

## CASE STUDIES

# CARDIOMYOPATHY IN PATIENTS WITH THE AMISH & MENNONITE VARIANT OF PROPIONIC ACIDEMIA

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## BACKGROUND

- Propionic acidemia (PA) is found in Amish & Mennonite populations throughout North America. *PCCB* c.1606A>G is a European variant with significant residual enzyme activity.
- Neonatal presentations are infrequent with this variant. Keto-acidotic crises with recurrent vomiting, tachypnea, encephalopathy, seizures, & metabolic strokes typically develop during infectious illnesses. An injured infant may become acutely hypotonic with poor head and trunk control followed by generalized dystonia. The basal ganglia are targeted & show increased T2-signal with low-diffusion immediately after crisis, then, volume loss, scarring, and worsening hypertonic dystonia.
- At least 25% patients develop heart failure, which often presents at the end of minor respiratory tract illnesses without keto-acidosis. End stage PA heart disease is a dilated, thin-wall myocardium with endocardial fibroestosis.

# BIOCHEMISTRY-1: DIAGNOSIS

- *PCCB* c.1606A>G has significant residual enzyme activity, blood propionyl-carnitine levels are low, but MS/MS Newborn Screening is usually positive.
- Propionyl-CoA forms within mitochondria by oxidation of 4-amino acids & odd chain fatty acids. Propionyl-carnitine is formed in mitochondria by an acyl-transferase & propionyl-CoA.
- The propionyl-carnitine levels in myocardium are >1000 times higher than blood. Amino acids & odd chain fatty acids transported into myocardium & oxidized.
- Urine organic acids by GC/MS have diagnostic levels of methylcitrate. 3-OH-propionate is present. Propionyl-glycine is usually absent. Glycine concentrations in plasma are high but, unlike methylcrotonylglycineuria, glycine conjugation is not a significant excretion pathway.

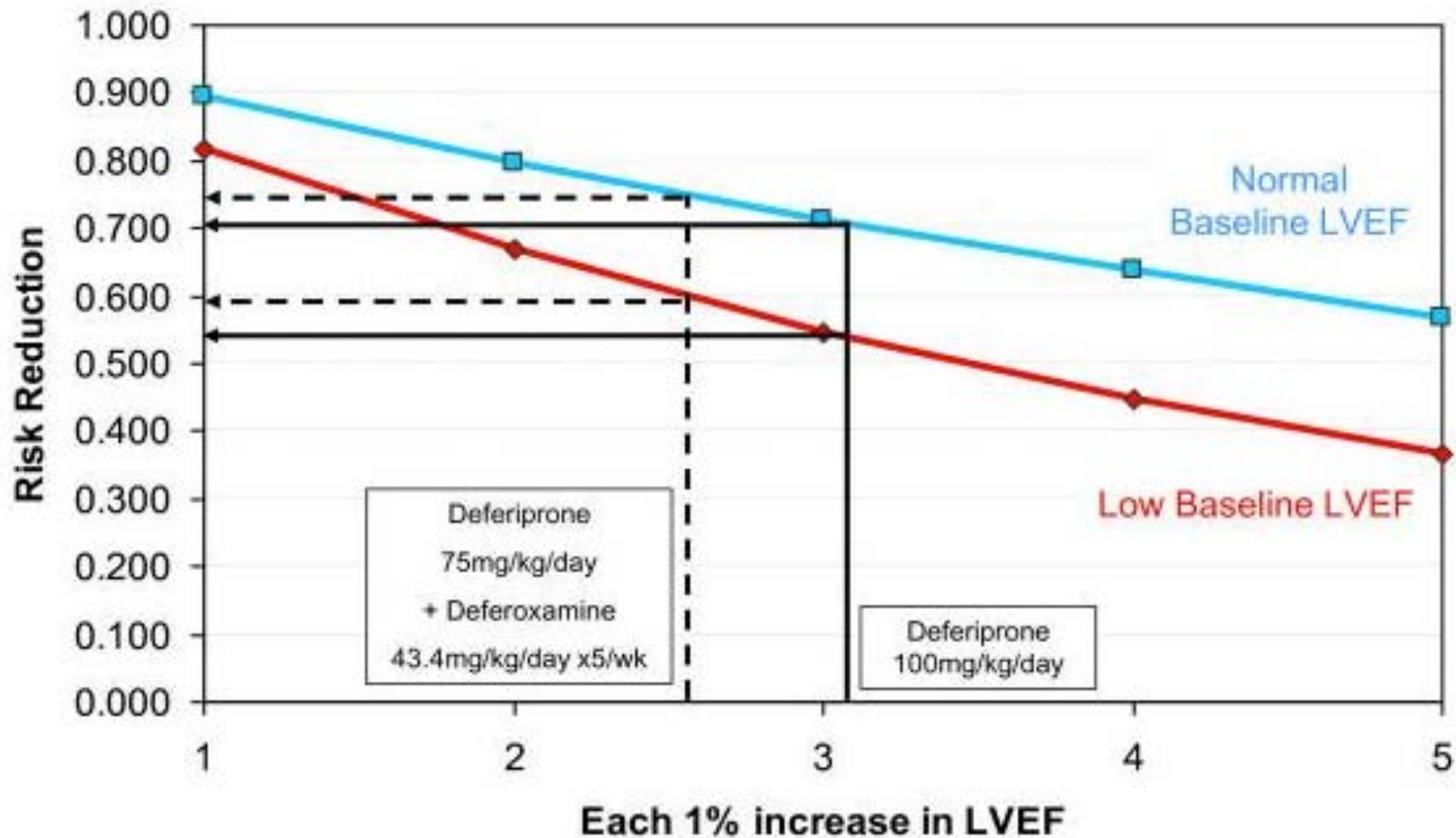
## METHODS & OBSERVATIONS

- Patients (age  $10.2 \pm 5.6$  years,  $n=33$ ) homozygous for *PCCB* c. 1606A>G were followed at our Clinic for 250 patient-years.
- We routinely monitored cardiac morphology, LV ejection fraction, QTc , selected citric acid metabolites by GC/MS, plasma amino acids by HPLC, propionyl-carnitine by MS/MS, and, more recently, serum Coenzyme Q levels.
- Two teenage brothers developed symptomatic cardiomyopathy were treated to lower tissue propionyl-CoA and restore citric acid cycle anaplerosis.

# PA: Underlying & Overt Myocardial Dysfunction

- Among PA patients LV ejection fraction ( $64 \pm 11\%$ ) was lower than controls ( $73 \pm 5\%$ ;  $p < 0.0001$ ).
- LV-EF  $< 3SD$  below controls) developed in 12 (36%) PA patients, ranged from mild (EF 58%) to severe (EF 7%).
- Heart failure was fatal in three children.
- In the 2 brothers with cardiomyopathy LV ejection decreased from normal to 18% and 42%.
- On the metabolic therapy outlined above, LV-EF increased from slowly from 18% to 56% over 4-6 months, and, from 42% to 63% over 1 month.

Reduction in the risk of Heart Failure is shown for each 1% increase in ejection fraction.



- **HOW CAN MYOCARDIAL DEPRESSION BE REVERSED?**
- A 32-year-old Amishman presented with dilated cardiomyopathy and was found to be homozygous for the PCCBc.1606G>A mutation. He was only able to achieve 81% predicted ETT. Two of his brothers died at ages 21 & 24 years undiagnosed – one suddenly, the other from CHF.
- Cardiac MRI confirmed 44% LV-EF, normal wall thickness, & an absence of myocardial fibrosis – despite long standing cardiomyopathy.
- Baseline Speckle-Track Echo showed diffuse wall movement abnormalities.

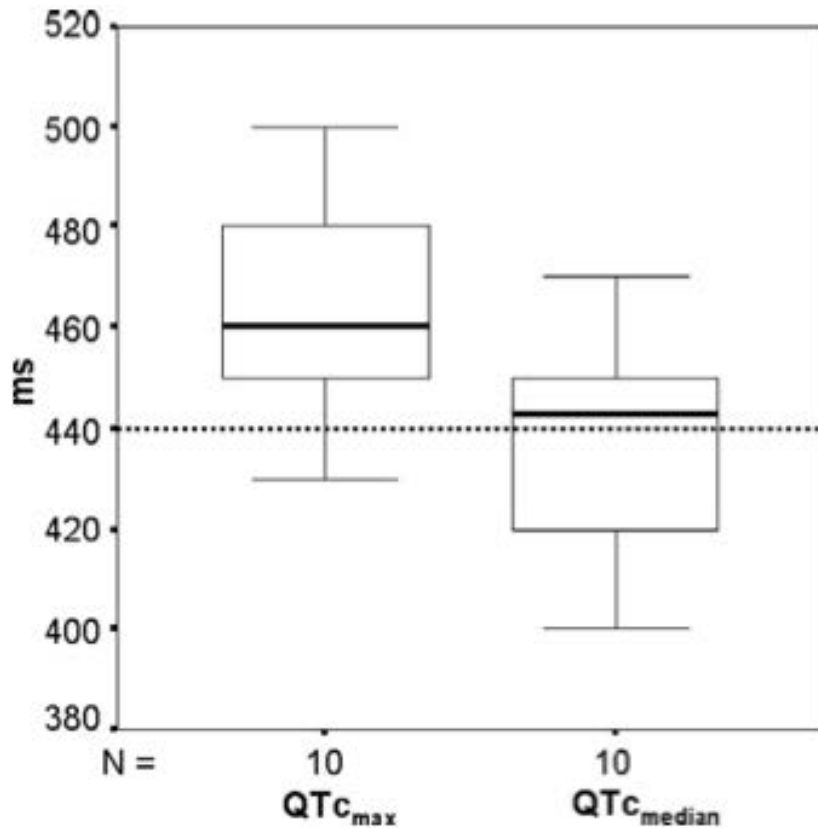
## RECOVERY

- Magnetic resonance imaging showed biventricular dysfunction with increased end diastolic volumes but myocardial interstitial volume fraction was normal 26%.
- After recovery of LV-EF, CMR documented normal biventricular function, cardiac volumes, and absence of fibrosis.
- Myocardial interstitial volume fraction was normal-29%.
- After recovery & on therapy the 17-year-old achieved predicted exercise - a targeted HR of 200 bpm.
- Before CoQ his EKG showed prolonged QTc, which shortened with exercise, and inverted T-waves in V5&6.

