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INVITED COMMENTARY: CURRENT ISSUES IN OBSTETRICS AND GENETICS

Prenatal diagnosis of disorders of fatty acid transport and mitochondrial oxidation

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Fatty acid transport and mitochondrial oxidation (FATMO) is a complex pathway that plays a major role in energy production during fasting or when illness and stressful situations require higher energy consumption. After long-chain fatty acids are mobilized from adipose tissue, taken up by liver and muscle cells, and activated to coenzyme A esters, they are transported into mitochondria by the sequential action of carnitine palmitoyltransferase (CPT) I, carnitine-acylcarnitine translocase, and CPT II (Bonnefont *et al*., 1999). Fatty acids are then oxidized in a cyclic fashion by four sequential reactions, each catalyzed by one of multiple enzymes with overlapping substrate chain-length specificity: FAD-dependent acyl-CoA dehydrogenase, enoyl-CoA hydratase, NAD-dependent L-3-hydroxyacyl-CoA dehydrogenase, and ketoacyl-CoA thiolase. Each cycle of the pathway produces a molecule of acetyl-CoA and a shortened fatty acid that re-enters the cycle until it is completely metabolized. In the liver, acetyl-CoA constitutes the building block for the synthesis of ketone bodies, which serve as fuels for production of energy in other tissues.

Inherited FATMO disorders represent a rapidly expanding class of metabolic diseases (Bennett *et al*., 2000). Signs and symptoms may vary greatly in severity and typically include hypoketotic hypoglycemia, transient to fulminant liver disease, skeletal myopathy, cardiomyopathy, and sudden and unexpected death in early life. Symptoms may appear at any age, from birth to adult life, and in variable combinations, frequently leading to life-threatening episodes of metabolic decompensation. To date, all known FATMO disorders are inherited as autosomal recessive traits, with a recurrence risk of 25%. Although prenatal 'screening' could occasionally be informative (Nada *et al*., 1996; Chalmers *et al*., 1997), the pursuit of a prenatal diagnosis after an index case with an unspecified diagnosis should be limited to special circumstances, recommending instead aggressive preventive treatment and a thorough evaluation of the newborn immediately after birth.

The prenatal diagnosis of FATMO disorders raises both ethical and medical issues. On one hand, parents face the option of abortion for a treatable disorder like MCAD deficiency or carnitine uptake defect, having experienced the sudden death of one or more children. At the other end of the clinical spectrum are disorders like glutaric acidemia type 2 with congenital anomalies and neonatal CPT II deficiency, characterized by very poor prognosis and outcome, and obvious candidates for prenatal diagnosis.

Another unique aspect of prenatal diagnosis for FATMO disorders is the associated risk of maternal complications. Although this association has been best characterized for fetal LCHAD deficiency and acute fatty liver of pregnancy (Ibdah *et al*., 1999), Table 1 shows that additional phenotypes have been reported in pregnant women carrying fetuses affected with at least five other FATMO disorders. Despite the possibly coincidental nature of isolated observations (Nelson *et al*., 2000), a detailed inquiry of the past obstetric history should be a required component of the clinical evaluation of all new patients with FATMO disorders. Furthermore, routine monitoring of subsequent pregnancies in known families should include measures to achieve early detection of the complications listed in Table 1.

Table 2 summarizes the current status of prenatal diagnosis of FATMO disorders using metabolite analysis of amniotic fluid, protein analysis in CVS or cultured amniocytes, and molecular analysis. Direct analysis of metabolites in amniotic fluid is based on positive identification of target metabolite(s) by gas chromatography/mass spectrometry or tandem mass spectrometry. The major advantages are the independence from tissue expression and the availability of results usually within 24 h. This approach, however, is limited only to the detection of glutaric acidemia Type 2 (Shigematsu *et al*., 1996). Detection of medium- and long-chain acylcarnitines is not reliable in amniotic fluid, but could be measured in the supernatant of cultured amniocytes after incubation with labeled fatty acids and carnitine (Nada *et al*.,

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Table 1—Disorders of fatty acid transport and mitochondrial oxidation causing maternal complications of pregnancy

	Disorders of fatty acid transport and mitochondrial oxidation						
Maternal complication	LCHAD	TFP	CPT I	CACT	MCAD	SCAD	Unknown[a]
Acute fatty liver of pregnancy	+++	+	(+)	–	–	(+)	+
HELLP syndrome	+++	(+)	–	–	(+)	(+)[b]	+
Pre-eclampsia	+++	–	(+)	(+)	–	–	–
Placental floor infarction	(+)[c]	–	–	–	–	–	–

[a]Mothers of children with unspecified disorders but with clinical manifestations and strong biochemical evidence *in vivo* and *in vitro* of an underlying FATMO disorder (P. Rinaldo*et al.*, unpublished observations).
[b]Niels Gregersen, personal communication, cited with permission.
[c]Matern D *et al.* Placental floor infarction complicating the pregnancy of a fetus with long-chain L-3-hydroxy acyl-CoA dehydrogenase deficiency (submitted for publication).
+++, Association reported in multiple cases; +, association reported in more than one case; (+) association reported in one case, possibly a coincidental event; –, association not reported.
CACT, Carnitine-acylcarnitine translocase; CPT, carnitine palmitoyltransferase; HELLP, hemolysis, elevated liver enzymes, low platelets; LCHAD, long-chain 3-hydroxy acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; SCAD, short-chain acyl-CoA dehydrogenase; SCHAD, short-chain 3-hydroxy acyl-CoA dehydrogenase; TFP, trifunctional protein.

