

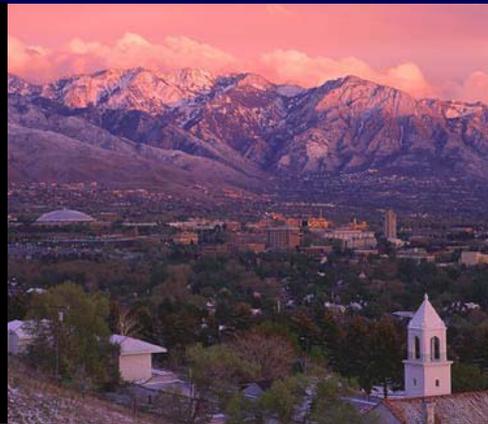
# Carnitine Transporter Deficiency at different ages (and similarities to other FODs)

**Nicola Longo MD PhD**

*Professor and Chief, Medical Genetics  
Depts. Pediatrics and Pathology  
ARUP Laboratories, University of Utah  
Salt Lake City, Utah, USA*



July 2016

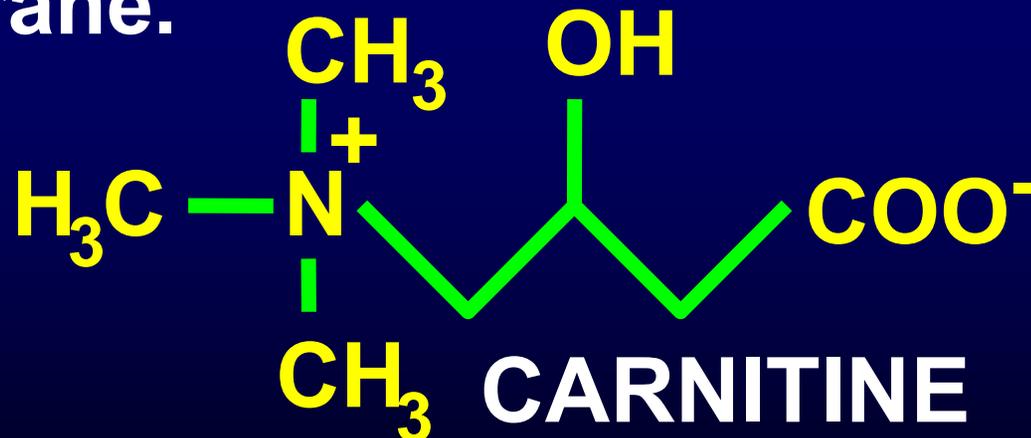


# OBJECTIVES

- Describe carnitine and its role in fatty acid oxidation
- How disorders of fatty acid oxidation are diagnosed
- Treatment for fatty acid oxidation defects.
- Role of newborn screen in the detection of fatty acid oxidation defects.

# CARNITINE

Carnitine (3-hydroxy-4-N-trimethylammonium butyrate) is essential for the transfer of fatty acids across the inner mitochondrial membrane.



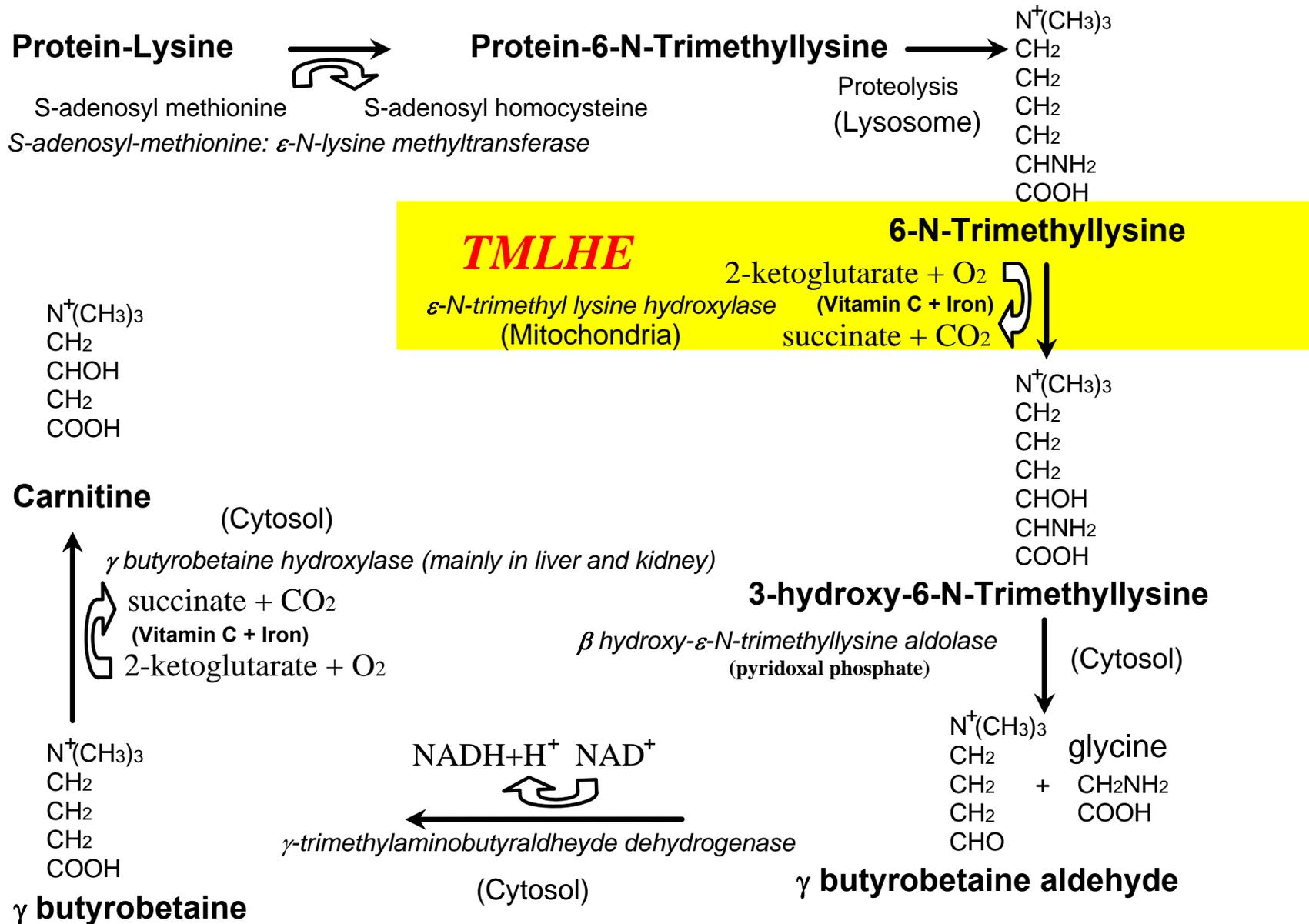
# Sources of carnitine

Synthesized by liver and kidneys, but not in heart or skeletal muscle, which depend on carnitine transport for fatty acid oxidation. Carnitine synthesis is low in newborns.

In the diet, most carnitine is supplied by red meat and dairy products, while fruits and vegetables contain insignificant amounts. About 75% of carnitine is provided by the diet in normal adults.

Carnitine is lost in the urine and secreted in the bile. Acute renal failure can result in high levels of plasma carnitine (100-300  $\mu\text{M}$ ). By contrast, chronic renal failure and dialysis can cause carnitine deficiency.

