BIOCHEMICAL AND MOLECULAR HETEROGENEITY IN CARNITINE PALMITOYLTRANSFERASE II DEFICIENCY

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The Newborn screening programme (NBS) by MS/MS – 2004 -2012 ➡ 737 902 newborns screened

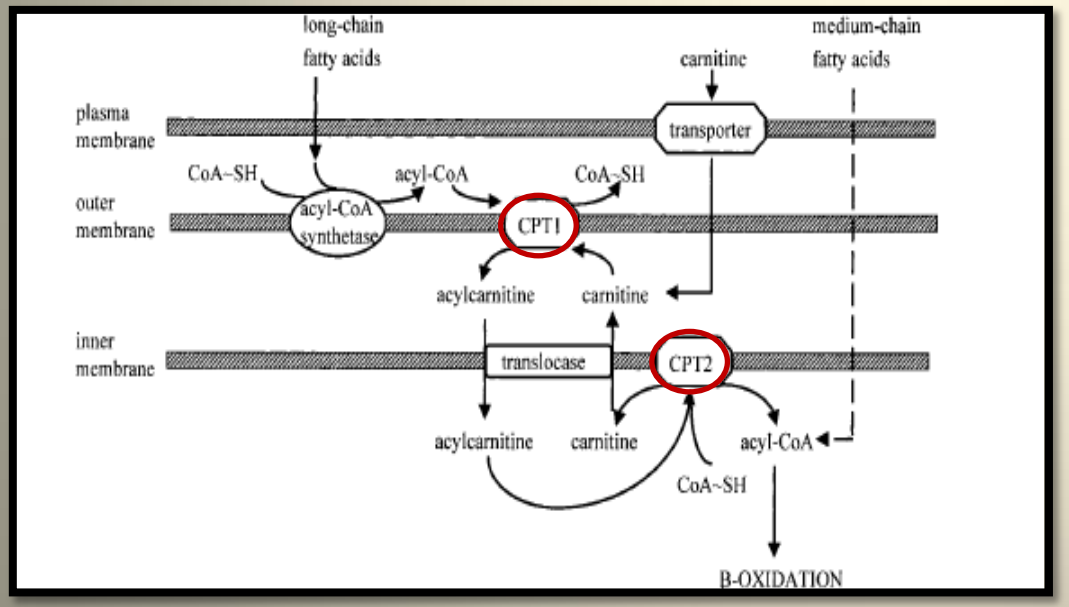
4 cases of Carnitine palmitoytransferase II deficiency (CPTII) were detected by NBS
1 remains without a definitive diagnostic.

Prevalence 1:245 967 in Portugal

Substimated prevalence ? The myopathic form may not be detected

METABOLIC PATHWAY

This enzymatic complex is made up of two distinct proteins named carnitine palmitoyltransferase I (CPTI) and II (CPTII)



The mitochondrial CPT system

CPTI — **Catalyzes the formation of acylcarnitine from carnitine and acil-CoA**

CPTII — **Catalyses the formation of acil-CoA from acylcarnitine and CoA for the β-oxidation process.**

CPTII DEFICIENCY

A CPTII deficiency is a **autosomal recessive inherited disorder** of fatty acid metabolism.

Organic acids profile - Acumulation of long chain acylcarnitines in mitochondrial matrix – metabolic pathway of peroxisomal b-oxidation**. Most of these cases with a normal profile.**

Histological investigation (muscle)
- Normal or shows mild unspecific myophatic changes - heterozygotes
- Lipid accumulation - homozygotes

Differencial diagnosis – carnitine-acylcarnitine translocase deficiency (CACT)

Existence of **symptomatic heterozygous patients**

Clinical heterogeneity

CLINICAL PHENOTYPES

Lethal neonatal form
MIM#608836

Liver failure

hypoketotic hypoglycemia

Cardiomyopathy

Seizures and coma after fasting or infection

Facial abnormalities or structural malformations (e.g., **cystic renal**)

Severe infantile hepatocardiomuscular form
MIM#600649

Seizures
hypoketotic hypoglycemia
Cardiomyopathy

Peripheral myopathy

Attacks of abdominal pain and headache

Myopathic form
MIM#255110

Attaks of **myalgia** accompanied by **myoglobinuria**

Rhabdomyolysis (triggered by extensive exercice, cold, fever or prolonged fasting or stress)

