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First Prenatal Diagnosis of the Carnitine Transporter Defect

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We report the first attempt at prenatal diagnosis of the carnitine transporter defect in a fetus at high risk of having the disorder. Analysis of cultured CVS after prolonged culture predicted that the fetus was not affected but might be heterozygous for the carnitine transporter defect, but chromosome 15 satellite DNA markers showed no paternal contribution, suggesting that the CVS cells assayed were of predominantly maternal origin. Subsequent assay of cultured amniocytes predicted that the fetus would be affected, and this was confirmed in the newborn period. We conclude that prenatal diagnosis of the carnitine transporter defect is possible, but where results depend on extended culture of CVS, molecular studies should be performed to confirm genetic contributions from both parents.

KEY WORDS: cardiomyopathy, carnitine, transport, fatty acid oxidation, inborn error of metabolism, prenatal diagnosis

INTRODUCTION

Carnitine (3-hydroxy-4-N-trimethylammonium butyric acid) plays a critical role in fatty acid metabolism, being involved in the transport of long-chain fatty acyl CoA esters across the mitochondrial membranes, and allowing β-oxidation of fatty acids to occur in the mitochondrial matrix [Stanley, 1987]. Carnitine is largely derived from dietary sources [Stanley, 1987]. There is an active transport system across cell membranes for carnitine in the small intestine [Shaw et al., 1983], kidney [Rebouche and Mack, 1984], skeletal muscle, and cultured skin fibroblasts [Rebouche and Engel, 1982]. A defect of the carnitine transporter, with autosomal recessive inheritance, can cause one of three abnormal phenotypes in infancy or early childhood: potentially fatal dilated cardiomyopathy, hypoketotic hypoglycemia or, less commonly, progressive skeletal myopathy [Treem et al., 1988; Stanley et al., 1991]. We report here what we believe is the first attempt at prenatal diagnosis of the carnitine transporter defect and highlight potential difficulties in prenatal diagnosis using enzymatic assay of cultured fetal cells in genetic metabolic disorders.

SUBJECTS AND MATERIALS

Methods

Total and free carnitine in plasma were estimated by using [1-14C]acetyl coenzyme A and carnitine acetyltransferase [de Sousa et al., 1990]. Skin fibroblasts were established and cultured using previously developed methods [Freshney, 1992], as were CVS and amniocytes [Priest, 1991]. The fibroblast carnitine transporter assay was performed as reported [Stanley et al., 1990].

Evaluation of fetal cells for the presence of both maternal and paternal DNA contributions was performed using multiplex PCR [Mutirangura et al., 1993]. Two pairs of primers were used to amplify two polymorphic CA microsatellites within chromosome 15 (15q11-q13), with the PCR products being run on a 6% polyacrylamide gel and bands visualized by silver staining.

Subjects

The proband, RL, the second child of unrelated Chinese parents, was born after an uncomplicated pregnancy and delivery. Her elder sister had apparently died of meconium aspiration at 12 hours of age in China. RL's early developmental milestones and health were normal, until the age of 6 years when she presented with acute cardiac decompensation following a viral illness. Her plasma total carnitine was unde-

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tectable (normal 35–65 mmol/L), which was highly suggestive of a carnitine transporter defect. The diagnosis was confirmed by direct assay in cultured skin fibroblasts (see Table I). Initial therapy consisted of frusemide, spironolactone, captopril, and carnitine at a dose of 200 mg/kg/day, with rapid resolution of symptoms. Her cardiac size and function, based on echocardiographic studies, returned to normal within 3 months. Three months later the antifailure drugs could be suspended, with carnitine therapy continuing. When last reviewed at age 9 years, she remained in good health, with normal exercise tolerance, and developmental progress continued to be normal.

RL's mother subsequently became pregnant and requested prenatal diagnosis. As prenatal diagnosis for this disorder had not been previously reported, the mother was counselled that an attempt at diagnosis would be initially made using cultured CV cells, but that if these studies predicted a normal fetus or were equivocal then studies of cultured amniocytes should also be performed.

RESULTS

Table I summarises the results of these enzymatic studies. Chorionic villus sampling was performed at 11 weeks gestation, with assay of cultured cells not being performed until 17 weeks gestation because of slow growth of cells. Carnitine transporter assay results predicted that the fetus would not be affected, but might be heterozygous. The karyotype was 46XX, and so chromosome 15 satellite marker analysis was performed. This however showed no paternal contribution, suggesting that the cells assayed were only of maternal origin. Amniocentesis was therefore performed. These cultured cells also had a 46XX karyotype, but this time chromosome 15 satellite markers clearly showed a paternal contribution (see Fig. 1). The carnitine transporter assay on amniocytes predicted that the fetus would be affected (Table I).