# CARNITINE SYNTHESIS

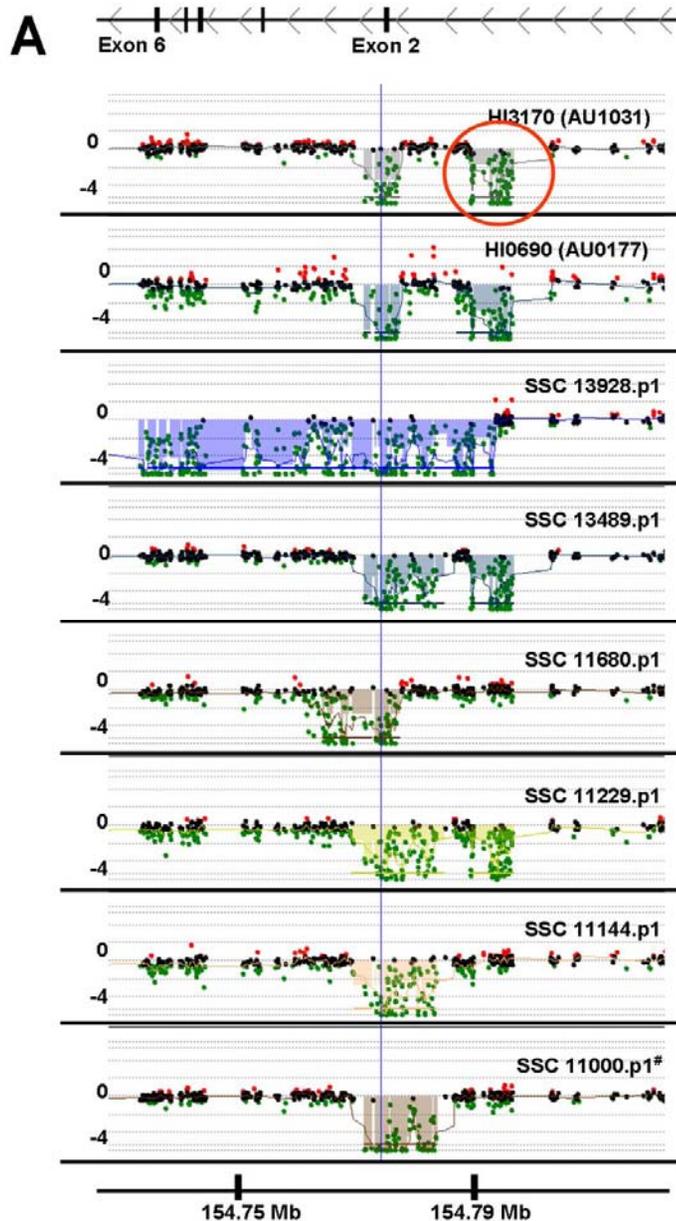


# Autism

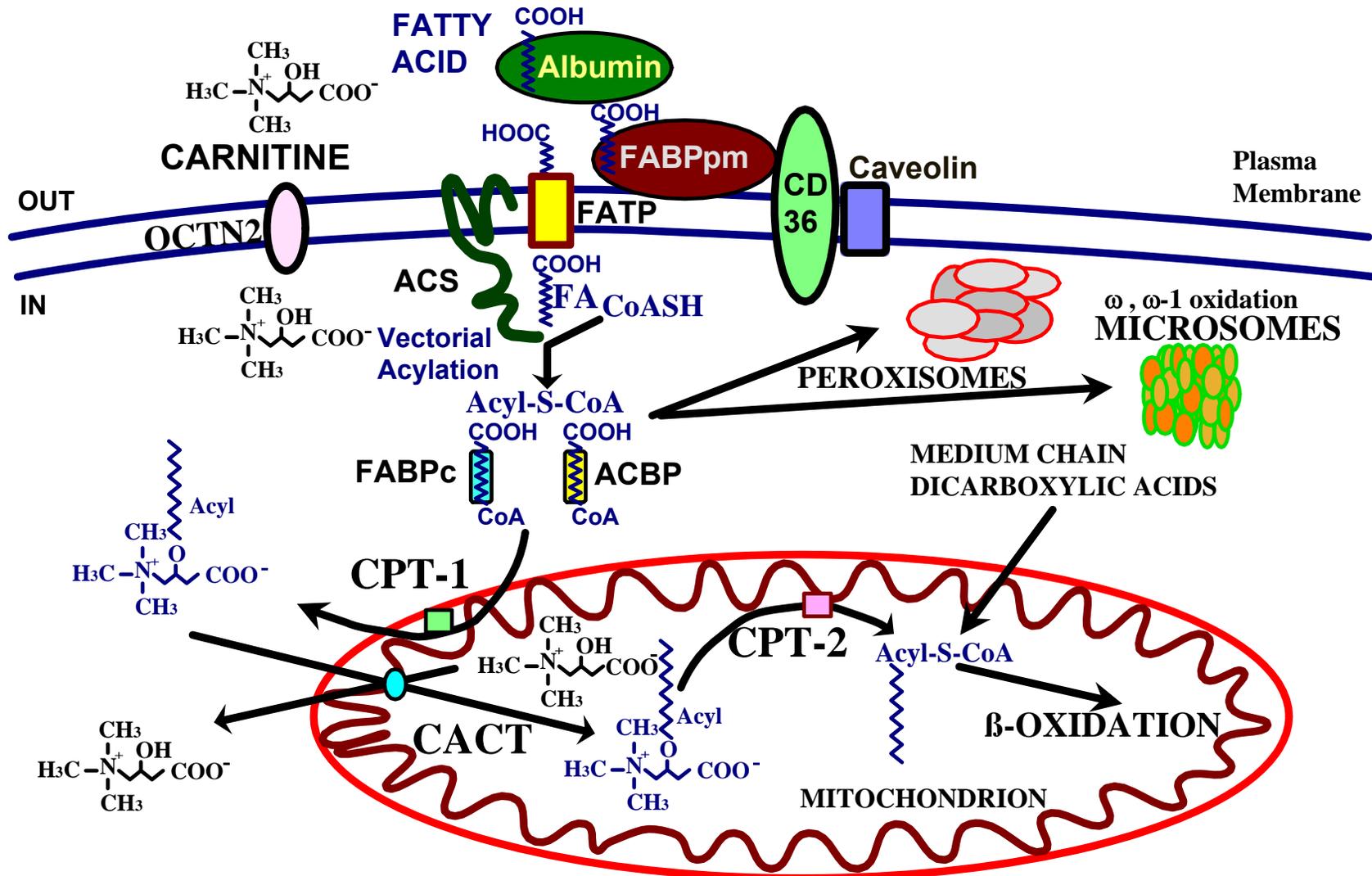
A common X-linked inborn error of carnitine biosynthesis may be a risk factor for non-dysmorphic autism.

Celestino–Soper et al. 2012. PNAS 109(21): 7974-7981

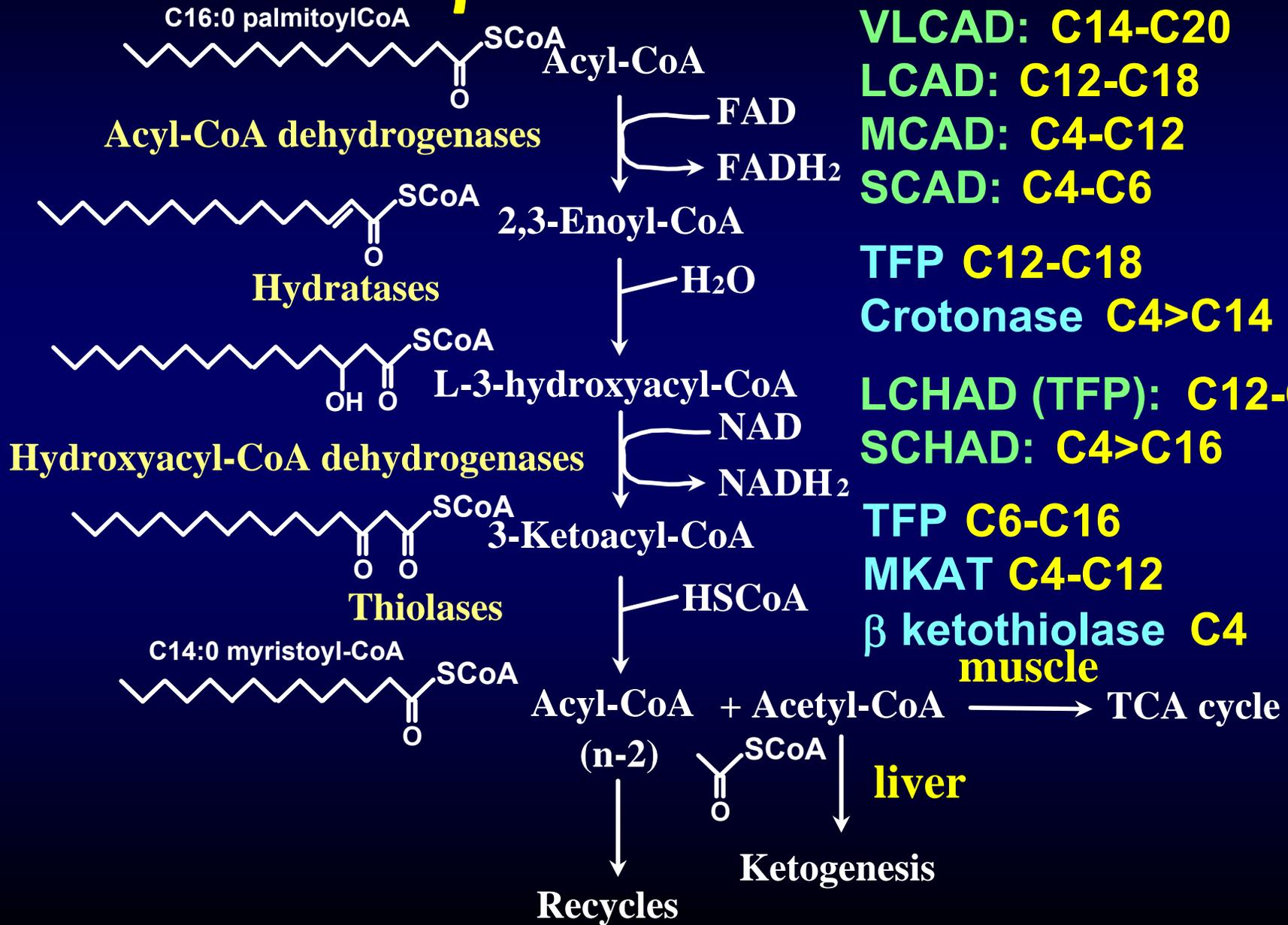
Exonic deletion of the *TMLHE* (trimethyllysine hydroxylase epsilon) that encodes the first enzyme in the biosynthesis of carnitine.



# THE CARNITINE CYCLE IN FATTY ACID OXIDATION



# β-OXIDATION



**VLCAD: C14-C20**

**LCAD: C12-C18**

**MCAD: C4-C12**

**SCAD: C4-C6**

**TFP C12-C18**

**Crotonase C4>C14**

**LCHAD (TFP): C12-C18**

**SCHAD: C4>C16**

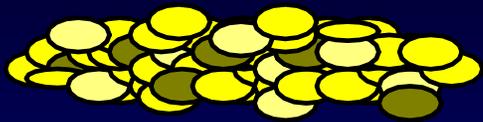
**TFP C6-C16**

**MKAT C4-C12**

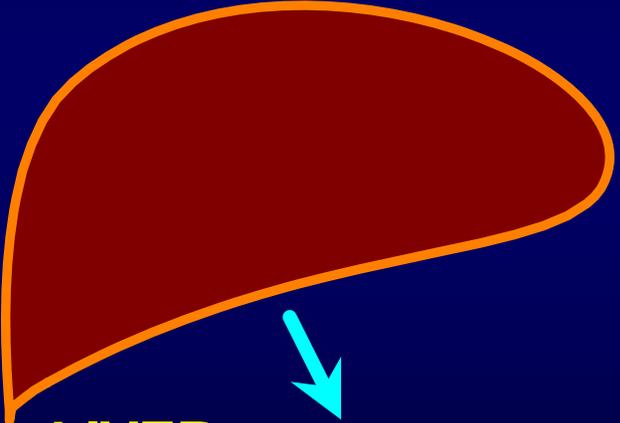
**β ketothiolase C4 muscle**

# FATTY ACID OXIDATION DURING FASTING

ADIPOSE TISSUE



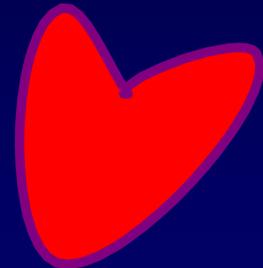
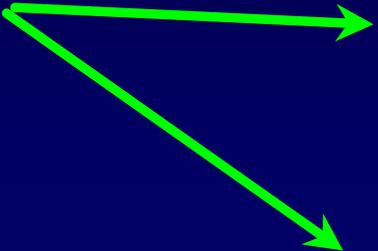
FATTY ACIDS