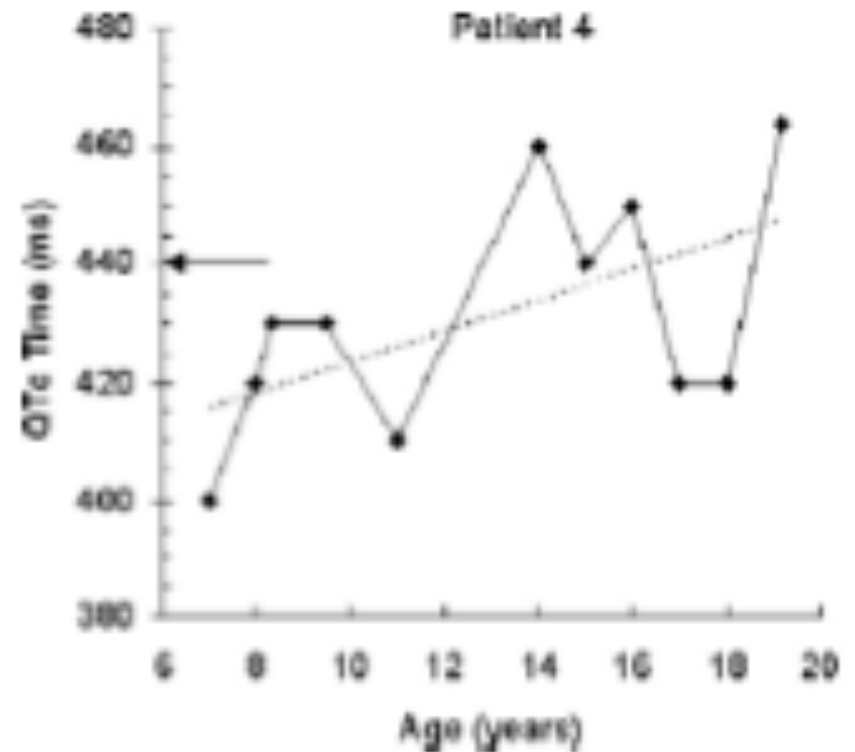


# QTC >460 MS

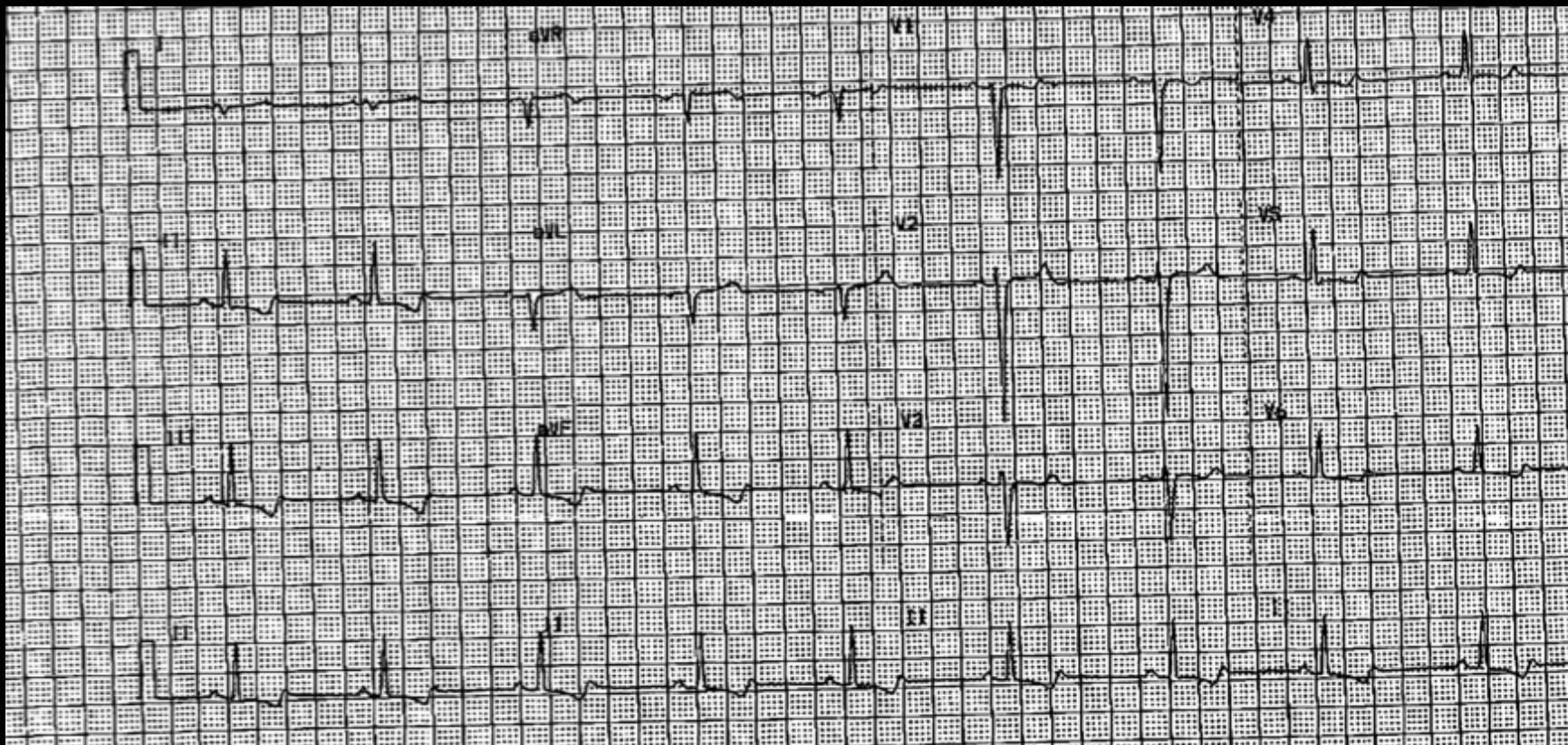
# INCREASED WITH AGE

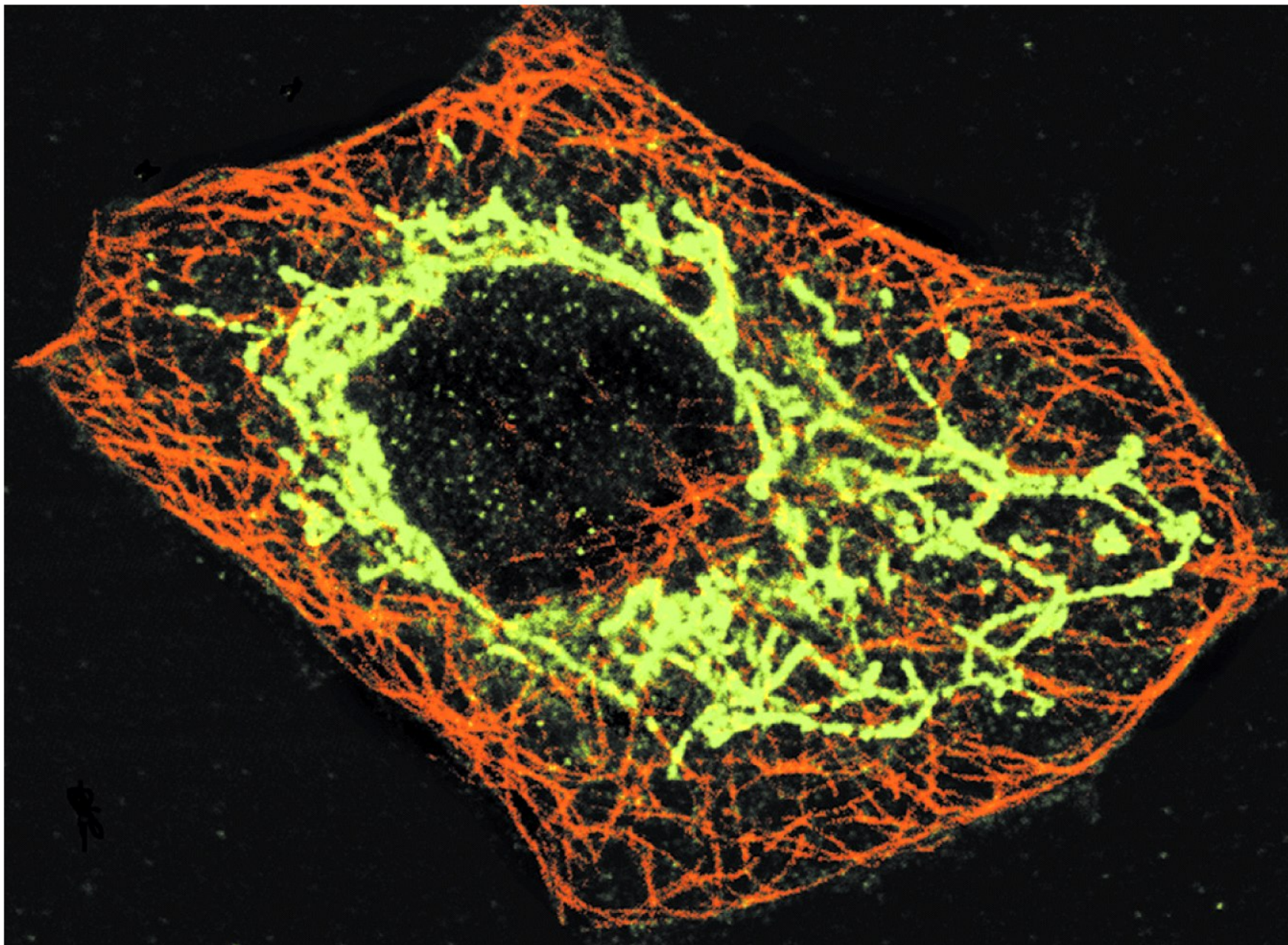


**Figure 1.** Box plot of maximum QTc (QTc<sub>max</sub>) and median QTc (QTc<sub>median</sub>) of all 64 standard 12-lead electrocardiograms of the 10 patients with PA. Median (black line inside the box), first and third quartile (margins of the box), minimum and maximum QTc are shown. The dotted line represents the upper limit of normal QTc. Note that the QTc<sub>max</sub> of most patients is above normal, and that QTc<sub>median</sub> of all sequential ECG recordings is normal in approximately half of the patients.