RL's mother was started on carnitine (50 mg/kg/day). She reported that she felt subjectively better on carnitine, no longer complaining of periodic dizziness, feeling less fatigued whilst taking carnitine, and experiencing no adverse effects. The pregnancy was continued to term, with a phenotypically normal female infant (EL) born following an uneventful labour. Birthweight was 3,040 gm, and Apgar scores were 9 and 9 at 1 and 5 minutes, respectively. The infant breast fed normally and experienced no problems in the neonatal period. Echocardiography on day 3 was normal. Plasma carnitine levels were measured serially and results are shown in Table II. As can be seen, by 1 week of age the plasma carnitine levels were very low, confirming that the infant was affected. Carnitine therapy (100 mg/kg/day) was commenced at 2 weeks of age. When last reviewed at 6 months of age, the infant was well, with normal growth, development, and physical examination, and with echocardigraphy showing normal heart size and function.

TABLE I. Carnitine Transporter Activity in Cultured Cells*

Tissue type	Patient[a] (pmol/min/mg protein)	Normal control[a] (pmol/min/mg protein)
Fibroblasts		
Proband (RL)	0.078	1.65
Chorionic villus cells		
Patient EL	0.45	1.50
Cultured amniocytes		
Patient EL	0.01	1.77

* Uptake of L-methyl-[^{3}H]carnitine in cultured cells from the patients and normal controls was measured as previously published [Stanley et al., 1990].
[a] Results are means of duplicate assays.

DISCUSSION

To date, over 20 cases of the carnitine transporter defect have been reported and, at least in the short term, patients have responded favourably to carnitine supplementation. However the long-term prognosis is uncertain [Stanley, 1992], and it is for this reason that prenatal diagnosis was requested by the proband's parents. Because the defect is expressed in cultured skin fibroblasts, it was felt that prenatal diagnosis would probably be possible using cultured fetal cells, although we recognised that this can be problematical in some inborn errors of metabolism [Fowler et al., 1989]. Assay of the carnitine transporter in cultured amniocyte cells clearly showed that the fetus was affected and this was confirmed by the demonstration of a dramatic decline of plasma carnitine in the first 2 weeks of life. We believe this is the first report of prenatal diagnosis in this inherited metabolic disorder.

The cultured CV cells, on the other hand, predicted that the fetus might be heterozygous for the disorder and chromosome 15 satellite DNA marker analysis showed that these cultured cells were of maternal origin. Dissection of maternal tissues from CV biopsies requires considerable expertise, making residual maternal contamination a small though real possibility. It has been estimated that CVS maternal contamination may occur in as many as 1.8% of procedures [Ledbetter et al., 1992]. Because prolonged culture of the CV cells was necessary to obtain an adequate number of cells for assay, it is possible that the culture conditions favoured cells of maternal origin, allowing presumed minor maternal contamination of the CVS to overrun the fetal cells, as has been previously reported [Besley and Broadhead, 1989], and accounts for our results in this case. This observation has significant implications wherever prenatal diagnosis relies on biochemical or molecular studies of cultured cells.

To our knowledge, diagnosis of the carnitine transporter defect has not previously been made in the neonatal period. We and others [Schmidt-Sommerfeld et al., 1988] have found that plasma carnitine levels in normal infants are lower in the first week than later in life, possibly because of decreased maternal levels [Marzo et al., 1994]. Females heterozygous for the carnitine transporter defect usually have low plasma carnitine levels [Stanley, 1992]. Because of this, and because individuals with the carnitine transporter defect may initially present with hypoglycemia in infancy in up to 45% of cases [Stanley et al., 1991], we elected to treat the mother with carnitine supplementation dur-

