Disorders of the Carnitine Cycle and Detection by Newborn Screening
Bridget Wilcken,1 AM, MB ChB, FRACP

Abstract
Carnitine is necessary for transport of long-chain fatty acids into mitochondria, to enter the β-oxidation cycle. Four carnitine cycle defects have been described. The carnitine transporter mediates carnitine transport across the plasma membrane. Symptoms include hypoketotic hypoglycaemia and cardiomyopathy. Some affected subjects are asymptomatic. Newborn screening detects very low levels of free carnitine in some but not all. Carnitine palmitoyltransferase type I A (CPTI) transports long-chain fatty acyl-CoAs across the outer mitochondrial membrane. Affected infants have hypoketotic hypoglycaemia with catabolic stress, but otherwise remain well. Newborn screening tests reveal elevated free carnitine, (elevated C0/C16+C18). Sensitivity is unclear and confirmation needs leukocyte or fibroblast assays. Carnitine-acylcarnitine translocase transfers fatty acylcarnitines across the inner mitochondrial membrane. The most common presentation is sudden death in the first days. Carnitine palmitoyltransferase type II (CPTII) converts long-chain acylcarnitines to long-chain acylCoAs for β-oxidation. Severe deficiency is lethal. Newborn screening for both disorders reveals elevated palmitoylcarnitine and enzymology or mutation analysis is needed for diagnosis. Late-onset CPTII is the most common disorder, presenting as muscle pain and rhabdomyolysis on severe exercise. All 4 disorders can be detected by newborn screening, with variable sensitivity. Late-onset CPTII probably cannot be detected. Carnitine transporter, CPTII and late-onset CPTII have proven treatment strategies.

Key words: Carnitine transporter, Carnitine palmitoyl, Transferase, Translocase

Introduction
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Four defects of the carnitine cycle have been described in man, all with autosomal recessive inheritance.2 Carnitine Transporter Defect
The carnitine transporter affects primarily the transport of carnitine across the plasma membrane and renal tubule, resulting in poor uptake of carnitine from the gut, and deficient renal reabsorption. The described defect is caused by mutations in the SLC22A5 gene which is located at 5q31 1-32 and encodes the high-affinity organic cation/carnitine transporter OCTN2.3 Some of these mutations affect failed maturation to the plasma membrane.4 Patients with the transporter defect may present in several ways: neonates and children may have hypoketotic hypoglycaemia; infants and children most commonly present with cardiomyopathy, which may be fatal during infancy, and skeletal myopathy

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Symposia

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is also recorded. At the other end of the spectrum, asymptomatic mothers have been found by newborn screening. Carriers may have left ventricular hypertrophy. The transporter defect can be detected by newborn screening because of low carnitine levels, usually less than 5 umol/L with an even lower level on repeat sampling. Oral carnitine therapy at a total dosage of 100 mg/kg daily, divided into 2 or 3 daily doses, usually gives a good result. In 900,000 newborns screened in the state of New South Wales, Australia (coverage over 99%) we detected 4 affected babies, and 3 mothers with less severe defects. Before screening, 3 patients had presented with cardiac failure secondary to cardiomyopathy at 18m (died before diagnosis), and 2 and 6 years. The survivors, as well as 2 affected siblings and the patients detected by screening are well on oral carnitine. We feel that newborn screening may possibly not detect all patients with this disorder, although there is as yet no definite evidence of missed cases.

**Carnitine Palmitoyltransferase Deficiency Type IA (CPTI)**

There are 3 isoforms of CPT I, a liver/kidney form, (IA), and isoforms that are present in heart and skeletal muscle, and in brain. Only CPT IA deficiency has been described in man. The enzyme is encoded by a gene located at 13q13.1-2. This rarely-described disorder usually presents with hypoketotic hypoglycaemic episodes mainly in the first 2 to 3 years of life. Affected subjects are well between episodes, and there appear to be no long-term sequelae. There have been reports of myopathy and a neonatal cardiac presentation, but these seem likely to have been chance associations. During attacks there are severe liver enzyme derangements and there may be renal tubular acidosis. There are 2 reports of acute maternal liver complications in pregnancy. The mainstay of treatment, as for other potential hepatic presentations of fatty acid oxidation, is the avoidance of fasting, especially during intercurrent illness. The role of carnitine or of medium-chain fats is unclear.