LIVER

KETONES

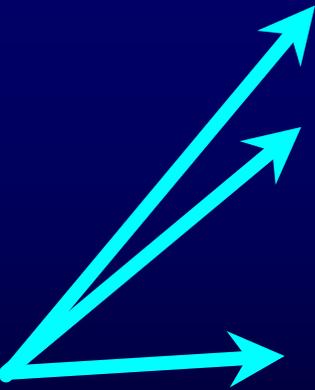
$\beta$ -hydroxybutyrate  
acetoacetate



HEART



SKELETAL  
MUSCLE



BRAIN

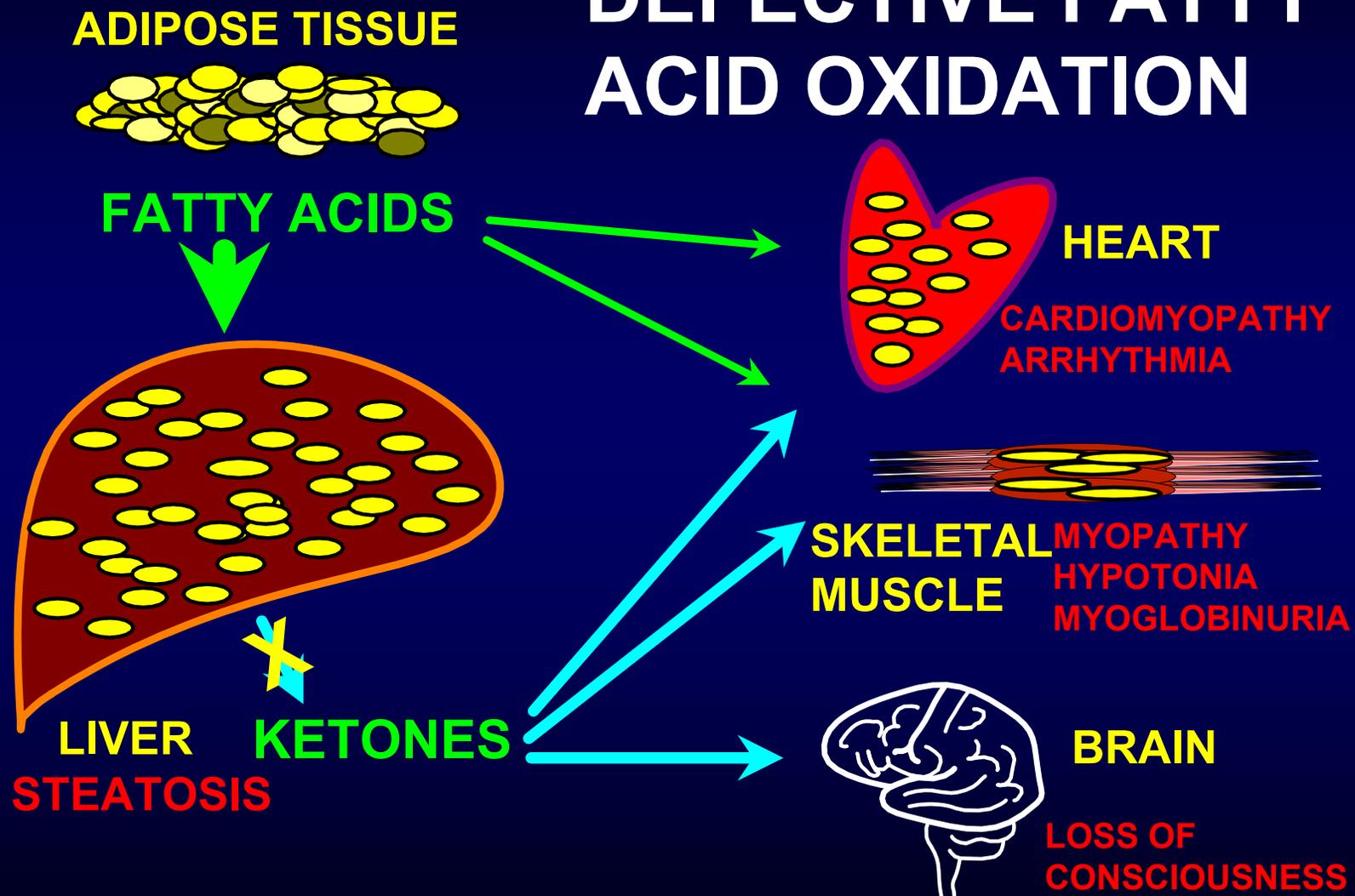
# **CONDITIONS REVEALING FATTY ACID OXIDATION DISORDERS**

**Most fatty acid oxidation defects are episodic and clinically silent when fat is not utilized.**

**Triggering conditions include fever, infections, gastroenteritis, reduced caloric intake.**

**Affected patients present at any age during an intercurrent illness causing catabolism.**

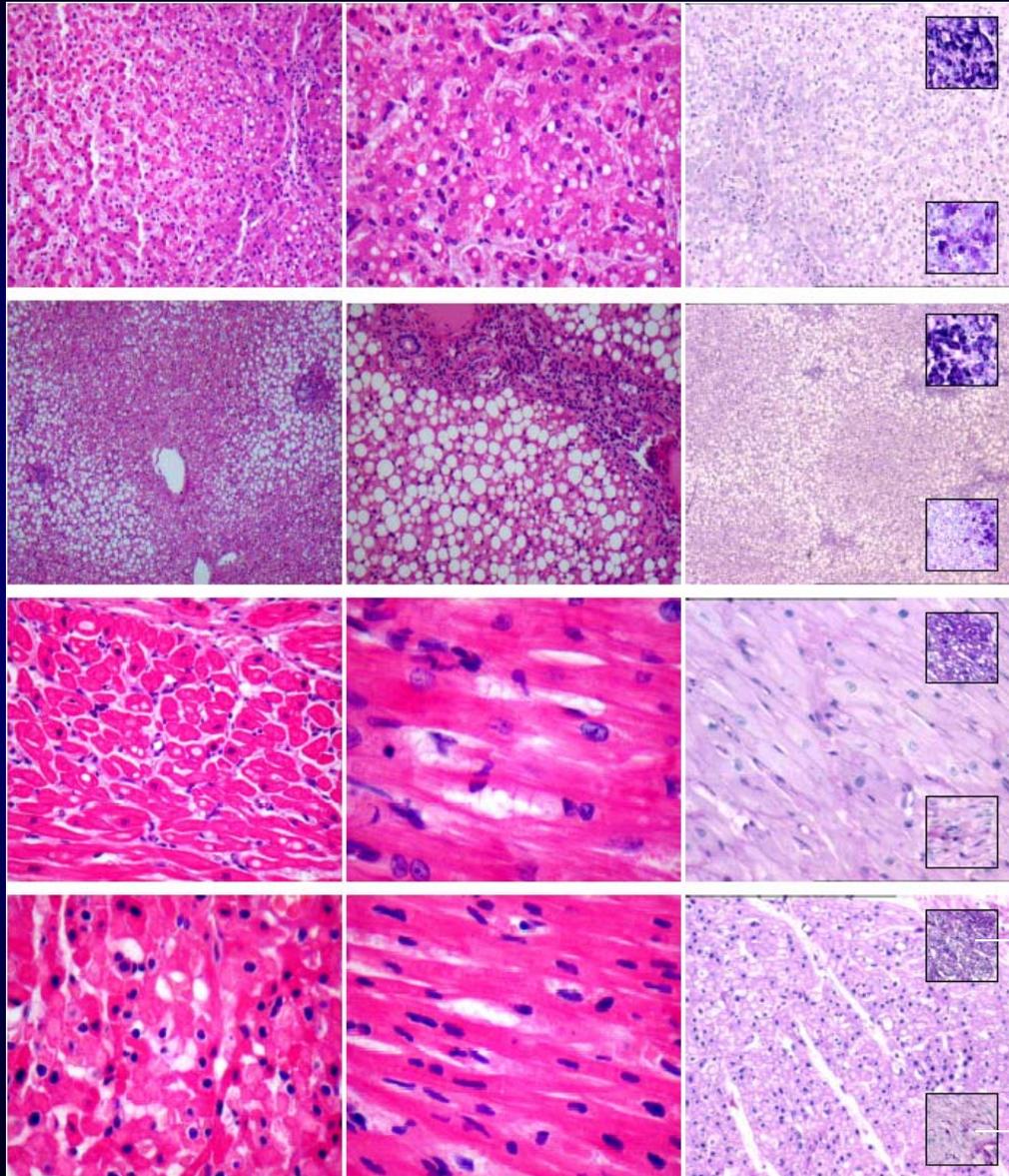
# DEFECTIVE FATTY ACID OXIDATION



# PATHOLOGY IN PRIMARY CARNITINE DEFICIENCY

## LIVER

Lipid deposition  
in peripheral  
areas of lobules



Exhaustion of  
glycogen  
reserve

## HEART

Focal lipid  
deposition exp. in  
subendocardium.  
Fiber/nuclei size  
variability  
(hypertrophy)