**ABRUPT DECREASE IN LVEF 58 > 43%  
SINUS BRADYCARDIA, POOR R-WAVE  
PROGRESSION, DEPRESSED ST SEGMENTS IN  
INFERIOR & LATERAL LEADS, QTC 431 MSEC**



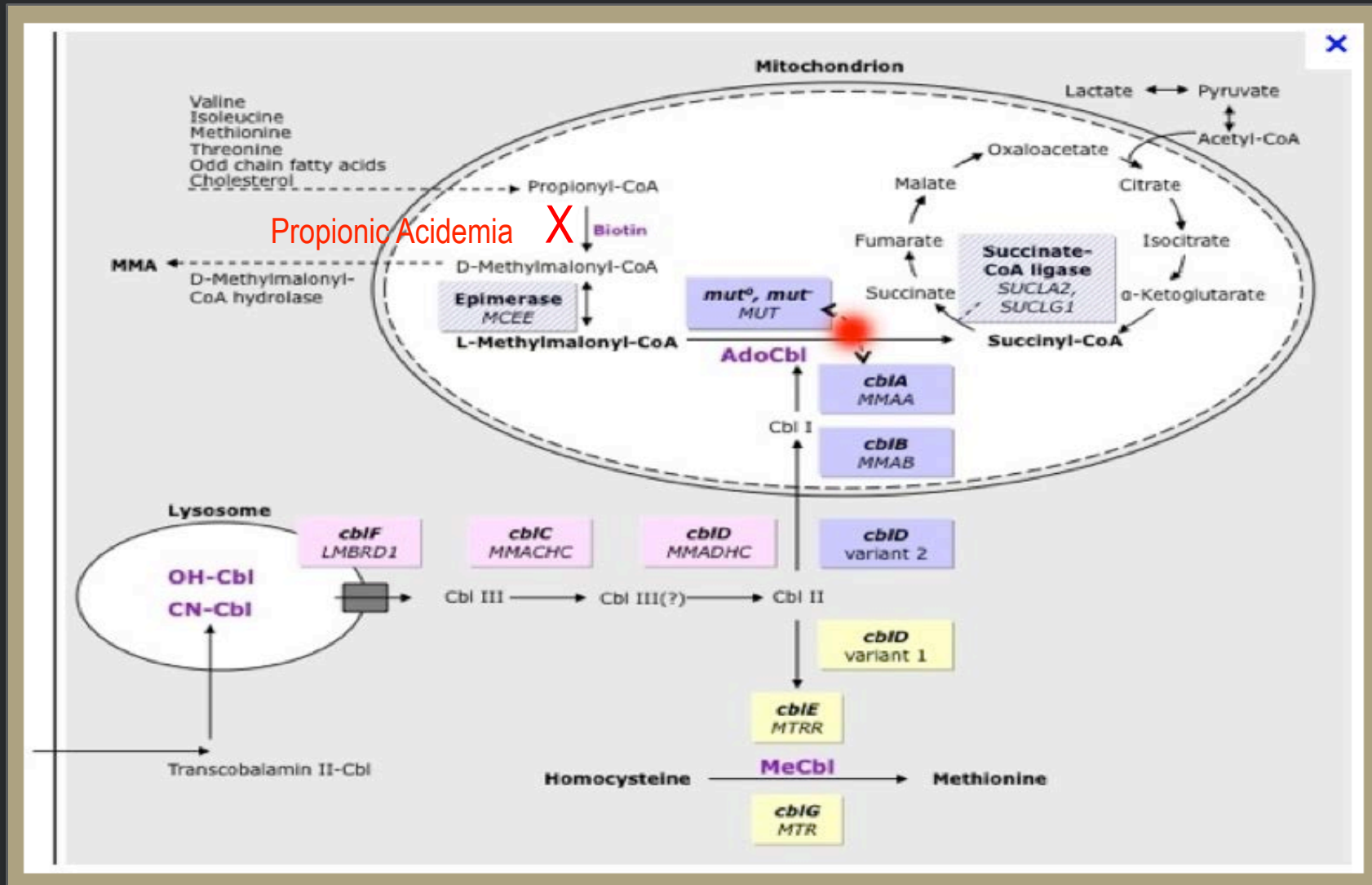


**Chapter 18 Opener part 1**  
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# PROPIONIC ACIDEMIA

## Why is this a "Disease?"

When & why does the disorder become an "Illness?"



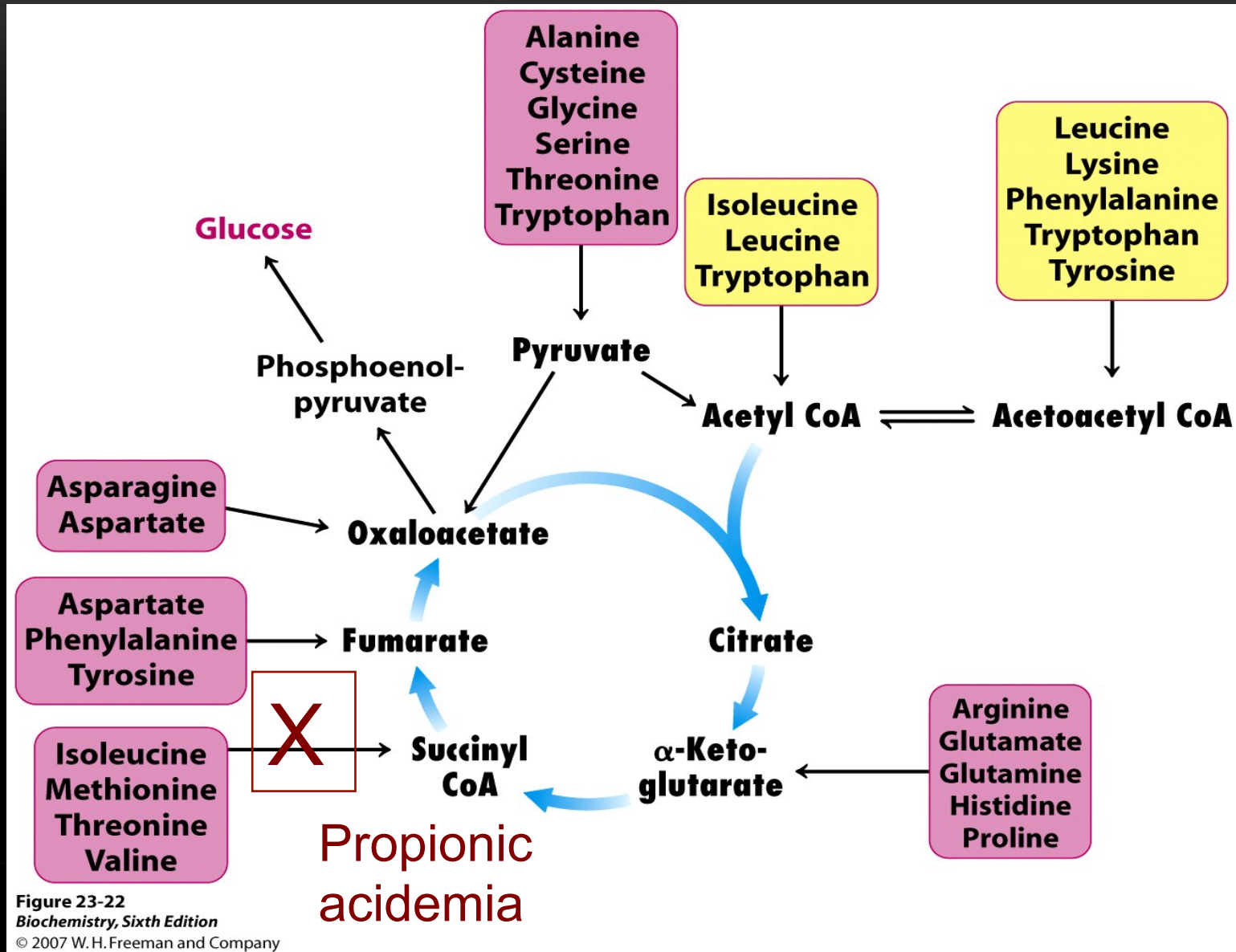
## BIOCHEMISTRY-2: PROPIONYL-COA

- Propionyl-CoA is a regulatory metabolite. At low concentrations it inhibits Pyruvate Dehydrogenase, slowing entry of glycolytic carbons into the citric acid cycle.
- Propionyl-CoA is an anti-metabolite of acetyl-CoA's reaction with oxaloacetate to form citrate.
- 2-Methylcitrate derives from the pathological reaction of propionyl-CoA & oxaloacetate. Methylcitrate is probably a competitive inhibitor aconitase, slowing the conversion of citrate to isocitrate.
- Biochemical pathology of propionyl-CoA is also directly related to failed anaplerotic synthesis of succinyl-CoA and the resulting decreased activity of succinyl-CoA dehydrogenase.