Usually no signs of myopathy (weakness, myalgia, elevation of serum creatine kinase [CK] concentration) **between attacks**

May be under-recognized

AGE OF ONSET

Myopathic form
MIM#255110

"Mild"

Variable 1st to 6th decade

Severe infantile hepatocardiomuscular form
MIM#600649

Severe

Sudden death (SIDS)

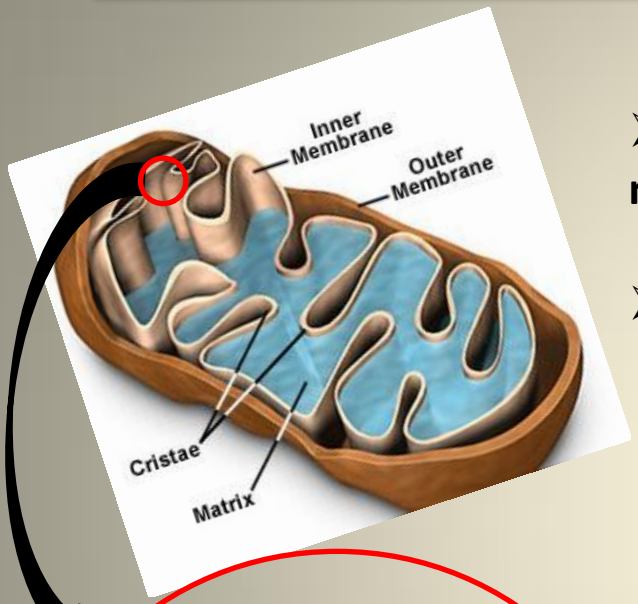
Lethal neonatal form
MIM#608836

Severe

S113L accounts for 60% of mutant alleles

Compound heterozygosity of mild and severe mutations has been reported whith this form

CPTII PROTEIN



- Localized in the inner mitochondrial membrane
- Catalytically active

Skeletal muscle
Liver
Fibroblasts
Lymphocytes

GENE *CPT2*

Cr.- 1p32

cDNA has 1974 bp

5 exons

658 amino acids

20 Kb

86 described mutations

PATIENTS AND METHODS

Patients

Over a 8 years period, 737 902 newborns were screened by blood spots

4 newborns were detected through newborn screening (NBS) by acylcarnitine profile