Table 2—Methods available for performing a prenatal diagnosis of disorders of fatty acid transport and mitochondrial oxidation

			Metabolite analysis			Protein analysis[a]		DNA analysis	
Disorder	First report	Approx. cases (*n*)	AC	AG	Others	CVS	Cultured amniocytes	Known mutations	Used prenatally
Disorders of membrane-bound enzymes									
Plasma membrane									
Carnitine transport defect	1988	> 30	N/A	N/A	–	–	+	+	–
Long-chain fatty acid transport defect	1998	8[b]	N/A	N/A	–	–	–	–	N/A
Mitochondrial membranes									
CPT-I deficiency (liver)	1988	> 10	–	N/A	N/A	(+)	(+)	+	–
CACT deficiency	1992	> 10	(+)[c]	N/A	N/A	+	+	+	–
CPT-II deficiency (neonatal onset)	1988	> 20	(+)[c]	N/A	N/A	(+)	(+)	+	+
CPT-II deficiency (late onset)	1973	> 100	(+)[c]	N/A	N/A	(+)	(+)	+	–
VLCAD deficiency	1993	> 50	+[c]	N/A	N/A	+	+	+	+
ETF-QO deficiency (GA2)	1985	> 30	+	+	+	+	+	+	–
Isolated LCHAD deficiency	1990	> 100	(+)[c]	N/A	–	(+)	+	+	+
α-TFP deficiency	1992	> 20	(+)[c]	N/A	–	(+)	+	+	+
β-TFP deficiency	1996	< 5	(+)[c]	N/A	–	(+)	+	+	–
Disorders of mitochondrial matrix enzymes									
MCAD deficiency	1982	> 300	+[c]	–	–	+	+	+	+
SCAD deficiency	1987	> 20	(+)[c]	–	(+)	–	+	+	–
α-ETF deficiency (GA2)	1985	> 30	+	+	+	(+)	+	+	–
β-ETF deficiency (GA2)	1990	< 5	+	+	+	(+)	+	+	–
Riboflavin responsive form(s) (GA2)	1982	< 10	(+)	(+)	(+)	–	(+)	–	N/A
SCHAD deficiency (muscle)	1991	2[b]	–	–	–	N/A[d]	N/A	–	N/A
SCHAD deficiency (fibroblasts)	1996	3[b]	–	–	–	–	–	–	N/A
SCHAD deficiency (liver)	1999	13[b]	–	–	–	N/A	N/A	–	N/A
M/SCHAD deficiency	2000	1	(+)[c]	N/A	–	–	–	+	–
MCKAT deficiency	1997	4[b]	(+)[c]	–	–	–	–	–	N/A
2,4-Dienoyl-CoA reductase deficiency	1990	1	–	N/A	N/A	–	–	–	N/A
HMG-CoA synthase deficiency	1997	2	N/A	N/A	N/A	N/A[d]	N/A	+	–
HMG-CoA lyase deficiency	1976	> 20	–	N/A	+[e]	–	(+)	+	+

+, Used to perform a prenatal diagnosis and/or exclusion; (+) not reported yet but likely to be informative; –, not reported; N/A, not applicable.
AC, Acylcarnitines; AG, acylglycines; CVS, chorionic villus sampling; ETF-QO, electron transfer flavoprotein ubiquinone-oxidoreductase; GA2, glutaric acidemia type II; HMG, 3-hydroxy 3-methylglutaryl; MCKAT, medium-chain 3-ketoacyl-CoA thiolase; M/SCHAD, medium/short 3-hydroxy acyl-CoA dehydrogenase; SCHAD, short-chain 3-hydroxy acyl-CoA dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase; other abbreviations as in the legend to Table 1.
[a]Includes enzyme assays, western blot, and metabolic flux studies with labeled substrates.
[b]Estimated number includes unpublished cases known to the authors.
[c]Informative by analysis of supernatant of cultured amniocytes after substrate loading.
[d]Tissue specific defect.
[e]Performed by analysis of maternal urine.

1996; Roe and Roe, 1999). This procedure has been tested only in a limited number of cases, but it could theoretically be applied to any disorder that is expressed in fibroblasts and associated with a characteristic acylcarnitine profile (Shen *et al.*, 2000).

Protein analysis implies use of western blot analysis and pulse-chase experiments (Yamaguchi *et al.*, 1991), conventional assays of enzyme catalytic activity (Chalmers *et al.*, 1997; Vianey-Saban *et al.*, 1998), uptake assays (Rinaldo *et al.*, 1997) and metabolic flux studies where the functional integrity of the whole metabolic pathway is tested by monitoring the turnover of labeled substrates (Bennett *et al.*, 1987) or by measuring the formation of soluble intermediates (Roe and Roe, 1999; Shen *et al.*, 2000). While experience is limited, analysis of six enzymes in CVS tissue without a culturing step is possible (Wanders *et al.*, 1999).

Molecular analysis is logically emerging as the method of choice for the prenatal diagnosis of FATMO and other metabolic disorders (Andresen *et al.*, 1999). To date, detection of several common mutations (for example, 1528G→C in LCHAD deficiency) is routinely available. However, several genes have yet to be characterized and molecular analysis is not widely available. A prenatal diagnosis should rely whenever possible upon detection of mutations using genomic DNA (Bennett *et al.*, 2000): although mutations can be determined using reverse transcriptase PCR amplification of mRNA, splice site and termination mutations may result in rapid degradation of precursor mRNA and these mutations could be missed. In summary, when the genomic structure of a gene is known, detection of mutations in these genes can and should be offered, allowing a prenatal diagnosis by CVS sampling in the early stages of pregnancy.

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