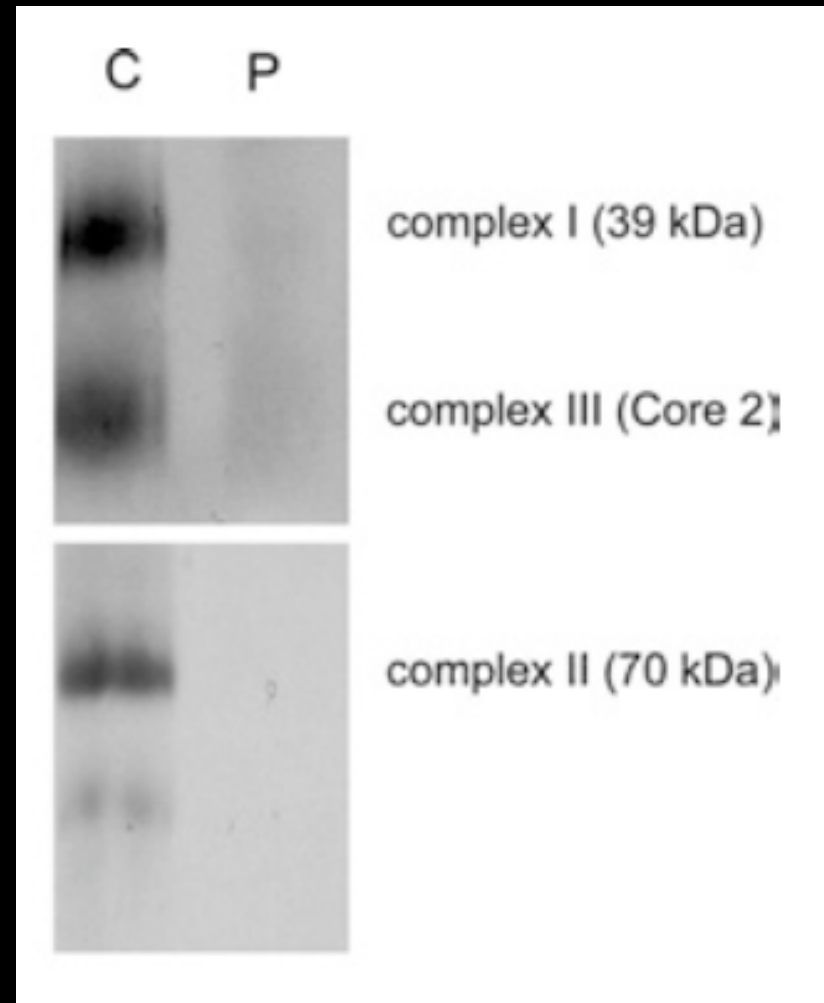
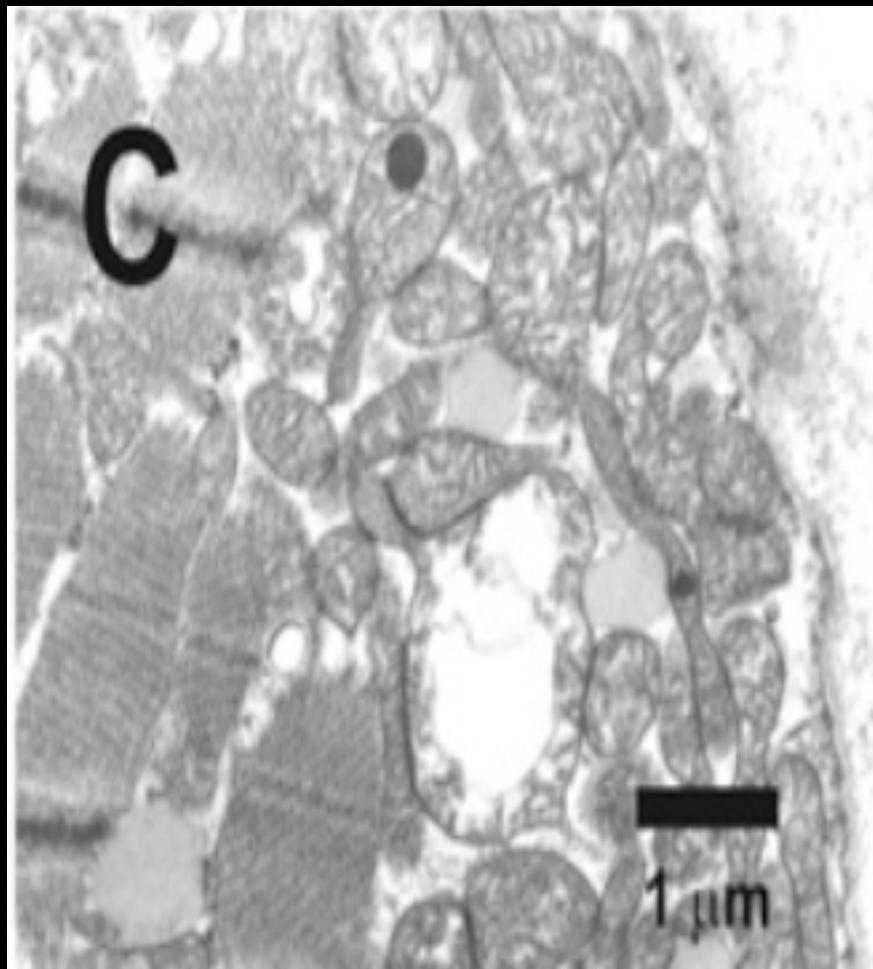
## BIOCHEMISTRY-3: SUCCINYL-COA

- Succinyl-CoA is a critical metabolite. The severity of PA of as a disease largely reflects the central role of succinyl-CoA in citric acid cycle function.
- Ketone body use during fasts and illnesses requires succinyl-CoA as a CoA donor. Pathological ketosis indicates pathological depletion of succinyl-CoA.
- Succinyl-CoA reacts with glycine to initiate mitochondrial heme synthesis. Chronic depletion of succinyl-CoA leads to loss of heme-dependent electron transport centers.
- Succinyl-CoA dehydrogenase:Coenzyme-Q is Complex-II of the electron transport system. Loss of function means loss of the proton gradient, oxidant damage to & degeneration of mitochondria.
- Succinyl-CoA dehydrogenase regulates mitochondrial replication through controlling the activity of NDPK.