Control

Control  
caloric-  
deprived

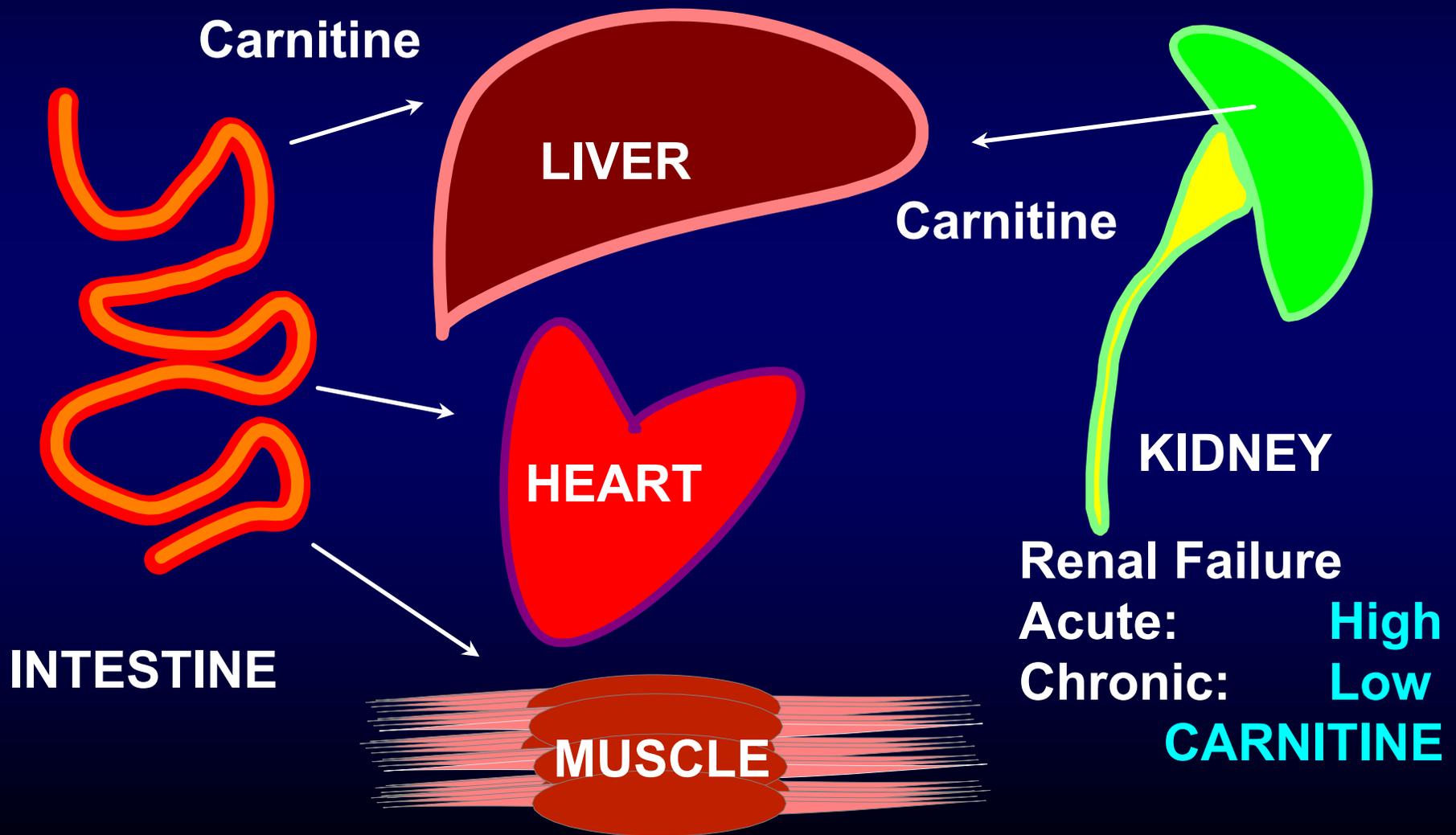
Hematoxylin-eosin

PAS

# Carnitine transporter deficiency (Primary carnitine deficiency MIM 212140)

- **Frequency** 1:142,000 (USA), 1:40,000 (Japan), 1:200 (Faroe Islands)
- **Cause:** Carnitine transporter (OCTN2) defect (*SLC22A5* gene)
- **Pathogenesis:** Loss of carnitine in urine reduces availability of carnitine in liver, muscle and heart, impairing FAO
- **Presentation:** Hepatic encephalopathy, hypoglycemia, cardiomyopathy in childhood, arrhythmia in adults, sudden death in children and adults
- **Diagnosis:** Plasma carnitine levels (very low, usually  $C_0 < 5 \mu\text{M}$ , can be higher in newborns), decreased urinary carnitine reabsorption, confirmed by transport studies in fibroblasts or DNA testing. Can be detected by newborn screening.
- **Therapy:** Carnitine 100-150 mg/kg up to 3 g per day PO divided into 3-4 daily doses
- **Monitoring:** Plasma carnitine free and total
- **Prognosis:** Excellent (with treatment)

# THE KIDNEY CONSERVES CARNITINE



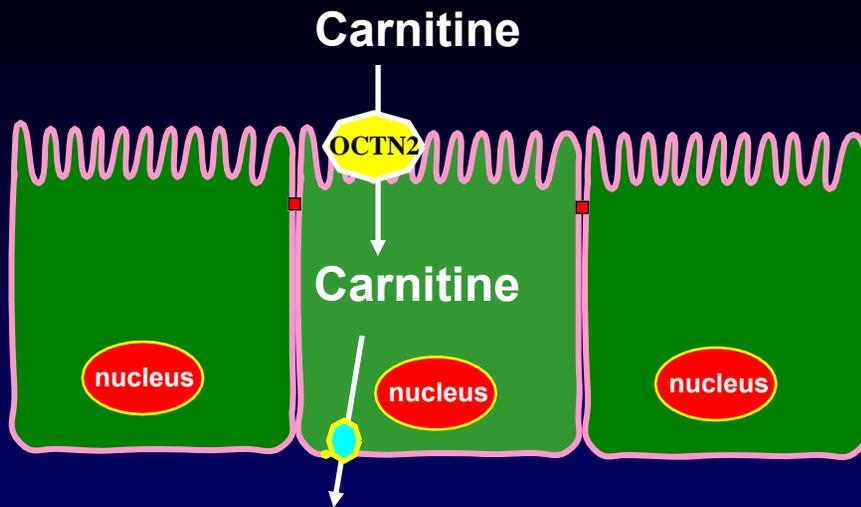
# CHILDHOOD HYPOGLYCEMIA

**Eight months old boy with history of frequent infections and vomiting presented with low oral intake and lethargy prompting hospital admission.**

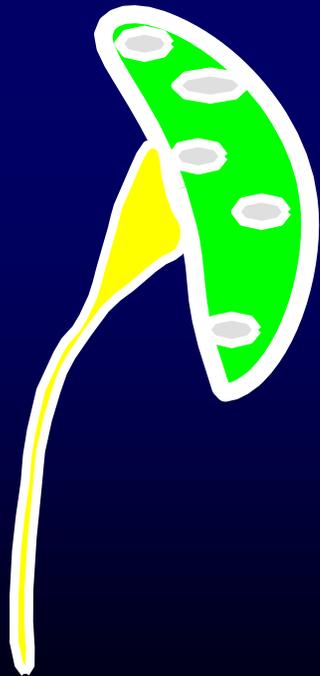
**Exam: hepatomegaly, lethargy**

**Labs: nonketotic hypoglycemia (glucose 35 mg/dL), hyperammonemia, and elevated liver function tests (Reye syndrome). Urine organic acids; mild dicarboxylic aciduria, Normal plasma amino acids were normal.**

**Therapy: he improved with intravenous fluids and glucose. The child was placed on a low protein diet in view of the hyperammonemia and referred to genetics.**



**DIAGNOSIS: Plasma carnitine**

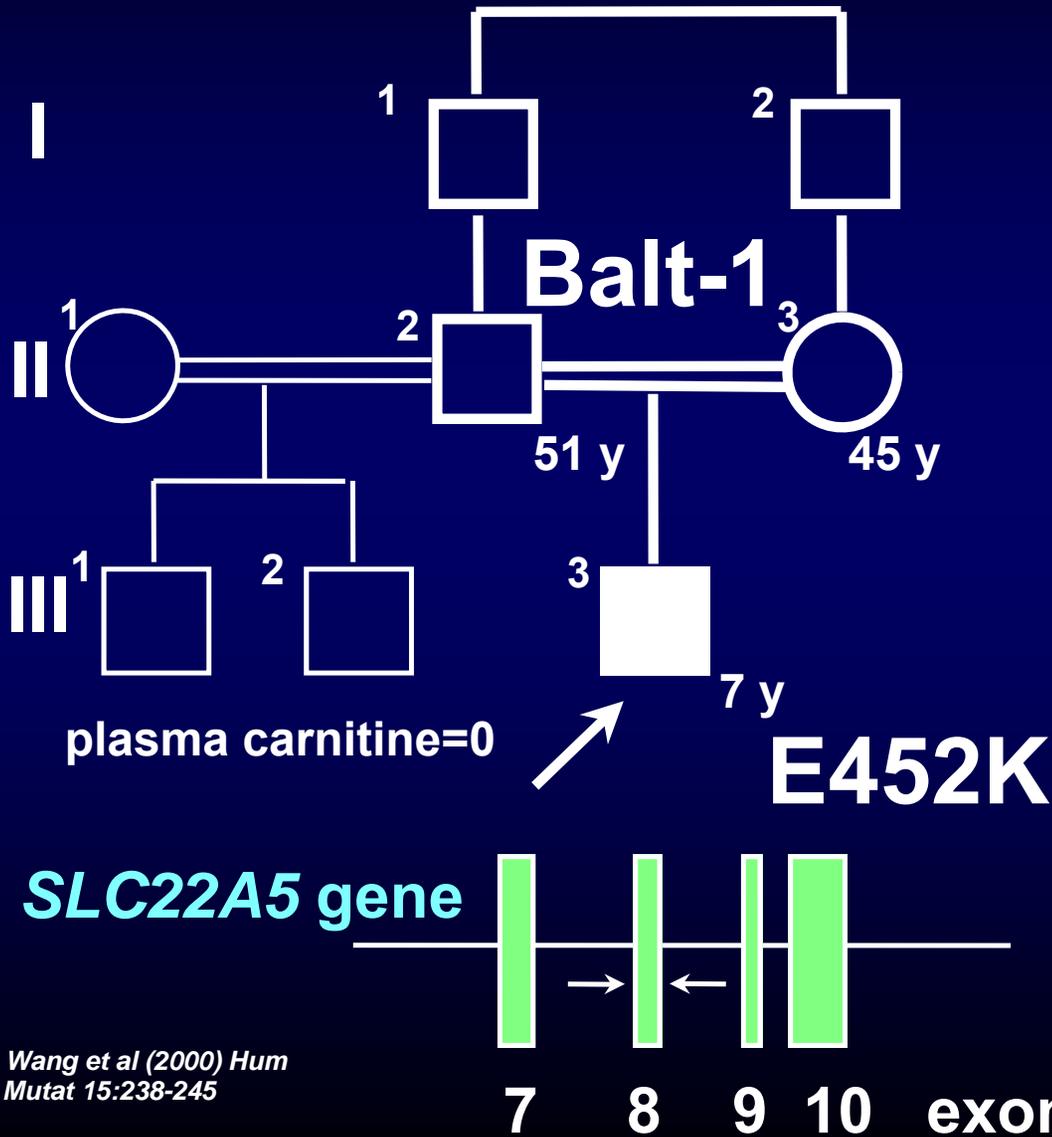


**Carnitine**

	Total carnitine μM	Free carnitine μM	Acyl-carnitine μM
Proband	1	1	0
Mother	21	16	5
Father	24	20	4
<b>Controls</b>	<b>30-70</b>	<b>24-56</b>	<b>6-14</b>

# CARDIOMYOPATHY

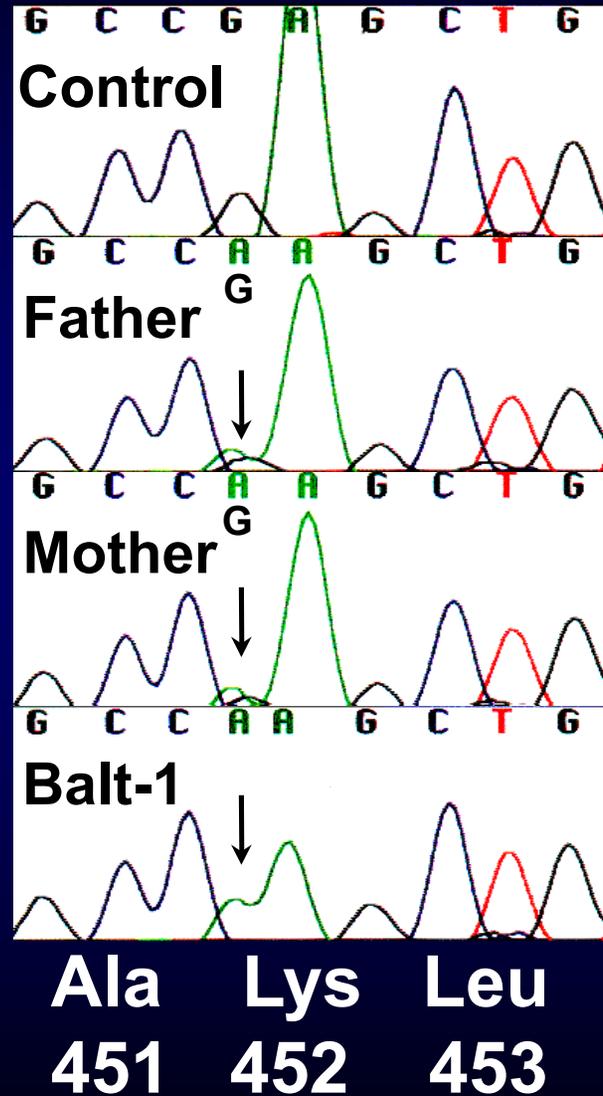
**A** 7 yo boy referred for cardiomyopathy resistant to therapy



451 452 453

Ala Glu Leu

**B**



Wang et al (2000) Hum Mutat 15:238-245

# CARDIAC ARRHYTHMIA

23 years old female presents with a syncopal episode caused by ventricular tachycardia, and a prolonged QT interval. Arrhythmias were poorly controlled by pharmacologic therapy and a defibrillator was installed. Syncopal episodes escalated during her first pregnancy. A positive NBS in the patient's child suggested a carnitine uptake deficiency, which was confirmed by reduced carnitine transporter activity and by molecular testing. After starting carnitine supplementation, no further syncopal episodes have occurred and the QT interval returned to normal.

