/dL)	Number <7 μmol/L	Number <5 μmol/L
Age 2 days	13,311	26.6	14.0-52.0	98 (0.73%)	4	0
Age 3 days	79,422	26.8	14.1–51.8	500 (0.63%)	46	3
Age 4 days	51,248	27.2	14.1–52.3	330 (0.64%)	30	2
Age 5-8 days	6,546	27.5	14.3–49.6	41 (0.74%)	7	1
Birth weight <2000 g	3,264	30.5	14.0–58.6	48 (1.47%)	17	2

Wales since 1990; the diagnosis was suspected after low levels of plasma free carnitine were found and was confirmed by uptake studies on cultured skin fibroblasts (Table I).[9]

Newborn screening blood samples from 3 patients were retrieved and tested retrospectively; one of them (patient 2) had had a prospective MS/MS test of the newborn screening sample during a pilot study.

Protocol

The MS/MS methods were those we routinely use in newborn screening. Briefly, free and acyl carnitines were extracted from blood samples dried on filter paper and converted to butyl esters before analysis by electrospray MS/MS (Micromass Quattro II).[10] Carnitine uptake in cultured skin fibroblasts was measured as described by Stanley et al.[11] Rates of fatty acid oxidation were measured by tritium water release assay as described by Manning et al.[12]

The low cutoff for free carnitine adopted for the newborn population was 10 μmol/L (0.16 mg/dL) for confirmation assay within the laboratory (to include at least the lowest 0.5% of values). A confirmed level of <5 μmol/L (0.08 mg/dL) generated a request for a second sample. The median level of dried blood spot acetyl carnitine was 44 μmol/L (0.9 mg/dL), and the low cutoff was 10 μmol/L (0.2 mg/dL). Our cutoff levels for other acylcarnitine species have been published.[10]

RESULTS

Table II shows the free carnitine levels measured by MS/MS of dried blood samples from the newborn population collected on days 2, 3 (the preferred day), 4, or 5 to 8. The percentage of samples with a level of <5 and <10 μmol/L (<0.08 and <0.16 mg/dL) was similar for each group. The distribution was skewed to the right. The median levels of free carnitine increased slightly from day 2 to days 5 to 8, from

26.6 to 27.5 μmol/L (0.43-0.44 mg/dL). Babies weighing <2000 g had somewhat higher median free carnitine levels, and only 2 of 3268 had a free carnitine level of <5 μmol/L (0.08 mg/dL), which was not significantly different from the proportion in normal birth weight babies. The affected baby (patient 1), identified by newborn screening, had a level of free carnitine below the cutoff on day 3, when the routine screening sample was taken. The affected baby (patient 2), born during the pilot study before our cutoff levels had been established, had a free carnitine level of 10.2 μmol/L (0.16 mg/dL) and so would not have been identified with our current protocol. Neither of the two babies retrospectively tested would have been diagnosed with the current protocol, although minor adjustments to this would have identified one of them. Reassay of the dried blood spot of patient 2 after an interval of 30 months showed that although the acetyl carnitine value had decreased greatly during storage, the free carnitine value was similar (Table I).

The carnitine uptake assay in cultured skin fibroblasts from all our patients with the carnitine transporter defect demonstrated very low uptakes of carnitine, 5% to 8% of control values. The tritiated water release assay on cultured skin fibroblasts, a general fatty acid oxidation screening assay, also provided abnormal results for patient 1, with low oxidation rates of both oleate and myristate of 21% of intra-batch control values for each.

DISCUSSION

We have shown that some cases of the carnitine transporter defect can be detected by expanded newborn screening. However, at least one and perhaps two of our clinically identified patients could not have been identified by measurement of free carnitine alone with any reasonable protocol. We have provided data on free carnitine levels in the healthy neonatal population for days 2 to 8 and for low birth weight babies. The measurements are of whole blood levels determined by MS/MS and do not equate with levels measured in plasma by this or other methods. Shenai et al[13] measured total carnitines in whole cord blood and found levels approximately 23% higher than our median level of free carnitine on day 2. These authors also substantiate that total carnitine levels are higher in red blood cells than in plasma. Because published plasma levels of free carnitine are similar to those we found in whole blood,[13] the excess is likely to be largely acetylcarnitine. There is not a sufficient change in whole blood free carnitine levels from day 2 to day 8 that would dictate different action levels for newborn screening dependent on day of screening.

In neonates, low free carnitine levels are seen principally in those who are sick. We found, in intensive care units, an over-representation of babies with levels <10 μmol/L (0.16 mg/dL). In neonates with a variety of inborn errors of organic acid or fatty acid metabolism, low free carnitine levels are usual, but generally other individual acyl carnitines will be elevated, and these elevations are readily detectable by MS/MS in the same assay. When there is very low free carnitine with no other elevated acylcarnitines, a carnitine transporter defect must be considered.

Of the two affected babies tested prospectively, one (patient 2) had a level well above our current cutoff for action. Of the two retrospectively studied, both had levels above this action level, although one level (patient 3) was only marginally so. The other retrospectively studied baby (patient 4) had been identified prenatally as being affected. Her mother was receiving carnitine medication during the pregnancy, and the baby was breast fed. This may have influenced her carnitine levels on day 3, when her screening sample was taken. The timing of the samples in relation to feeding is not known, but feeding regimens are an important determinant of postnatal carnitine levels.[13] Moreover, carnitine is actively transported across the placenta,[14] and maternal levels influence the neonatal load.[15] Babies with a defect in the carnitine transporter fail to reabsorb carnitine in the proximal renal tubule, and blood levels fall rapidly after birth.[9] In stored blood samples, acetyl carnitine apparently degrades, and measured levels are low. This is not accompanied by a significant rise in free carnitine measured by MS/MS.