# ANAPLEROSIS OF THE CITRIC ACID CYCLE

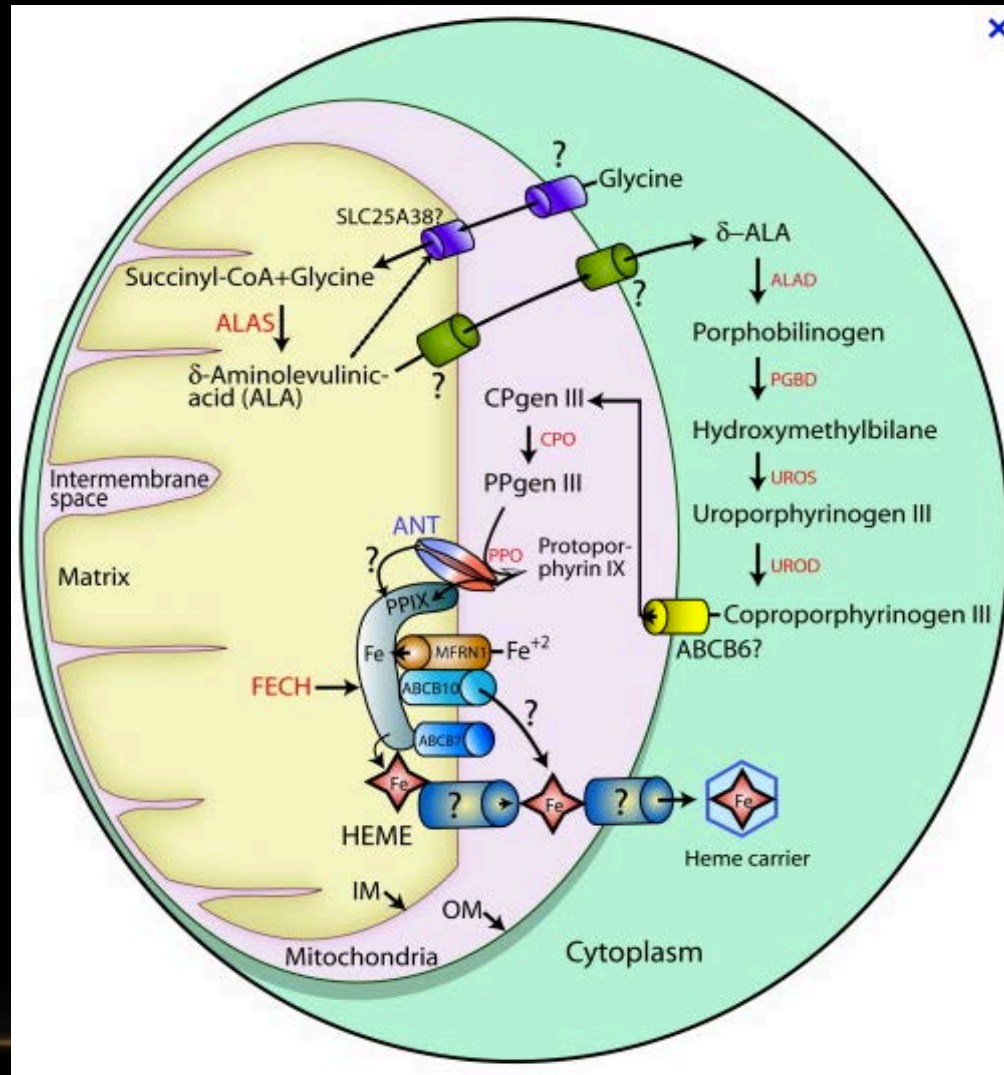


# Muscle: Mitochondrial Degeneration Loss of Electron Transport Activity





# SUCCINYL-COA + GLYCINE >>> HEME SYNTHESIS



# Succinyl-CoA Dehydrogenase & CoQ-10

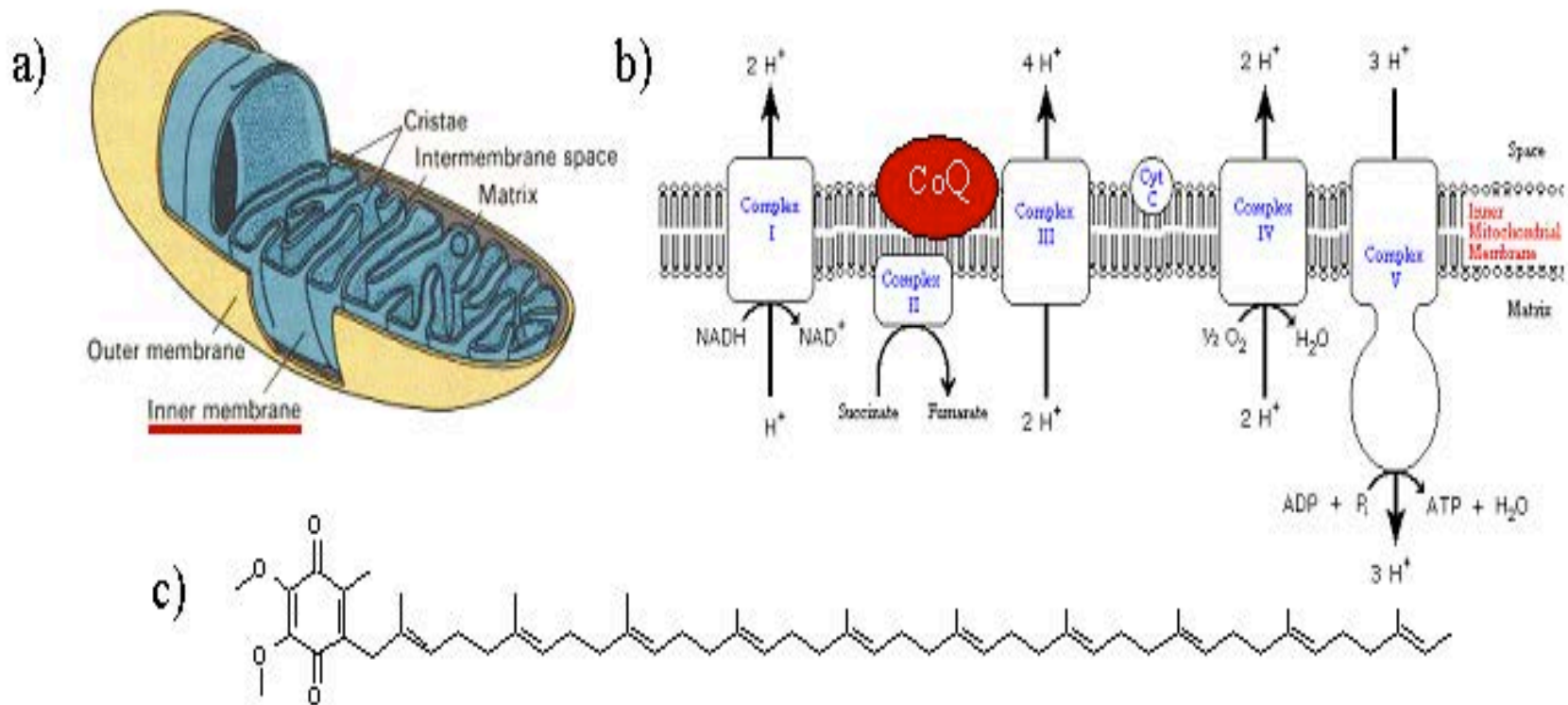
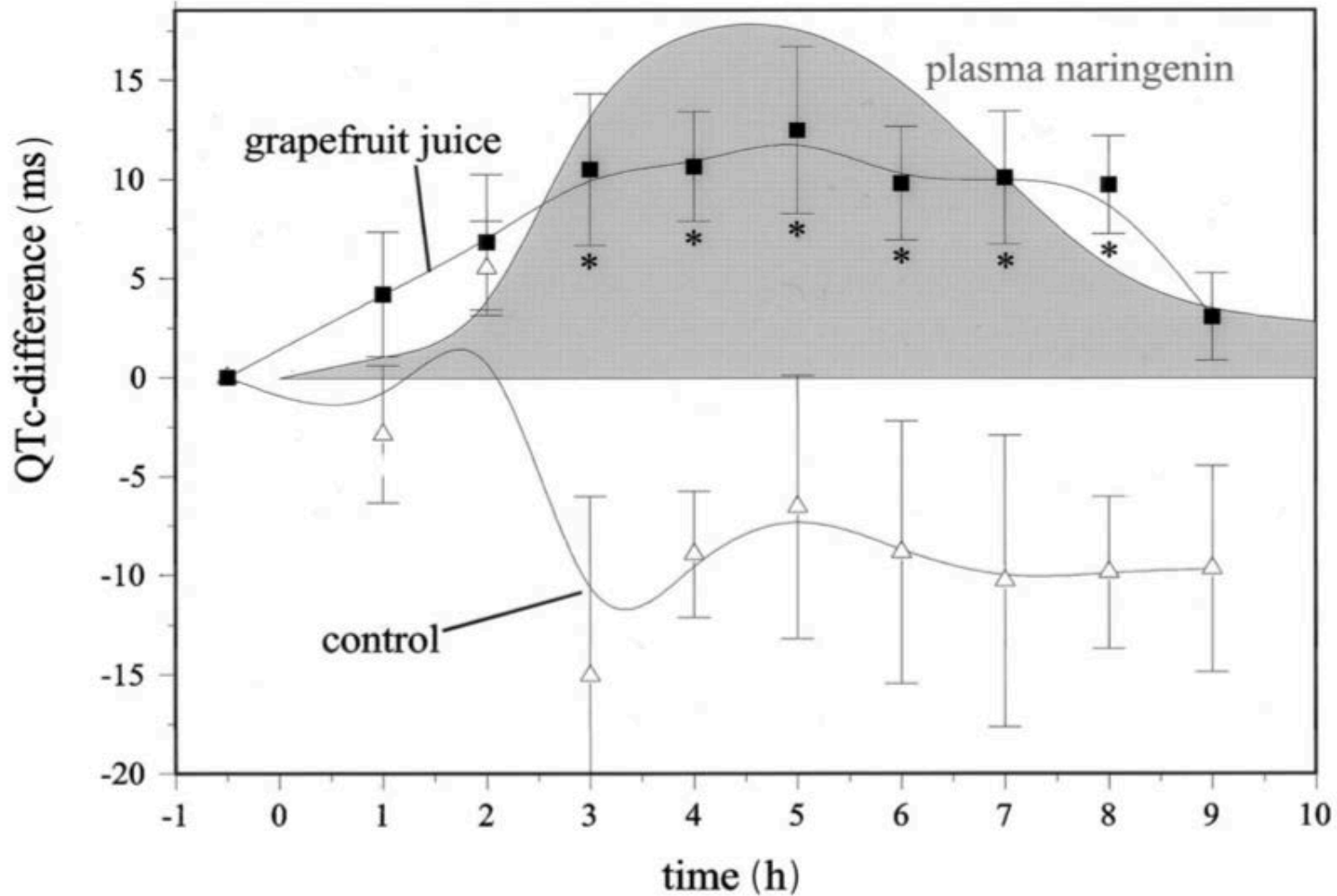


Figure 1.  $\text{CoQ}_{10}$  is an essential component of the electron transport chain within the mitochondria.<sup>2,3</sup>

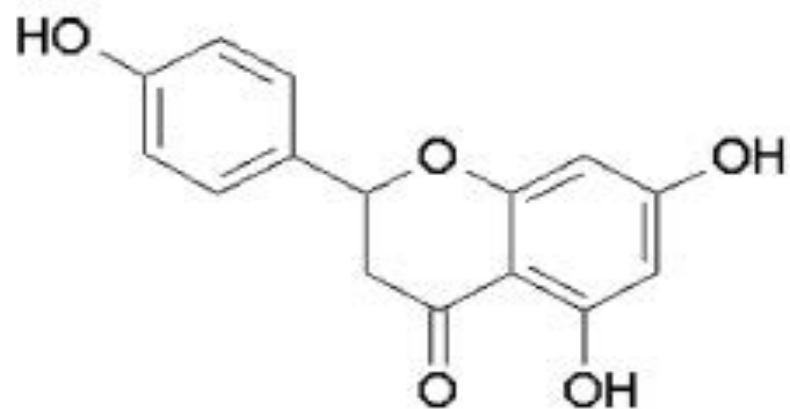
# Effect of flavinoids from grapefruit upon QTc in normals. QTc Reserve

Repolarization reserve and proarrhythmia

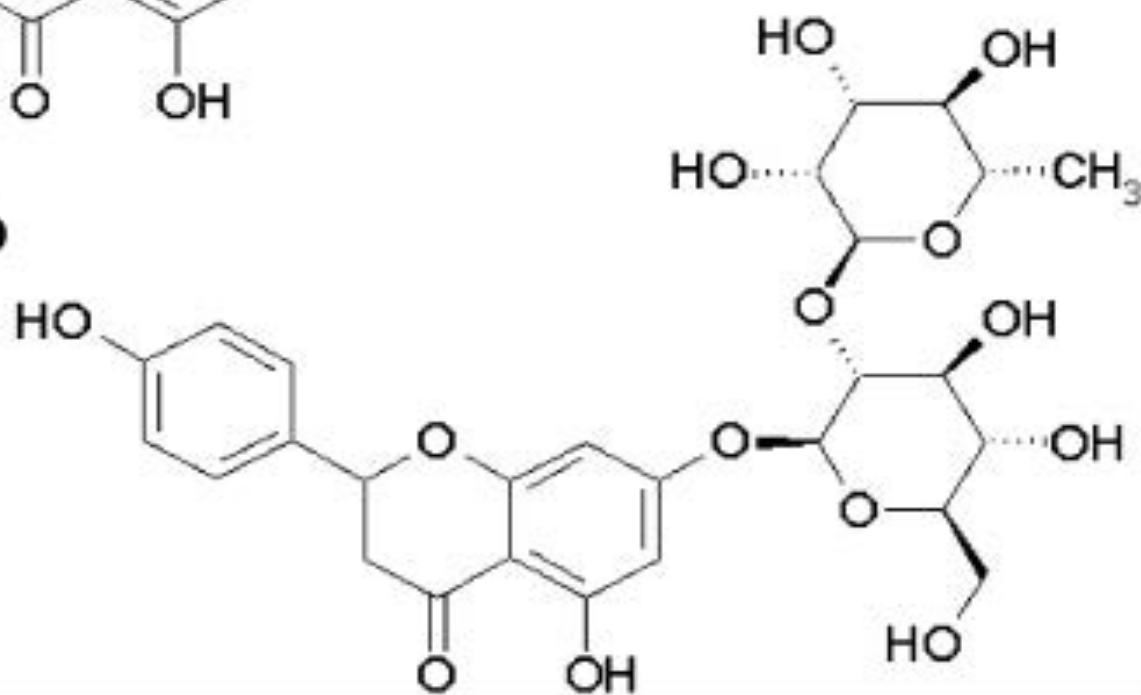


Chemical structures **(a)** of (+/-)-naringenin (4',5,7-trihydroxyflavanone) and **(b)** of its 7- $\beta$ -neohesperidoside, naringin.