5 cases with clinical symptoms suggestive of CPTII deficiency

Methods

Tandem mass spectrometry (MS/MS) by API 2000 triple quadrupole

Molecular analysis of *CPT 2* gene was done by direct sequencing

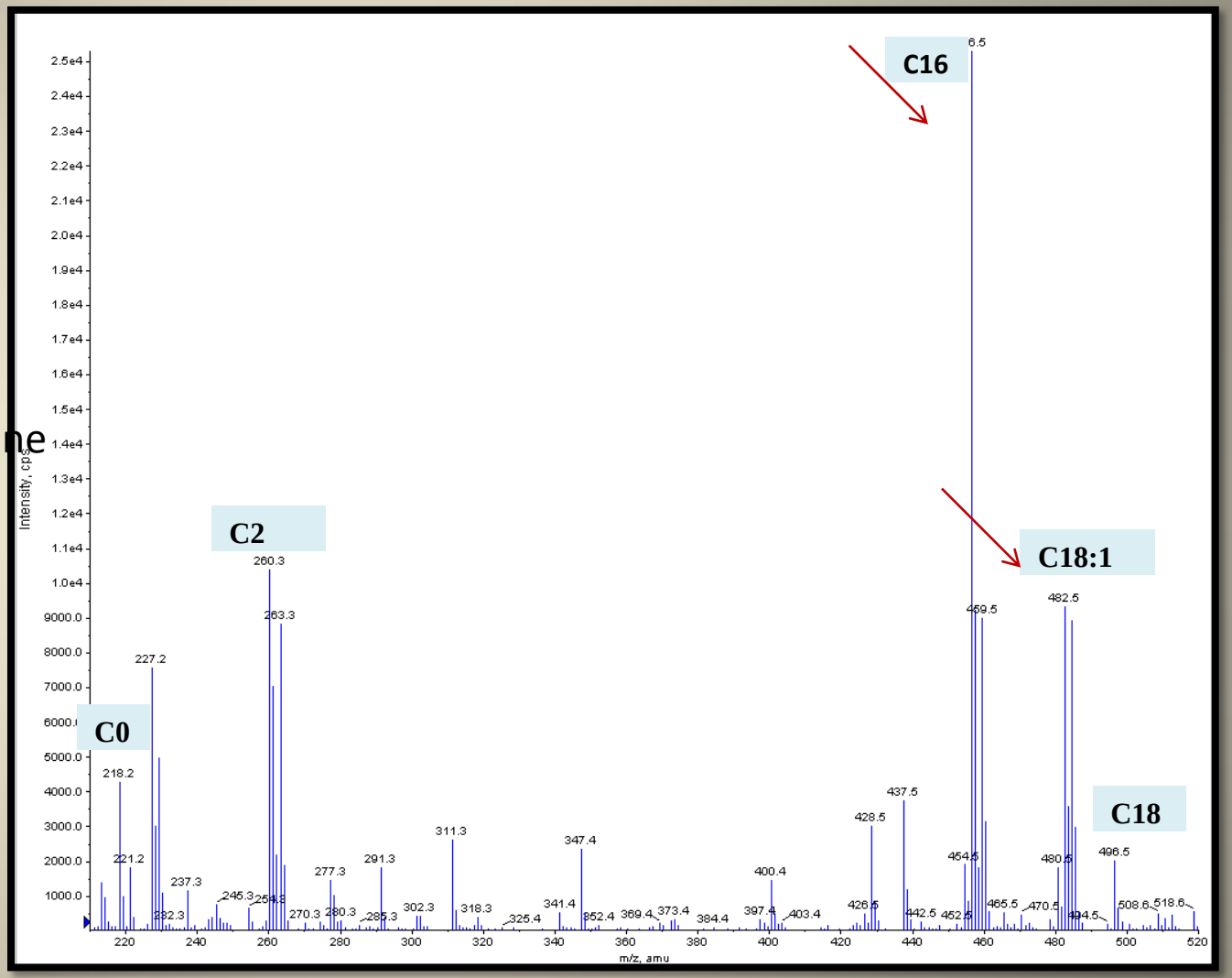
MASS SPECTROMETRY OF ACYLCARNITINE PROFILE

↑ **C16** Palmitoylcarnitine

↑ **C18:1** Octadecenoylcarnitine

↓ **C0/(C16+C18)**

↑**(C16+C18:1)/C2**



A typical profile of CPTII deficiency

May be ambiguous for myophatic form

RESULTS

Newborn screening cases

Case		Age	C0 µM	C2 µM	C16 µM	C18 µM	C18:1 µM	C0/ C16+18	C16+ C18:1/C2	Allele 1	Allele 2
			Rf>9.13	Rf>7.0	Rf<7.99	Rf<2.28	Rf<3.42	Rf>3.0	Rf<0.5		
1	M	4 d	15.1	7.02	7.02	2.42	2.7	1.6	1.38	c.430_435delGAGTAT (p.E144-Y145del)	c.725_726insA (p.H242Qfs*14)
2†	M	2 d	17.7	9.1	31.07	9.24	11.6	0.44	4.76	c.110_111dupGC (p.36_38insGC)	c.110_111dupGC (p.36_38insGC)
3	M	6 d	14.94	10.71	7.22	3.13	4.21	1.44	1.07	Under study	Under study
4	M	5 d	10.3	7.7	6.8	2.9	4.4	1.06	1.5	c.680C>T (p.227L)	c.1106A>G (p-H369R)