To have a better chance of identifying all babies with the carnitine transporter defect, the cutoff level would need to be increased, at the cost of having more false-positive cases. It is arguable to what extent this is acceptable, especially for such an apparently rare disorder. In our program a cutoff level of 5 μmol/L led to a recall rate of only 0.004%, whereas adopting a higher cutoff of 10 μmol/L (0.16 mg/dL) would have generated a recall of 0.65%. This in itself is modest, but screening by MS/MS involves the analysis of 20 or more analytes for potential detection of a large number of disorders.[10] Each analyte will have an individual cutoff level, and sometimes ratios of two analytes may be used in addition. Because of this, the overall recall rate could easily become unacceptably high. For detecting the carnitine transporter defect, there is a case for a modest increase in the cutoff level of free carnitine to 6 or 7 μmol/L (0.1-0.11 mg/dL). A cutoff of 7 μmol/L (0.11 mg/dL) would have generated a recall rate of 0.058% in our series and would probably have identified one of the clinically diagnosed patients in addition.

It is known that obligate heterozygotes for the carnitine transporter defect may have levels of plasma carnitine below the normal range.[6] This implies that carriers, particularly when the mother is also a carrier, might be identified in newborn screening programs. It is not yet known whether the false-positive cases, babies with initially low

levels of carnitine but who do not have the transporter defect, will turn out to be carriers. The prevalence of carriers is not known in our population. Extrapolating from all cases diagnosed in New South Wales from 1990 to 1999, we would anticipate a carrier rate of 1:240 (95% CI 1:150-1:480).

The detection of the carnitine transporter defect in a neonate, reported here, helps to affirm the usefulness of the new trend in newborn screening, use of MS/MS to detect in a single test a wide range of disorders that are too rare to merit individual testing procedures. The carnitine transporter defect is but one of many rare but treatable disorders that have been detected by this form of screening.[10,16]

REFERENCES

1. Stanley CA. New genetic defects in mitochondrial fatty acid oxidation and carnitine deficiency. Adv Pediatr 1987; 34:69-88.
2. Shaw RD, Li BU, Hamilton JW, Shug AL, Olsen WA. Carnitine transport in rat small intestine. Am J Physiol 1983; 245:G376-81.
3. Rebouche CJ, Mack DL. Sodium gradient-stimulated transport of L-carnitine into renal brush border membrane vesicles: kinetics, specificity, and regulation by dietary carnitine. Arch Biochem Biophys 1984;235:393-402.
4. Rebouche CJ, Engel AG. Carnitine transport in cultured muscle cells and skin fibroblasts from patients with primary systemic carnitine deficiency. In Vitro 1982;18:495-500.
5. Stanley CA, DeLeeuw S, Coates PM, Vianey-Liaud C, Divry P, Bonnefont JP, et al. Chronic cardiomyopathy and weakness or acute coma in children with a defect in carnitine uptake. Ann Neurol 1991;30:709-16.
6. Tamai I, Ohashi R, Nezu J, Yabuchi H, Oku A, Shimane M, et al. Molecular and functional identification of sodium ion dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 1998;273:20378-82.
7. Nezu J-I, Tamai I, Oku A, Ohashi R, Yabuchi H, Hashimoto N, et al. Primary systemic carnitine deficiency is caused by mutations in a gene encoding sodium ion-dependent carnitine transporter. Nat Genet 1999;21:91-4.
8. Pierpont ME, Breningstall GN, Stanley CA, Singh A. Familial carnitine transporter defect: a treatable cause of cardiomyopathy in children. Am Heart J 2000;139:96-106.
9. Christodoulou J, Teo SK, Hammond J, Sim KG, Hsu BYL, Stanley CA, et al. First prenatal diagnosis of the carnitine transporter defect. Am J Med Genet 1996;66:21-4
10. Wiley V, Carpenter KH, Wilcken B. Tandem mass spectrometry in the New South Wales Newborn Screening Programme. Acta Pediatr 1999;(Suppl 432):48-51.
11. Stanley CA, Treem WR, Hale DE, Coates PM. A genetic defect in carnitine transport causing primary carnitine deficiency. Prog Clin Biol Res 1990;321:467-64.
12. Manning NJ, Olpin SE, Pollitt RJ, Webley J. A comparison of [9,10-^{3}H]palmitic and [9,10-^{3}H]myristic acids for the detection of defects of fatty acid oxidation in intact cultured fibroblasts. J Inherit Metab Dis 1990;13:68-68.
13. Shenai JP, Borum PR, Mohan P, Donlevy SC. Carnitine status at birth of newborn infants of varying gestation. Pediatr Res 1983;17:579-82.
14. Tamai I, Ohashi R, Nezu J, Yabuchi H, Shimane M, Sai Y, et al. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter. J Biol Chem 1998;273:20378-82.
15. Sachan DS, Smith RB, Plattsmier J, Lorch V. Maternal, cord, and neonatal carnitine correlations and lipid profiles of various birthweight infants. Am J Perinatol 1989;6:14-7.
16. Levy HL. Newborn screening by tandem mass spectrometry: a new era. Clin Chem 1998;44:2401-2.