Total carnitine: 3.0  $\mu\text{M}$  (26–69  $\mu\text{M}$ )

Free: 1.9  $\mu\text{M}$  (16–60  $\mu\text{M}$ ).

Carnitine uptake in fibroblasts: 4.8% of normal controls.

DNA Testing: c.95 A>G (p.N32S) and c.136 C>G (p.P46S)

# OTHER SYMPTOMS

**Fatigability, Decreased stamina, Exercise intolerance**

**Fasting intolerance**

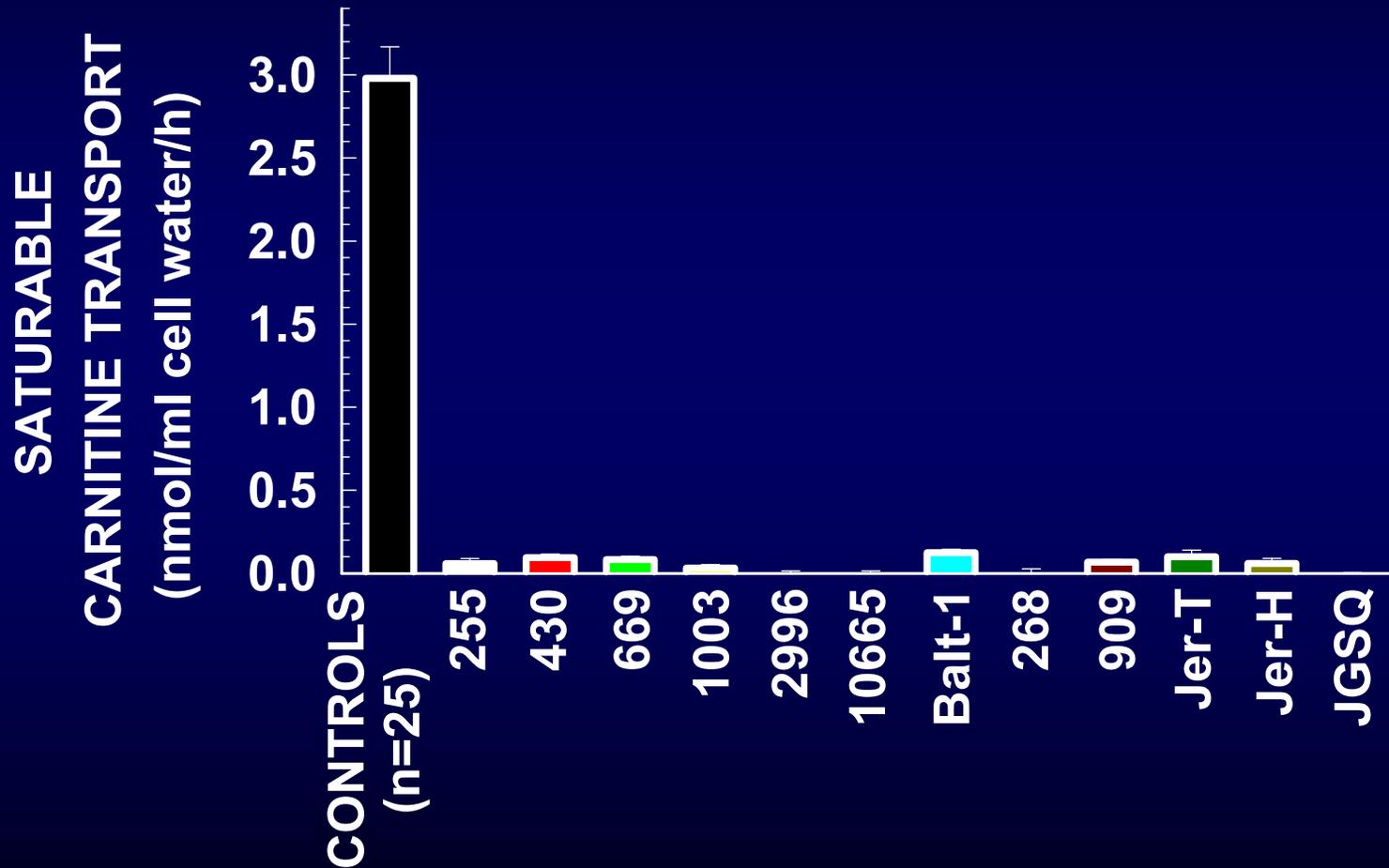
**Cardiomyopathy, Palpitations, sinus tachycardia**

**Ventricular arrhythmias, sudden death**

**ECG Abnormalities: ventricular premature beats (VPCs), nonspecific T wave abnormality, prolonged QT interval, PR, short QT interval with increased T waves.**

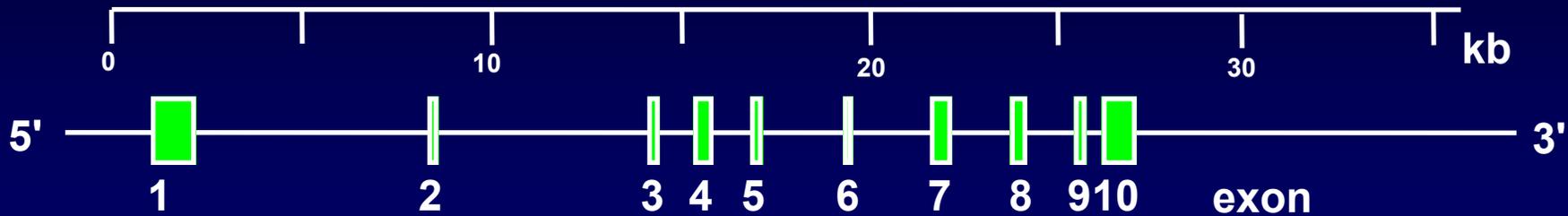
**Most adult patients have no symptoms with normal values in cardiac studies and can have sudden death as the first event.**

# CONFIRMATION OF DIAGNOSIS: CARNITINE TRANSPORT IN CULTURED FIBROBLASTS



# HUMAN OCTN2 (SLC22A5)

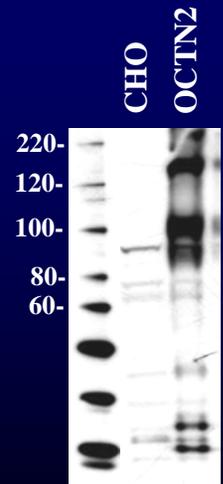
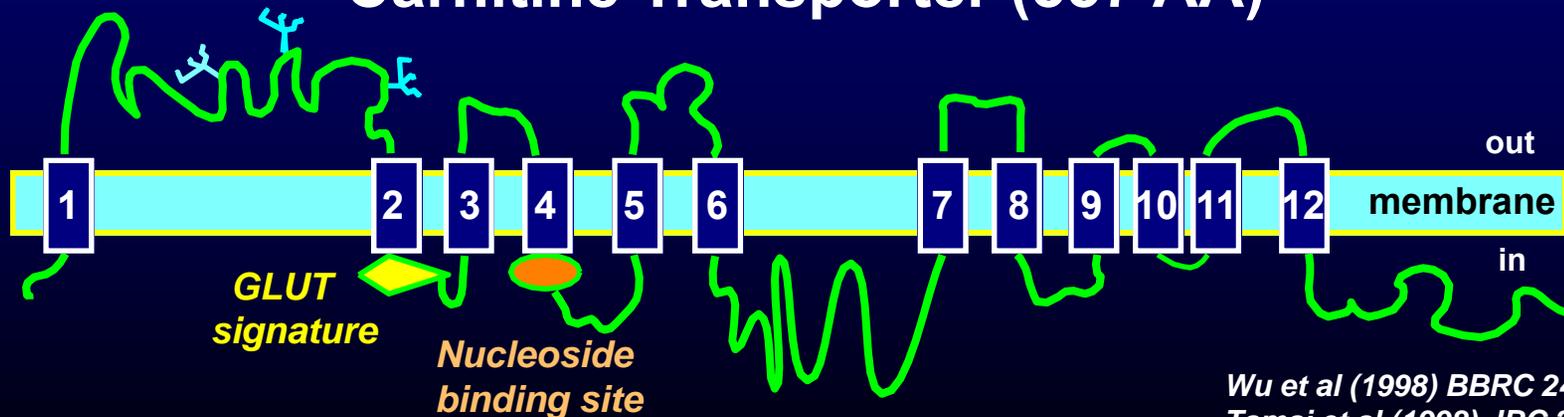
5q31.1-32



ORF (1,674 nt)