- Novel mutations

All cases - ↑C18 and C18:1
↓Co/C16+C18 and ↑C16+C18:1/C2

RESULTS

- Novel mutations

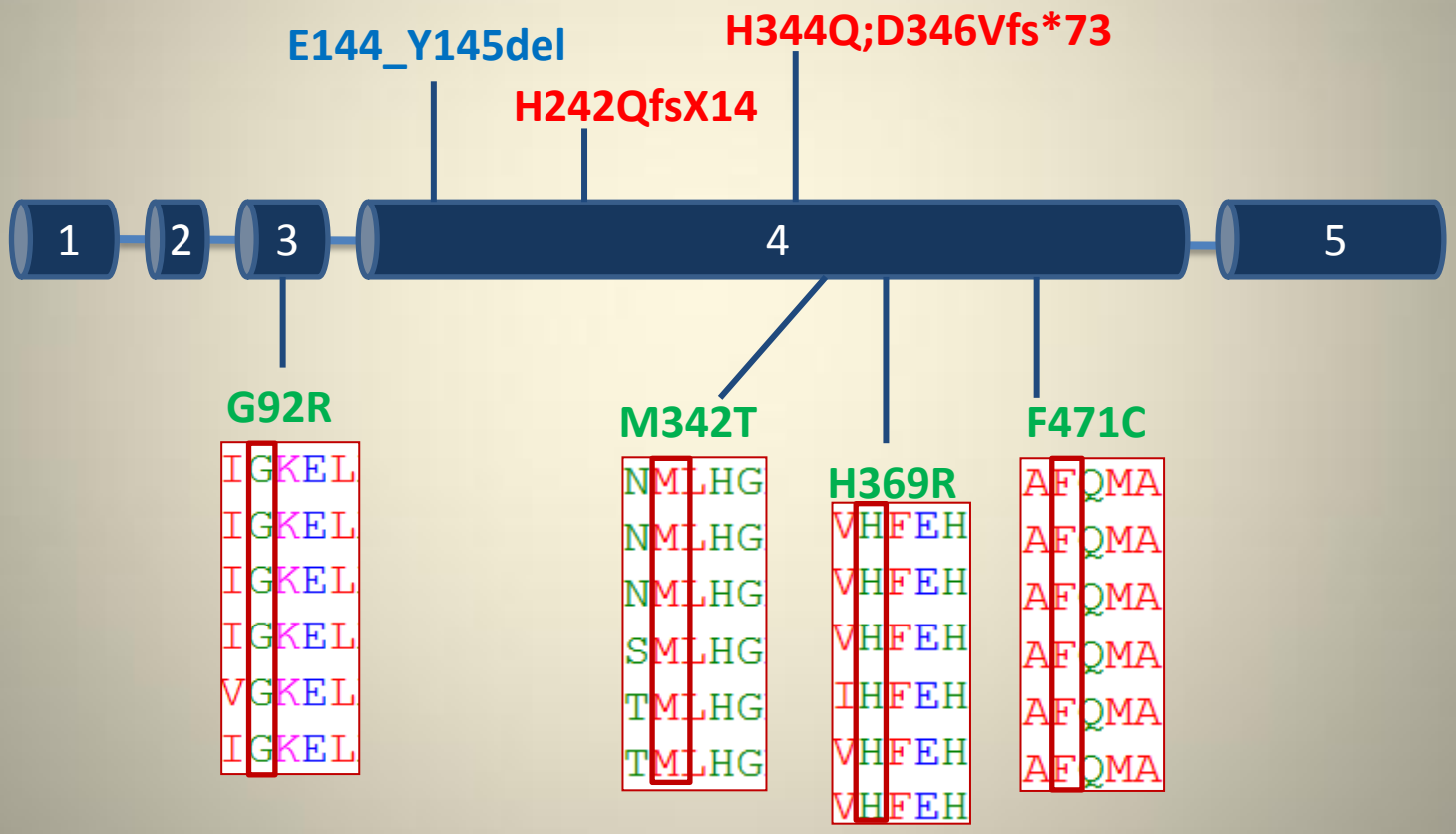
Symptomatic cases

Case		Age of Onset (years)	C0 µM	C2 µM	C16 µM	C18 µM	C18:1 µM	Allele 1	Allele 2
			Rf>19.24	Rf>8.06	Rf<2.09	Rf<1.36	Rf<2.33		
5	M	52	55.01	14.55	0.8	0.67	1.25	c.1025T>C (p.M342T)	Wild-type
6	M	2	1.75	3.42	1.18	0.85	1.11	c.359A>G (p.Y120C)	c.1412T>G (p.F471C)
7	M	35	na	na	na	na	na	c.338C>T (p.S113L)	c.274G>A (p.G92R)
8	F	15	19.2	4.71	0.75	0.69	0.86	c.338C>T (p.S113L)	c.[1032T>G;1037delA] (p.H344Q;D346Vfs*73)
9	F	2	12.3	4.42	2.63	1.38	2.85	c.338C>T (p.S113L)	c.338C>T (p.S113L)