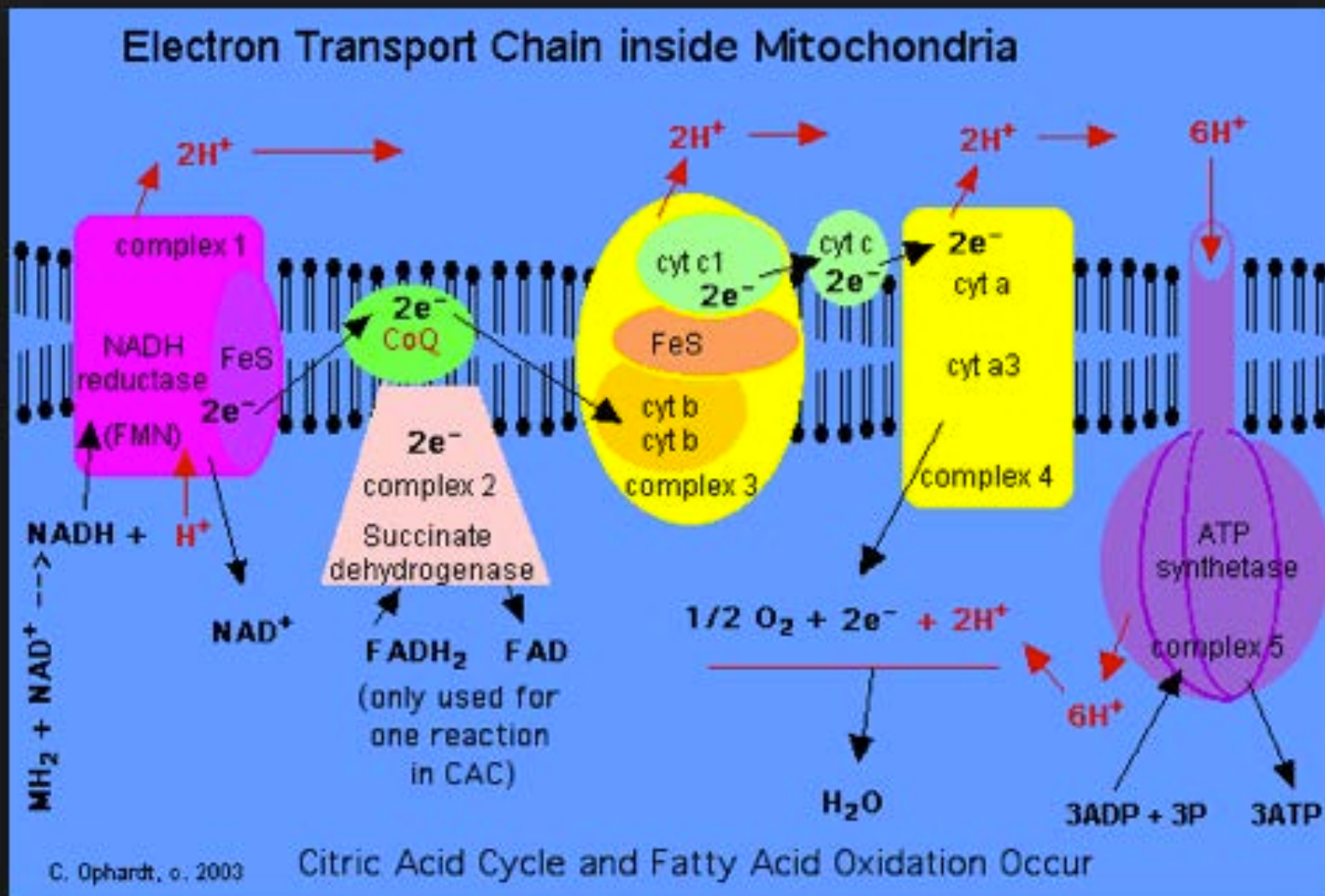
**a**



**b**



# Succinyl-CoA Dehydrogenase FADH<sub>2</sub> & CoQ-10



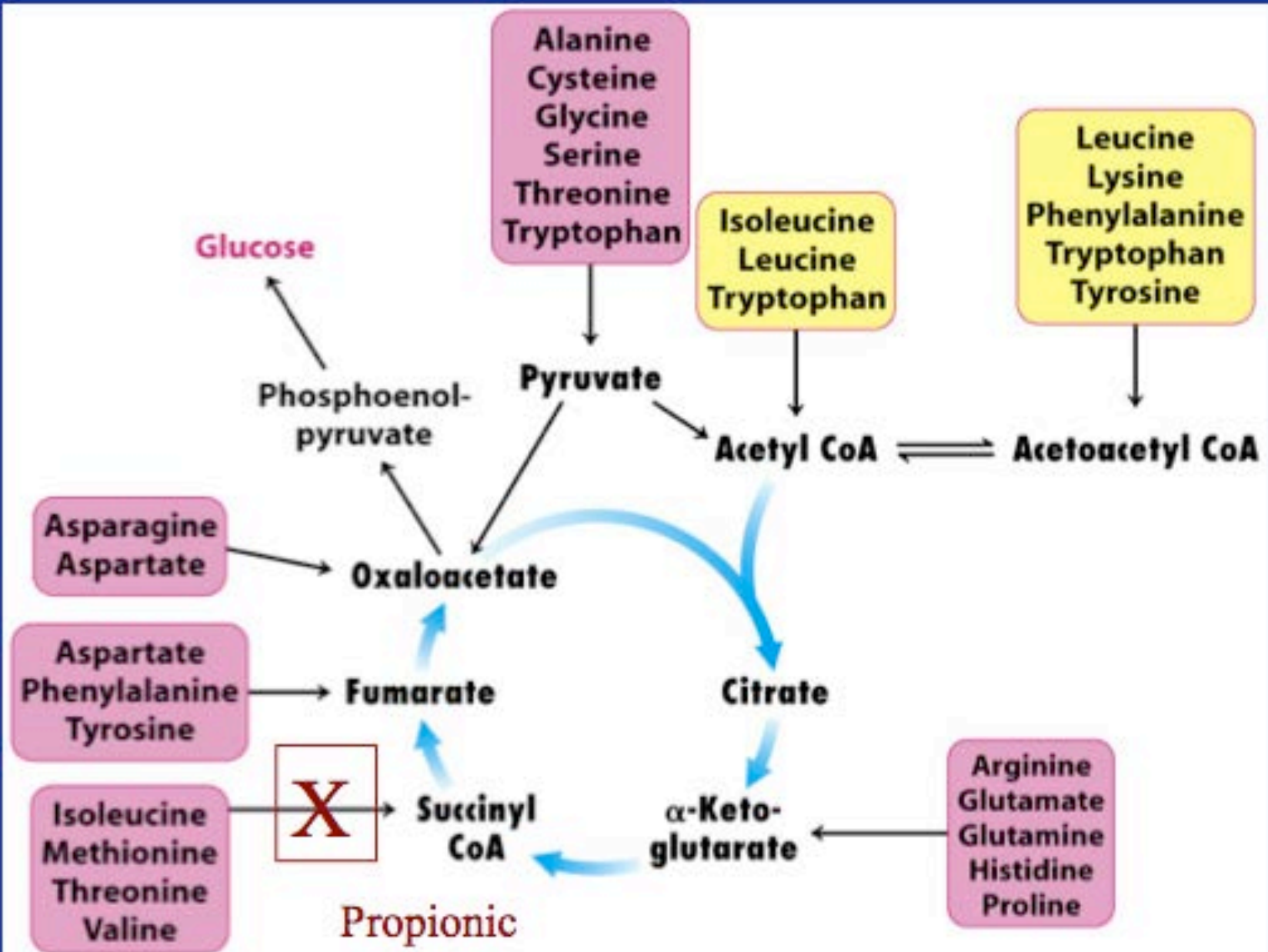


Figure 23-22  
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## BIOCHEMISTRY-4: THERAPEUTICS

- Patients with the Amish/Mennonite variant of propionic acidemia are often stable for many years, undiagnosed and untreated.
- The natural tolerance of the disorder likely involves adaptations that limit propionyl-CoA accumulation, supply succinyl-CoA through alternative anaplerotic pathways, and protect the function of succinyl-CoA dehydrogenase:CoQ.
- Dietary protein excess, fasts and starvation, carnitine or CoQ depletion, hypoxia or drugs that further impair genetically compromised mitochondrial energetics can set-off a cascade of events that lead to failed energetics in the myocardium or brain.