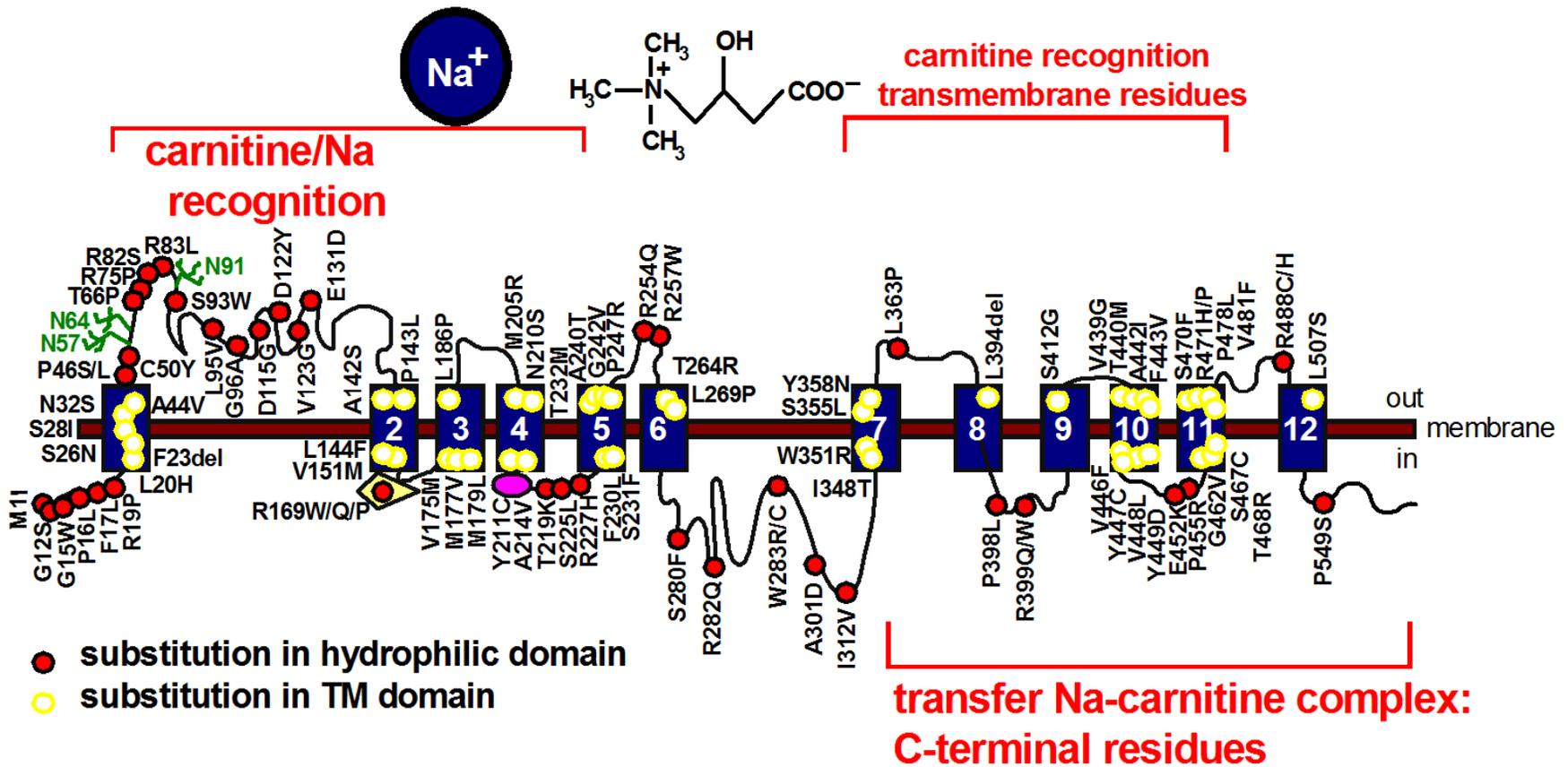


Carnitine Transporter (557 AA)



Wu et al (1998) BBRC 246: 589-595  
Tamai et al (1998) JBC 273: 20378-82

# MUTATIONS IN THE OCTN2 CARNITINE TRANSPORTER IN PRIMARY CARNITINE DEFICIENCY



Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency

YUHUAN WANG\*, JING YE\*, VADIVEL GANAPATHY†, AND NICOLA LONGO\*‡

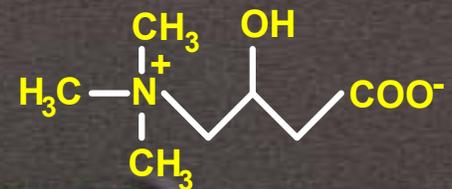
*Proc. Natl. Acad. Sci. USA*  
Vol. 96, pp. 2356–2360, March 1999

July 2016

# OCTN2 OPERATES A SODIUM/ CARNITINE COTRANSPORT

$[\text{Na}^+] = 140 \text{ mM}$

$\text{Na}^+$  Carnitine



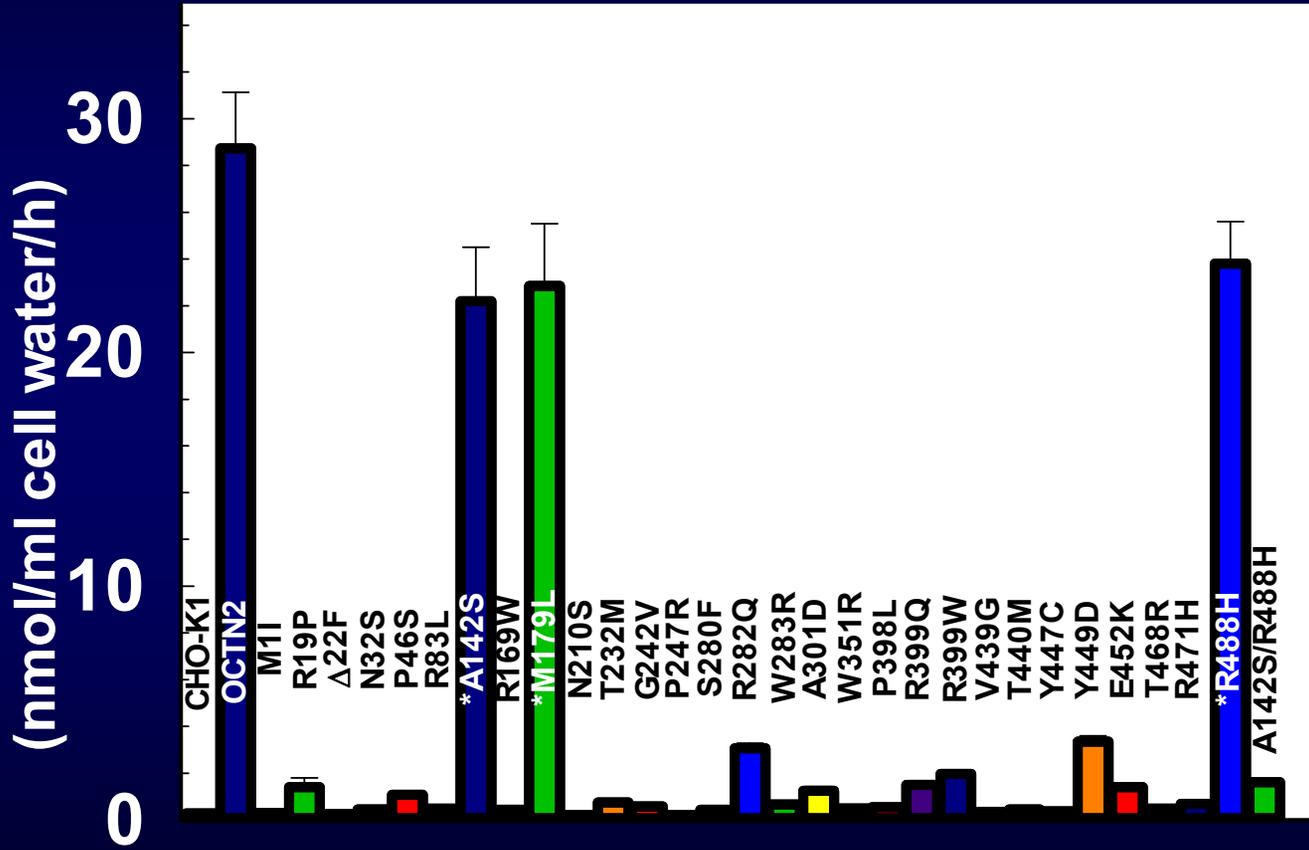
OCTN2

+ -  $[\text{Na}^+] = 20 \text{ mM}$   
 $\Delta\psi = -65 \text{ mV}$

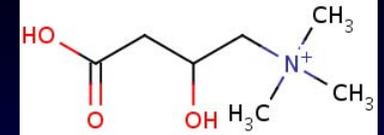
Carnitine can be accumulated up to 100 fold inside tissues

# CARNITINE TRANSPORT IN CHO CELLS EXPRESSING NORMAL AND MUTANT OCTN2 CARNITINE TRANSPORTERS

SATURABLE CARNITINE TRANSPORT



# THE THERAPY: CARNITINE



L-carnitine is indicated in the treatment of primary and secondary carnitine deficiency.

**MONITORING:** Plasma free and total carnitine

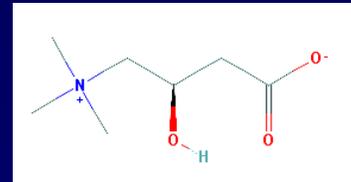
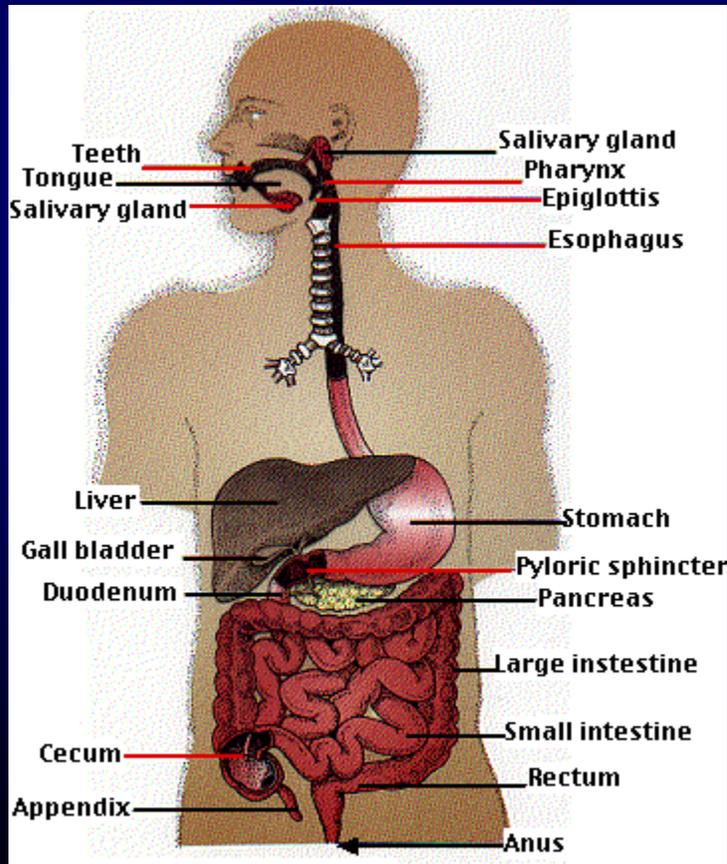
**SIDE EFFECTS:** usually well tolerated.

Nausea, vomiting, stomach upset, heartburn, diarrhea, and seizures. It can also cause the urine, breath, and sweat to have a “fishy” odor.

Used extensively in children, during pregnancy and breastfeeding.

# CARNITINE ABSORPTION

Only about 15% of L-carnitine supplements taken by mouth are absorbed in the gut compared to 50-75% absorption of carnitine present in foods. This is likely due to the higher load of carnitine in supplements.