- All cases with two mutations - ↓ C2 (β-oxidation)
- Case 5 → C2 normal: **symptomatic heterozigotic** (dominant-negative mutation / Synergistic heterozygosity)
- Case 9 → NBS normal. 2 years – C2 ↓ and ↑C16 C18 C18:1 ⇨ Myopathic form (father is S113L/S113L)

MUTATIONS IN *CPT2* GENE

- 11 mutations: 7 novel mutations - 2 frameshift, 1 deletion and 4 missense + 4 reported
- V368I and M647V polymorphism found in must patients (may impair the enzymatic activity)



4 missense novel mutations were classified as pathogenic by prediction softwares – Polyphen 2, SIFT and Mutation taster

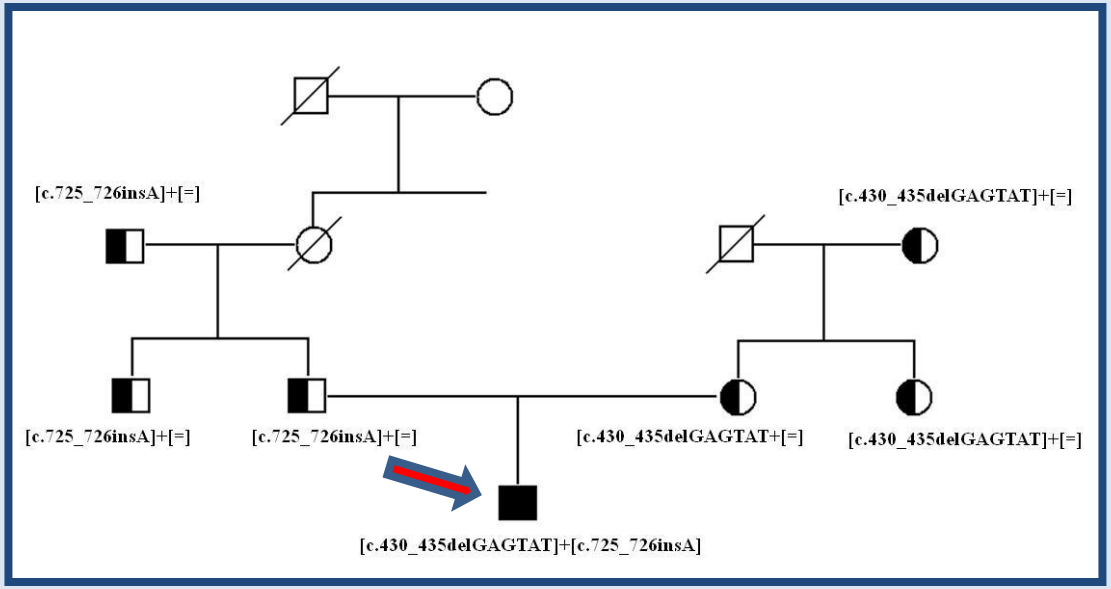
Molecular characterization – additional relevance

Example

- **Case 1**
- **two novel mutations**
  p.E144-Y145del/p.H242Qfs*14

256 amino acids

- **Enzymatic act. ↓(8,5%)**
- **4 days asymptomatic**



The identification of severe mutations should alert the clinicians for the careful follow-up of these patients

CONCLUSIONS

Our results support that newborn screening can efficiently detect infantile CPT II deficiency and allows an early intervention.

NBS may be less effective in detecting myophatic forms.

The molecular characterization allow the definitive diagnostic in CPTII deficiency and may help the clinicians to define the prognostic of the clinical phenotype and prenatal diagnosis.

Thank you!