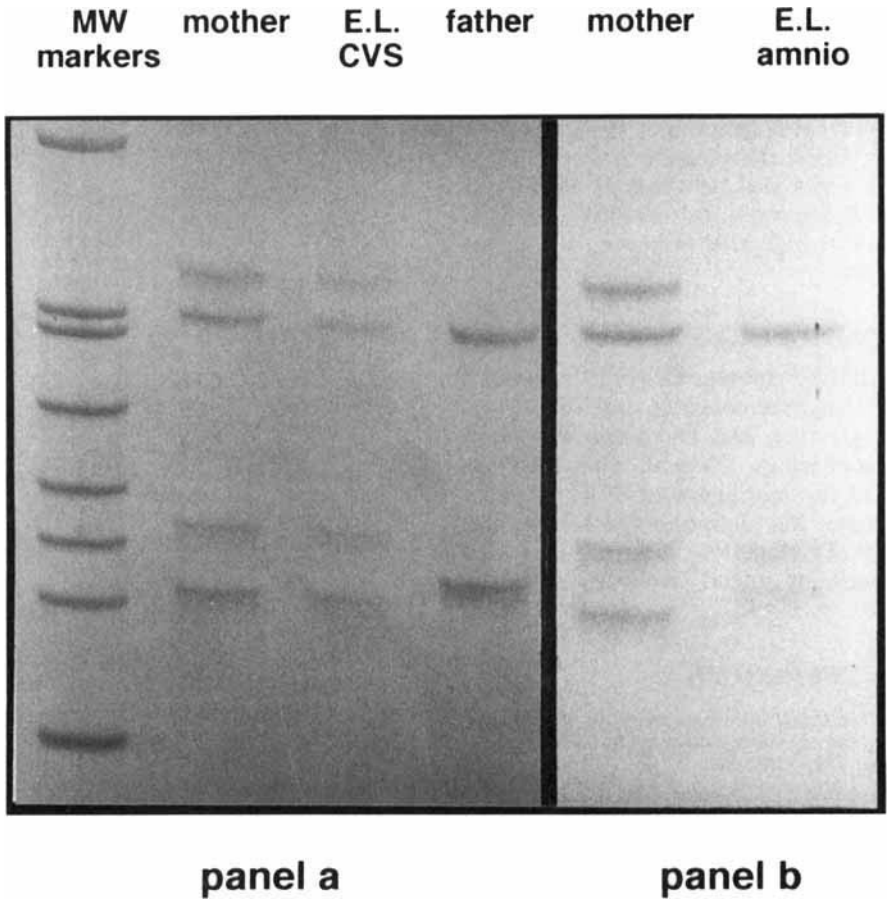

Fig. 1. Molecular analysis of genetic origins of the cultured CVS and amniocyte cells. Multiplex PCR of highly polymorphic CA repeats at chromosome 15q11-q13 was used to determine whether the cultured cells had contributions from both the maternal and paternal genomes. **a:** Amplified DNA from the mother and cultured CVS cells showed the same pattern, with no obvious genetic contribution from the father. **b:** The cultured amniocyte cells had genetic contributions from both parents. These results suggest that the carnitine transporter activity of the cultured CVS cells was largely a reflection of the maternal levels, whereas the activity in the cultured amniocyte cells was a true reflection of the fetus.

ing the second half of her pregnancy. It is of interest that the mother felt subjectively better on carnitine. These subjective feelings however cannot be readily attributed to a positive effect which carnitine may impart on fatty acid oxidation in a heterozygous individual, and is more likely to be due to a placebo effect. More gratifying was the cord blood carnitine level being just above the upper limit of normal for age. It is postulated that prenatal carnitine therapy in our patient has minimised the likelihood of development of any features of the disorder, and it is hoped that ongoing therapy will facilitate her continued well-being.

In conclusion, prenatal diagnosis of the carnitine transporter defect is possible using cultured amnio-

TABLE II. Plasma Carnitine Levels in Patient EL

	Oral carnitine treatment	Plasma carnitine level (μmol/L)	
		Total	Free
Cord blood		53	35
Day 1	Nil	30	17
Day 2	Nil	15	7
Day 7	Nil	6	4
Day 14	Nil	5	3
3 months	100 mg/kg/day[a]	44	23
Normal			
Day 1–7 (N = 15)		15–51	8–35
Beyond day 7		35–65	30–60

[a] Commenced at 14 days.

cytes, and it remains to be determined for cultured chorionic villus cells. We suggest that where cultured fetal cells are used in biochemical analyses for prenatal diagnosis, molecular studies to confirm that there are genetic contributions from both parents should be performed, particularly when the biochemical results do not predict that the fetus will be affected. Finally, it may be worth considering prenatal carnitine therapy for those pregnancies at high risk of having the carnitine transporter defect.

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