## CARNITINE ABSORPTION

**Supplements: 15%**  
**Meat and dairy: 50-75%**

Rebouche CJ. Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann N Y Acad Sci.* 2004;1033:30-41.

Rebouche CJ, Chenard CA. Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites. *J Nutr.* 1991;121(4):539-546

# CARNITINE ABSORPTION

**In people without primary or secondary carnitine deficiency, peak carnitine level in plasma is observed about 3.4 hours after oral administration.**

**Terminal elimination half life of intravenous carnitine is 12-24 hours, meaning that carnitine levels return to baseline after about 1 day, even though part of the carnitine received goes inside the muscle. Entry of carnitine into the muscle requires very long time (months) and also loss of carnitine from the muscle is slow. The best way to fill the muscle and the heart with carnitine is by taking it every day consistently.**

Evans AM, Fornasini G. Pharmacokinetics of L-carnitine. Clin Pharmacokinet. 2003;42(11):941-67.  
Cao Y, Wang YX, Liu CJ, Wang LX, Han ZW, Wang CB. Comparison of pharmacokinetics of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine after single oral administration of L-carnitine in healthy volunteers. Clin Invest Med. 2009 Feb 1;32(1):E13-9.

# CARNITINE ELIMINATION

**Most carnitine is lost in urine (70-90% within 24 h after IV administration). Carnitine is conjugated to different chemicals (fatty acids, organic acids, drugs) in the human body, but is not chemically transformed.**

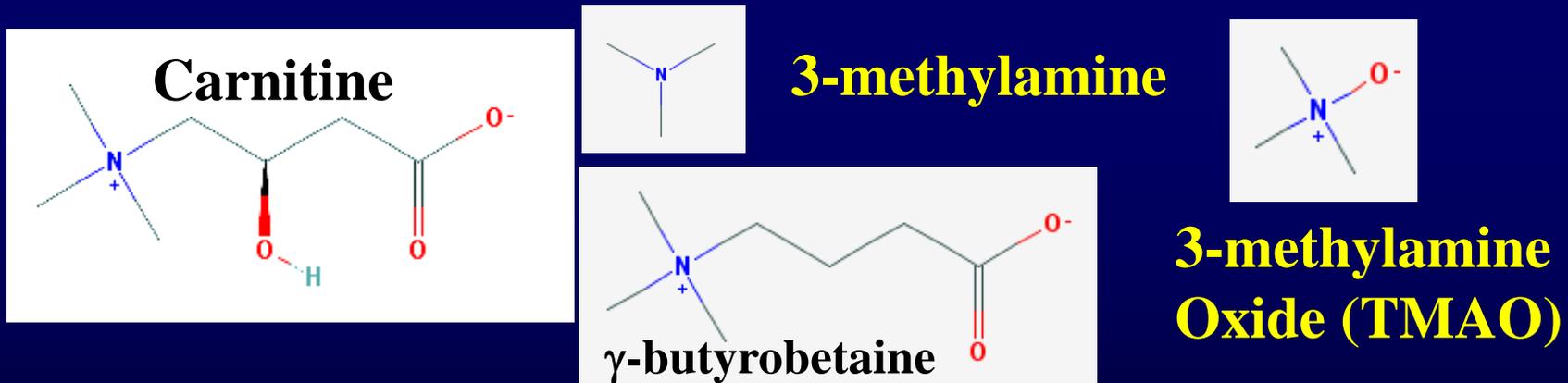
**There are no pharmacokinetic data in patients with primary carnitine deficiency, but carnitine is likely to be more rapidly eliminated than in control subjects.**

**We know that infants with the disease do not present before a few months of age, indicating that carnitine stores require quite some time to be depleted.**

Evans AM, Fornasini G. Pharmacokinetics of L-carnitine. *Clin Pharmacokinet.* 2003;42(11):941-67.  
Cao Y, Wang YX, Liu CJ, Wang LX, Han ZW, Wang CB. Comparison of pharmacokinetics of L-carnitine, acetyl-L-carnitine and propionyl-L-carnitine after single oral administration of L-carnitine in healthy volunteers. *Clin Invest Med.* 2009 Feb 1;32(1):E13-9.

# CARNITINE BODY ODOR

L-Carnitine can be converted to 3-methylamine and butyrobetaine by gut bacteria. They can be reabsorbed and 3-methylamine oxide can be produced by the liver and then eliminated in urine.



**3-Methylamine is responsible for the unpleasant smell.**

# INTESTINAL SIDE EFFECTS

Carnitine can cause intestinal discomfort: diarrhea, stomach pain, body odor.

**THERAPY:** no single strategy:

1. Divide carnitine dosage into several daily administrations: 3-6 times per day.
2. Lactobacillus (yogurt with active yeast): replaces gut bacteria.
3. Short course (7 days) of oral metronidazole: kills anaerobic germ in the gut.
4. Stop carnitine for up to 4 days if else fails.
5. Strong deodorants (used by hunters) can reduce body odor.

# DRUGS THAT INHIBIT CARNITINE TRANSPORT

## CARDIAC

Verapamil

Quinidine

Mildronate

## ANTIBIOTICS

Cephaloridine

## CHEMOTHERAPY

Imatinib

Oxaliplatin

## ANTIHISTAMINIC

Pyrilamine

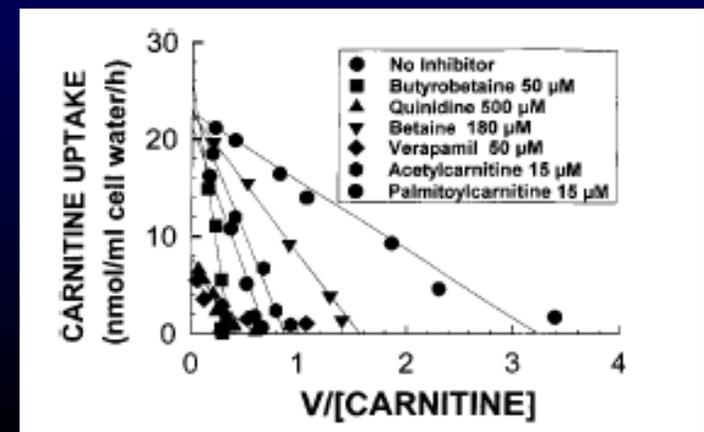
## DIURETIC

Spiroinolactone

## ANTISEIZURES

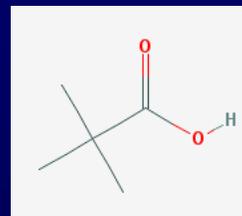
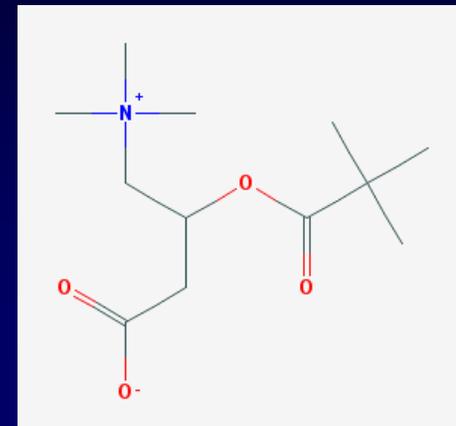
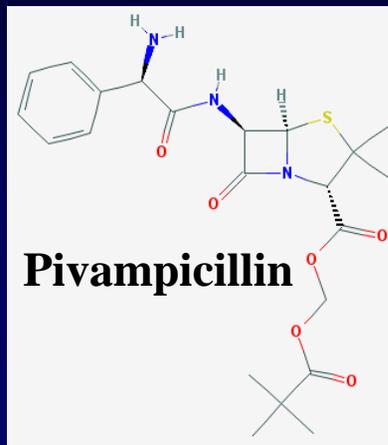
Valproic acid

Scaglia F, Wang Y, Longo N. Functional characterization of the carnitine transporter defective in primary carnitine deficiency. Arch Biochem Biophys. 1999 Apr 1;364(1):99-106.



# THERAPIES THAT CAUSE CARNITINE DEFICIENCY

Valproic acid  
Ketogenic diet  
Dialysis  
Pivalic acid-derived antibiotics



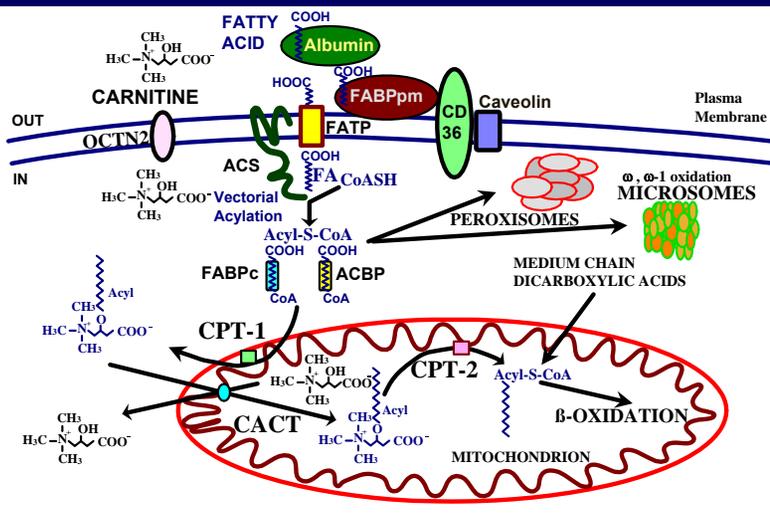
**Pivalic acid**

Pivampicillin  
Pondocillin  
Selexid

**Avoid these drugs**  
**If necessary, be very strict with carnitine supplements**

Rasmussen J, Nielsen OW, Lund AM, Køber L, Djurhuus H. Primary carnitine deficiency and pivalic acid exposure causing encephalopathy and fatal cardiac events. *J Inher Metab Dis*. 2013 Jan;36(1):35-41.

Todesco L, Bodmer M, Vonwil K, Häussinger D, Krähenbühl S. Interaction between pivaloylcarnitine and L-carnitine transport into L6 cells overexpressing hOCTN2. *Chem Biol Interact*. 2009 Aug 14;180(3):472-7.



# Gene: *SLC22A5*

*SLC22A5* solute carrier family 22 (organic cation/carnitine transporter), member 5

Number of variants 496 (Including filtered: 547)

UCSC Browser [5:131705444-131731306](#)

GeneCards [SLC22A5](#)

ExAC Browser: <http://exac.broadinstitute.org/gene/ENSG00000197375>

We examined the frequency of mutations (nonsense, splicing and expressed missense only) in 60,000 normal individuals (carriers) and extrapolated the frequency of affected individuals. This is a minimum frequency since most missense mutations were not expressed.