## BIOCHEMISTRY-5: THERAPEUTICS

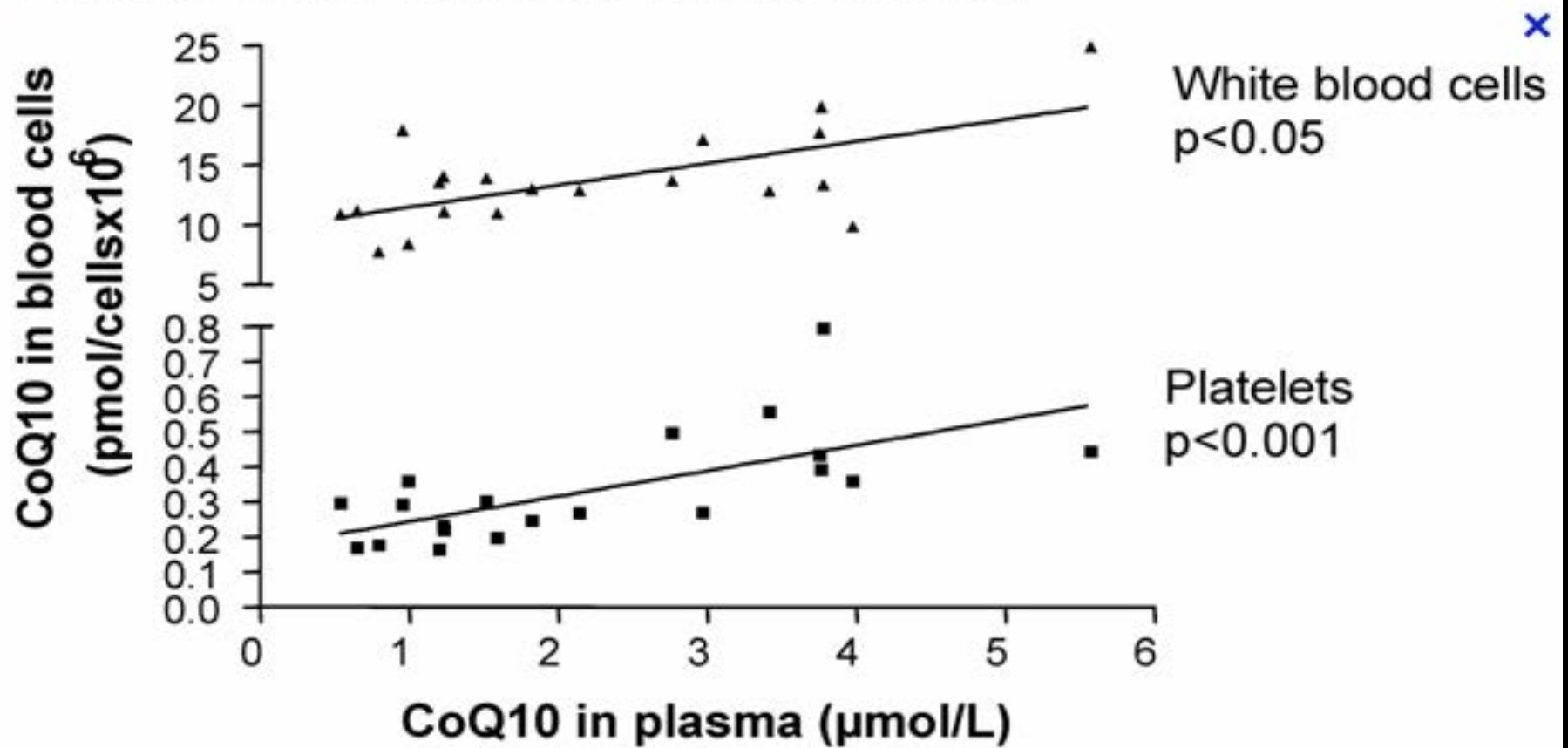
- Propionyl-CoA accumulation is limited by restriction of dietary protein to 0.75-1.25 g/kg-day, by the administration of carnitine 25-100 mg/kg-day & by limited transport of isoleucine, valine & threonine into the brain and myocardium through competitive LAT-1 amino acid transport kinetics. Effects of therapy are monitored by plasma amino acids, free carnitine & propionyl-carnitine in whole blood, serum CoQ, & the urine citrate/methylcitrate ratio.
- Succinyl-CoA formation & succinyl-CoA dehydrogenase function are supported by supplemented amino acids that form 2-ketoglutarate, fumarate, oxaloacetate, and by K-citrate. The effect of these supplements upon citric acid cycle repletion is monitored by measuring the citrate/methylcitrate ratios in urine using GC/MS.
- Oral glucose, anaplerotic amino acids, and potassium-citrate can be used to prevent or reverse ketosis during illnesses.



## BIOCHEMISTRY-6: THERAPEUTICS

- Propionyl-CoA accumulation is also limited by the propionyl-CoA cofactor biotin. High-dose biotin 5 mg twice daily is given to stabilize the residual enzyme activity. This vitamin is used with the same rationale as a “chaperone” drug. Betaine is being investigated as a possible chaperone drug for propionic acidemia.
- The function of Complex-II succinyl-CoA dehydrogenase:CoQ will be compromised if iron, sulphur, riboflavin, niacin, or CoQ deficiencies develop. Serum CoQ levels have recently been found low in several of our PA patients. We now supplement with 100-200 mg/day of CoQ10 and aim to have serum levels 3 times about the upper limit of the normal range.

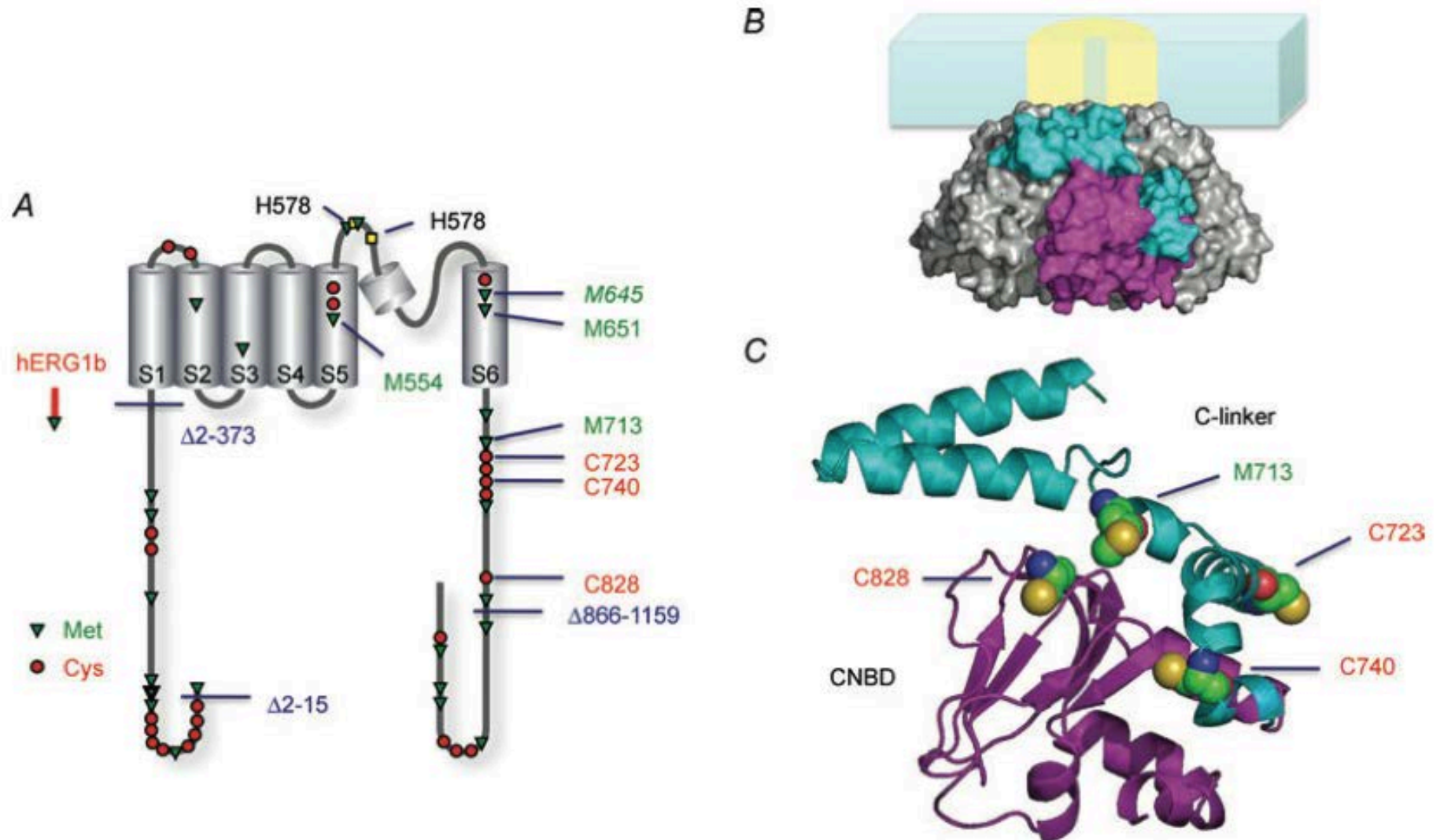
# CoQ10 plasma & cellular concentrations



## BIOCHEMISTRY-7: THERAPEUTICS

- Acquired long QTc syndrome is seen in the majority of patients but is uncommon in infants & increases with age. The etiology and treatment this acquired long QTc disorder is being studied.
- An interesting hypothesis about the cause of acquired long QTc in propionic acidemia is that it arises from oxidation of cysteine & methionine SH groups in hERG1 potassium channel and is a “biomarker” for poor Complex-II function & inadequate myocardial CoQ10 protection.
- Studies of the effect of improved anti-oxidant protection upon QTc are in progress.
- Sudden cardiac death in this variant of PA is common. Management protocols should be developed to protect at risk PA patients from drugs and dietary compounds that are known to increase the risk of life threatening events.