CPT 1A can be detected by newborn screening, since carnitine levels are often elevated. In newborns, there is elevation of the ratio of free carnitine to the long chain species C16 plus C18. The sensitivity overall is not yet clear, but may be very high. The birth incidence is very low, except in the Hutterite community and the Inuit in Alaska, presumably due to founder effects.

**Carnitine Acylearnitine Translocase Deficiency (CACT)**

The carnitine acylcarnitine translocase enzyme transfers fatty acylcarnitines into the mitochondria across the inner mitochondrial membrane, in exchange for free carnitine. CACT is coded for by the SLC25A20 gene which is located at 3p21.31, and is expressed mainly in heart, skeletal muscle and liver. The clinical expression of the disorder is usually very severe, with neonatal death in the first 1-3 days being the most common presentation. Babies may have hypoketotic hypoglycaemia, seizures, hypotonia, brady-cardia or other arrhythmias, and cardiac failure. There is usually significant hyperammonaemia, more prominent than with other fatty acid oxidation defects during a crisis. Some patients have survived on treatment with a high calorie low-fat diet, with carnitine and medium-chain fat supplements, but the early presentation in severe cases often precludes effective therapy being instituted in time in unsuspected cases.

Cases of CACT can readily be detected by tandem mass spectrometry newborn screening, as there is a significant elevation of palmitoylcarnitine (C16). It is likely that the sensitivity of newborn screening is very high. CACT cannot be distinguished by screening from carnitine palmitoyltransferase deficiency type II (see below), as C16 is also elevated in that condition. Elevated C16 levels can be confirmed by acylcarnitine profiling in leukocytes or cultured skin fibroblasts. To distinguish between CACT and CPTII, enzyme assay or mutation analysis is needed. In our own experience of newborn screening in New South Wales, Australia, we detected only 2 cases in over 900,000 babies; both had very high C16 levels, but died, at 22 and 72 hours after birth, before the newborn screening result was available.

**Carnitine Palmitoyltransferase Deficiency Type II (CPT II)**

The late-onset form of this disorder was the first disorder affecting fatty acid metabolism to be described, in 1973. This late-onset form presents with exercise-induced muscle...
cramps or frank rhabdomyolysis with myoglobinuria and very high creatine kinase levels in plasma. Typically, a young man undertakes a cross country run or similar activity, and becomes symptomatic. CPT II has been mapped to chromosome 1p32. There is a common mutation frequently seen in late onset CPT II, c.388C>T, (Ser113Leu) which is present in around 60% of disease-causing mutations. More severe mutations lead to neonatal-onset disease, which is usually fatal in the first 1 to 4 days of life, and may be associated with structural abnormalities of the brain and kidney 12 or an infantile form that presents predominantly with hypoketotic hypoglycaemia and its usual secondary manifestations. 12 There is no successful treatment for the neonatal presentation, but otherwise treatment is on the same principles as for other defects affecting long-chain fatty acids: avoidance of fasting, limiting the intake of long-chain fat and using supplementation with medium-chain triglycerides and carnitine. 12 Medium-chain triglyceride loading before exercise has been found successful in late-onset disease.

The more severe forms of CPT II can be detected by newborn screening, but it is not clear that this strategy will detect most late onset disease. Patients with late-onset CPT II, in our experience, do not have an abnormal plasma acylcarnitine profile except at times of acute stress. Elevated dried blood spot levels of C16 on newborn screening point to either CPT II or CACT, and as discussed above, enzymology or mutation analysis is required to differentiate these 2 disorders.

In summary, the 4 carnitine cycle disorders described in man can all be detected by newborn screening. However, late-onset CPT II seems unlikely to be detected, and the sensitivity is not known for CPT I (this may be quite high) or for the transporter defect, where probably not all cases can be detected without a large false positive rate. Mothers with mild transporter deficiency may also be detected, and specific uptake assays in cultured skin fibroblasts will be needed. Treatment of the carnitine transporter defect and of CPTI and late-onset CPT II is very effective. Treatment may also be effective in some cases of the translocase defect and later infantile onset CPT II, but neonatal presentations of both these disorders generally precludes successful treatment.

REFERENCES

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