Carriers: 1:141

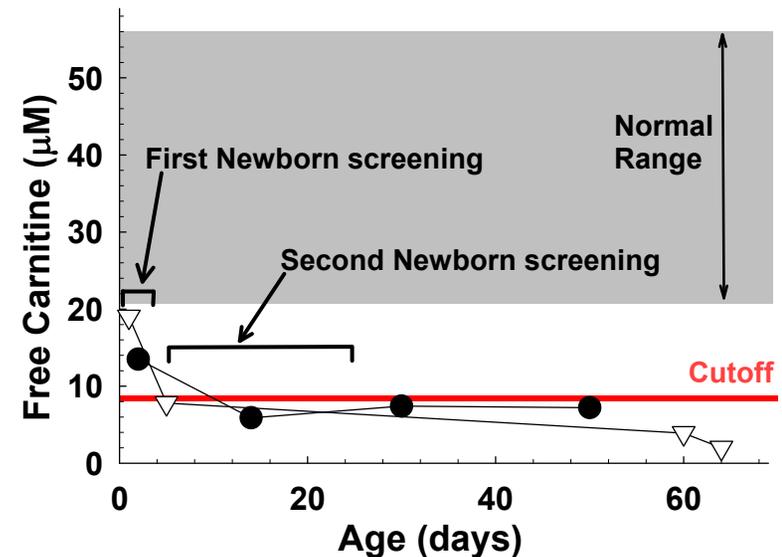
Predicted frequency: 1 : 79,910

USA NBS Frequency: 1 : 142,236

Many cases are missed by newborn screening.

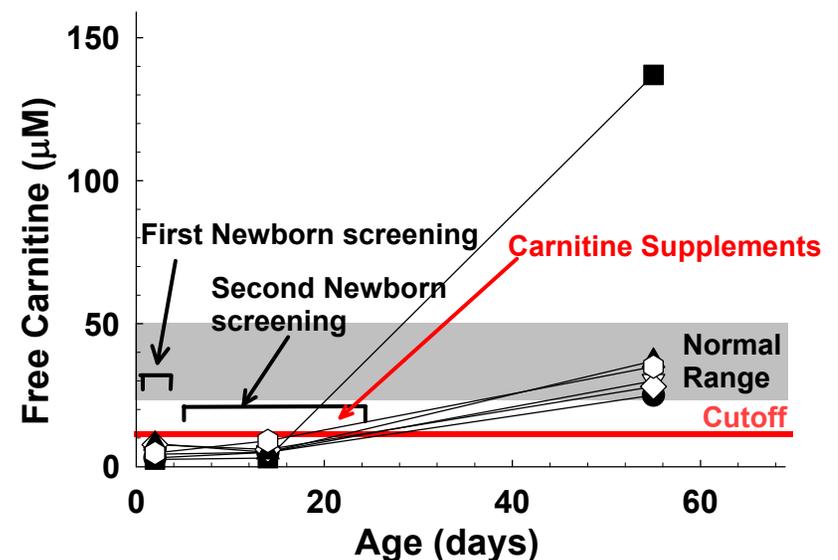
# Newborn screening for primary carnitine deficiency

- Carnitine is transferred from the mother to the fetus during pregnancy
- At birth, plasma carnitine levels reflect those of the mother
- Affected babies can have normal plasma carnitine levels at birth (0-7 days of age) that decline with time
- The longer from birth, the more likely that newborn screening identifies babies with primary carnitine deficiency



# Maternal primary carnitine deficiency identified by newborn screening

- In maternal carnitine deficiency, the levels of carnitine in the baby will be very low at birth
- With breast-feeding, carnitine levels will remain low in the baby, but increase briskly with supplements
- Most mothers are asymptomatic, but at risk of sudden death
- Their free carnitine levels (1-5  $\mu\text{M}$ ) are similar to those of classic patients



# Newborn Screening for primary carnitine deficiency

- Screening before 7 days of age can miss infants with primary carnitine deficiency (even when using different algorithms such as  $[C0 + C2 + C3 + C16 + C18:1 + C18] / CIT$ ).
- Affected mothers are more easily identified than infants.
- **POSSIBLE SOLUTIONS**
- Repeat screen at 14 days
- DNA testing common mutation(s)

Pasquali M, Longo N. Response to Chen et Al.: carnitine uptake defect (primary carnitine deficiency): risk in genotype-phenotype correlation. Hum Mutat. 2013 Apr;34(4):656.

**Table 1. Free Carnitine (C0) Levels in Dried Blood Spots of Infants with Primary Carnitine Deficiency as Compared with Infants of Mothers with Primary Carnitine Deficiency**

	Age at first screening (days)	C0 ( $\mu\text{mol/L}$ )	Age at second screening (days)	C0 ( $\mu\text{mol/L}$ )
Infant with CUD (4)	1.5 $\pm$ 0.6	13.7 $\pm$ 7.1	14.0 $\pm$ 1.8	7.2 $\pm$ 1.7
Maternal CUD (6)	1.8 $\pm$ 0.4	4.3 $\pm$ 2.0*	13.2 $\pm$ 1.2	5.0 $\pm$ 1.3*

Note the lower levels of carnitine in the first screening in infants of mothers with primary carnitine deficiency. Three out of four infants with primary carnitine deficiency had levels of C0 above the laboratory cut off at the time of the first screening.

\* $P < 0.05$  versus infants with carnitine uptake defect (first or second screen) using  $t$ -test. CUD, carnitine uptake defect.

# **SUMMARY**

**Lack of carnitine cycle can impair fatty acid oxidation and can present at any age when energy from fat is needed (fasting, infections, fever).**

**Patients are perfectly normal between episodes.**

**Carnitine uptake defect can cause very low carnitine levels, hypoglycemia in young children, cardiomyopathy and sudden death (at any age).**

# **SUMMARY**

**Carnitine therapy continued for life can prevent clinical symptoms in primary carnitine deficiency.**

**Newborn screening can identify affected infants or mothers. Infant identification requires a sample at several days of age (14 or more) or DNA testing.**

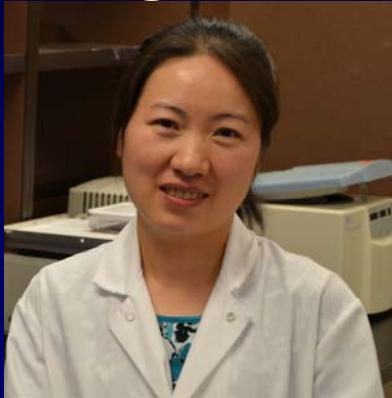
**Some drugs can inhibit the carnitine transporter or result in secondary carnitine depletion.**

**Carnitine supplements can prevent exacerbation of carnitine deficiency.**

# FATTY ACID OXIDATION

University of Utah

Longo's lab



Xue Yin



Marta Frigeni



ARUP Laboratories

Marzia Pasquali

Biochemical Genetics Service

Lorenzo Botto

Sharon Ernst

Krista Viau

Carrie Bailey

Ash Vollenweider

Hunter Underhill



All patients and their families



# QUESTIONS

**How important is it that mild CUD/CTD-variants are identified during the neonatal period?**

The newborn period is the only one on which all children will be seen in a hospital and is ideal to collect samples from all of them. Symptoms of CUD usually do not appear before a few months of age (the time necessary for tissues to become depleted of the carnitine that they received during intrauterine life through the placenta). Testing in the late neonatal period (20-30 days of age) might be more efficient in identifying all affected infants.

**Which symptoms can be expected in adults with untreated/undiagnosed milder variants?**

Fatigability, Decreased stamina, Exercise intolerance, Fasting intolerance.  
Cardiac symptoms: Cardiomyopathy, Palpitations, sinus tachycardia  
Ventricular arrhythmias, sudden death. ECG Abnormalities: ventricular premature beats (VPCs), nonspecific T wave abnormality, prolonged QT interval, PR, short QT interval with increased T waves.

Most adult patients have no symptoms with normal values in cardiac studies and can have sudden death as the first event.

# QUESTIONS

- **Which evidence do we have that carriers with low plasma-carnitine can be symptomatic?**

**None. Each one of us is a carrier of at least 50-100 bad genes and carriers of CUD only know one of them. In theory, being a carrier for CUD could aggravate other primary genetic diseases, but in the majority of cases CUD carrier status is a benign variant. CUD carrier status was NOT more frequent in adult patients with cardiomyopathy.**

- **Is it enough to control plasma carnitine for follow-up? Or would it be better add other numbers (like total carnitine)?**

**Plasma free and total carnitine is the best test. Total carnitine= free carnitine+ esterified carnitine. Presence of some esterified carnitine tells us that carnitine enters the cells and does its job by binding fatty acids.**

# QUESTIONS

- **What about correlation of plasma carnitine and carnitine levels in muscle cells?**
- **Why does it take such a long time until cardiac symptoms like palpitations disappear after initiation of carnitine supplementation?**

**Entry of carnitine into the muscle requires very long time (months) and also loss of carnitine from the muscle is slow. The best way to fill the muscle and the heart with carnitine is by taking it every day consistently.**

- **What happens if one stops taking carnitine? How long does one tolerate a “break” in carnitine supplementation?**

**Nothing happens acutely after stopping taking carnitine. The muscle, heart, and liver have sufficient stores for 1-2 weeks (if carnitine was taken consistently before). However, if there is an acute event for which fatty acid oxidation is required (prolonged fasting, surgery, fever, infection, vomiting), carnitine might become necessary to allow energy production.**

**In case of surgery, oral carnitine can be suspended. I recommend giving intravenous glucose (D10) instead of normal saline during procedures, while one is unable to eat. Intravenous carnitine can also be given, specially if compliance with carnitine was not stellar in the months before surgery.**

# QUESTIONS

- **How much does a (free) carnitine level tell if the blood sample is taken more than 12 hours after last carnitine dose (a typical problem for those who have to travel some distance to the lab)?**

**It still gives us an idea of how rapidly carnitine disappears from the body. Carnitine levels peak about 4 h after oral intake and stay around for 12-24 h. During the peak of carnitine, carnitine gets inside cells. If the interval is too long between doses (more than 12 h) it starts getting out of cells. It is important to note the time of the last carnitine dose to allow interpretation of results. It would be important to obtain pharmacokinetic data on patients with CUD to better understand the best time at which carnitine levels should be monitored.**

# QUESTIONS

- **How can it be that undiagnosed/untreated CUD/CTD-patients can practice sports (soccer, volleyball, long-distance running) for years without problems?** Their body still has some carnitine inside tissues that allows fatty acid oxidation. In addition, many people use sugary drinks before and during exercise. The metabolism of sugars or proteins does not require carnitine.

- **Are there any risks if your carnitine dose is too high?** None. When we give carnitine IV (we do that in patients with certain organic acidemias) carnitine levels go in the hundreds without any side effect.

**Is there any correlation between high carnitine dose and gain of weight?** I do not know of one. Some people eat more while taking carnitine because it might cause intestinal upset. I recommend taking carnitine with food to avoid extra calories.