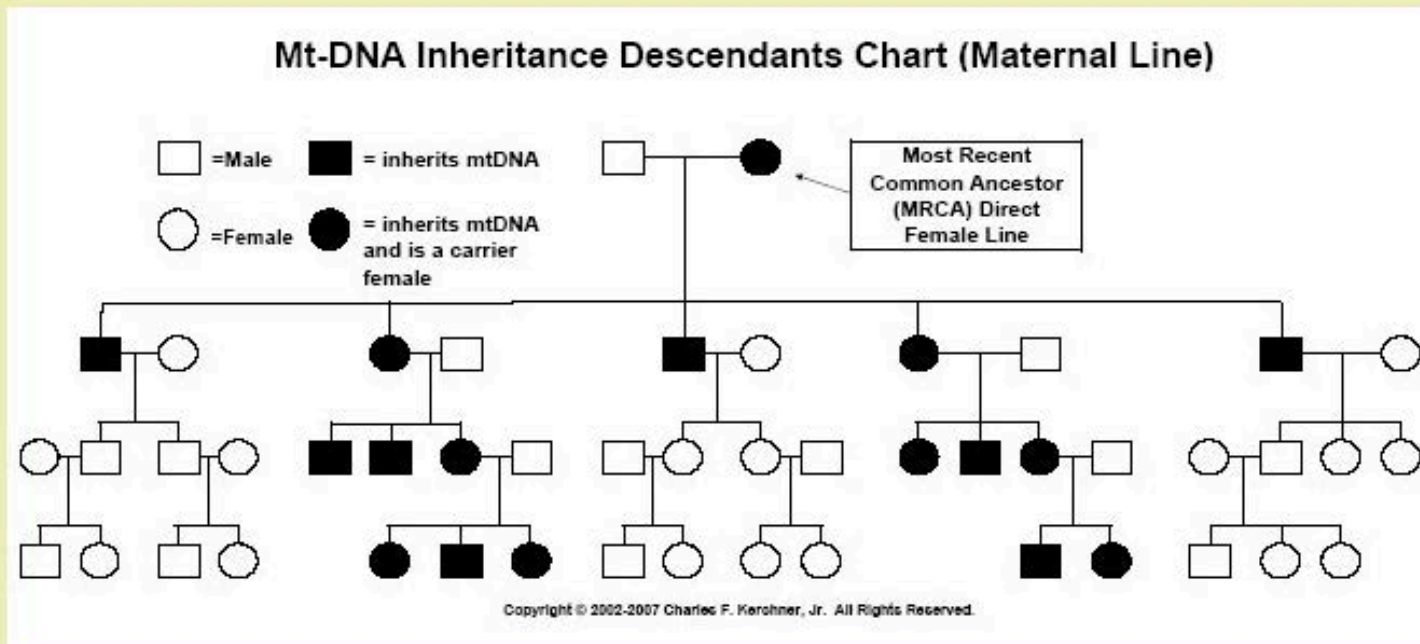
# MODELS OF THE HERG1-POTASSIUM CHANNEL SHOWING CYSTEINE-723 & METHIONINE-713 OXIDANT SENSITIVE -SH GROUPS



# Does mtDNA haplotype affected PA expression?

(sample mtDNA pedigree from google images, not a PA pedigree)

## mtDNA Inheritance Chart

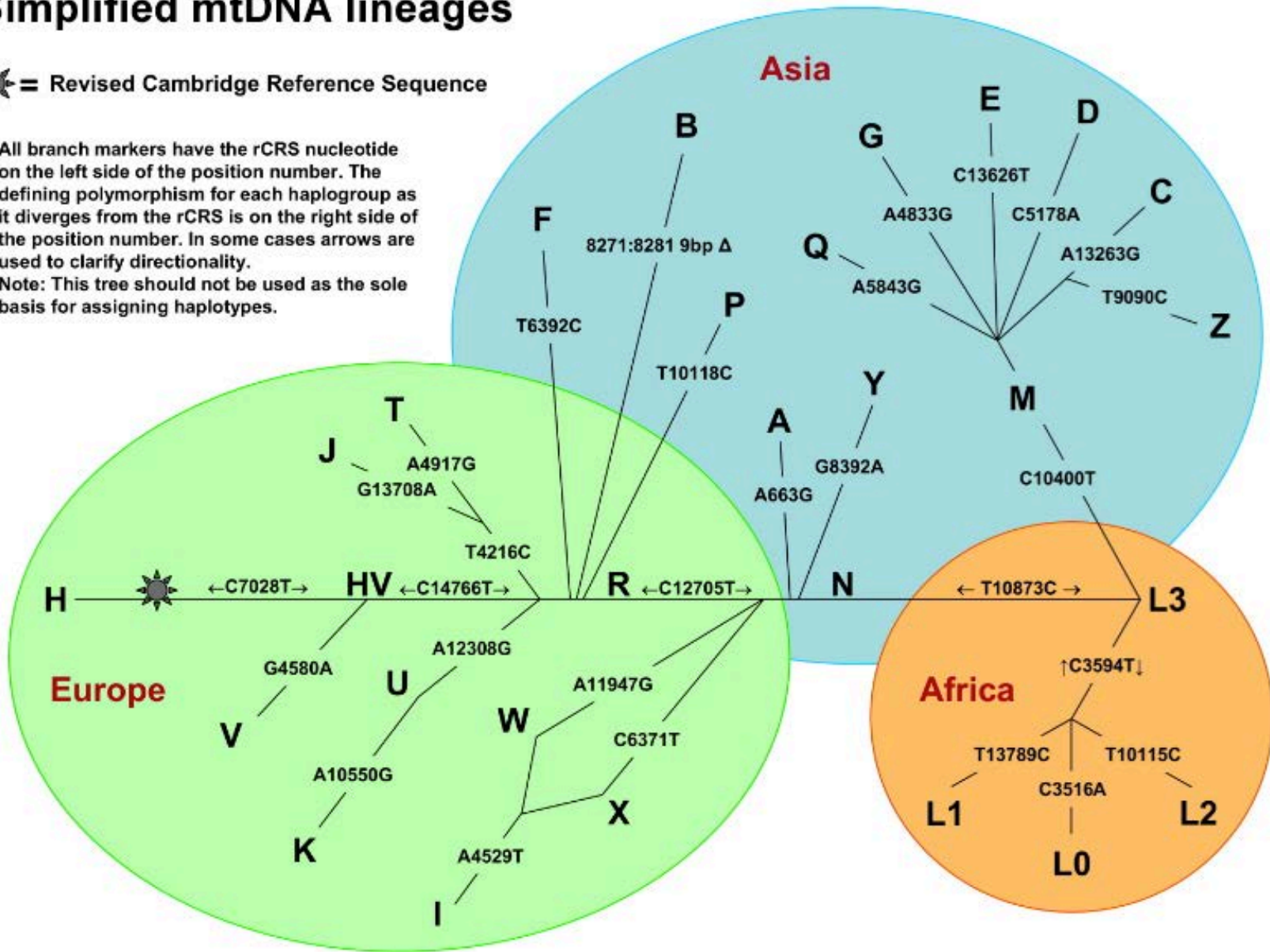


# Simplified mtDNA lineages

☼ = Revised Cambridge Reference Sequence

All branch markers have the rCRS nucleotide on the left side of the position number. The defining polymorphism for each haplogroup as it diverges from the rCRS is on the right side of the position number. In some cases arrows are used to clarify directionality.

Note: This tree should not be used as the sole basis for assigning haplotypes.



**Table 2** A frequency distribution of surnames and mitochondrial hypervariable region (HVR1) haplotypes among four Pennsylvania Amish and Mennonite demes<sup>1</sup>

	Groffdale Mennonites	Weaverland Mennonites	Lancaster Amish	Juniata and Mifflin Amish
<b>Surname</b>				
Martin	18%	21%	0%	0%
Zimmerman	18%	9%	0%	0%
Hoover	8%	6%	0%	0%
Nolt	7%	5%	0%	0%
Weaver	5%	7%	0%	0%
Stoltzfus/Stoltzfoos	0%	0%	26%	0%
King	0%	0%	12%	0%
Fisher	0%	0%	11%	0%
Beiler/Byler	0%	0%	11%	7%
Peachey	0%	0%	0%	29%
Swarey	0%	0%	0%	13%
Kanagy	0%	0%	0%	11%
Yoder	0%	0%	1%	11%

# Mitochondrial Haplotypes in 4 Pennsylvania Demes

	Groffdale Mennonites	Weaverland Mennonites	Lancaster Amish	Juniata and Mifflin Amish
mtDNA haplotype				
16298C.16311C	20%	5%	0%	0%
1669T.16126C.16261T	13%	19%	0%	0%
Cambridge reference	8%	0%	0%	38%
16304C	7%	5%	0%	8%
1693C.16304C	6%	11%	0%	0%
16294T.16304C	2%	11%	3%	4%
16126C.16294T.16296T	4%	8%	0%	0%
16129A.16311C.16316G	2%	8%	0%	0%
16209C.16304C	1%	8%	0%	0%
16260T.16356C	0%	0%	36%	0%
16051G.16092C.16129C.16183C.16189C.16362C	4%	0%	26%	0%
16129A.16264T.16311C	0%	0%	15%	4%
16124C.16189C.16356A	0%	0%	5%	15%
16100C	0%	0%	0%	10%



# PREVENTION

## EARLY DETECTION BY NEWBORN SCREENING

- In the absence of myocardial fibrosis, the cardiomyopathy of PA may be reversible by nutritional therapy that:
- 1) limits propionyl-CoA accumulation in mitochondria through dietary protein restriction, high-dose biotin, and carnitine
- 2) supports citric acid cycle function through daily intake citrate, malate, and anaplerotic amino acids
- 3) supports mitochondrial complex-2 function by an anaplerotic supply of succinyl-CoA & prevents oxidant damage to mitochondria by use of Coenzyme Q10 & vitamin E

## CONCLUSION

- Cardiomyopathy is common in patients with the PCCB c.1606A>G variant.
- Heart failure and arrhythmia cause of untimely death.
- Propionic acidemia should be considered in patients of any age who present with “idiopathic” cardiomyopathy.
- Monitoring of heart function by cardiac ECHO & EKG shows sub-clinical myocardial dysfunction and long QTc are present in the majority of patients with this otherwise relatively benign PCCB variant.

## FIND A MEDICAL HOME FOR PATIENTS WITH PROPIONIC ACIDEMIA

- Common infections cause severe metabolic illnesses – prevention, early detection and treatment of infections are essential. **WHO WILL PROVIDE THIS CARE?**
- Ketonuria can be detected by parents and reversed at home. **CONTROL OF VOMITING, ORAL REGIMENS OF GLUCOSE, POLYCITRA, AMINO ACIDS, AND CARNITINE CAN REVERSE KETOSIS.**
- Dietary monitoring prevents the accumulation of propionyl-CoA & depletion succinyl-CoA. **ROUTINE MONITORING OF DIET & BIOMARKERS.**

# Pennsylvania Demographics

Patients > 2 hours from CSC – 500 patients with 91 different genetic disorders.

