GOVERNO DE PORTUGAL
MINISTÉRIO DA SAÚDE

Instituto_Nacional de Saúde Doutor Ricardo Jorge

35 Aniversário Serviço Nacional Saúde
A cuidar dos portugueses

9th ISNS European Neonatal Screening
Birmingham UK
12th-15th October 2014

Inborn errors of metabolism and expanded newborn screening in Portugal: 2004-2014

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PORTUGAL

Location: Southwestern Europe

Borders: Spain and Atlantic Ocean

Includes Azores and Madeira Islands (Atlantic ocean)

Total area: 92,090 sq km

Population: 10,487,289 persons (December 2012)

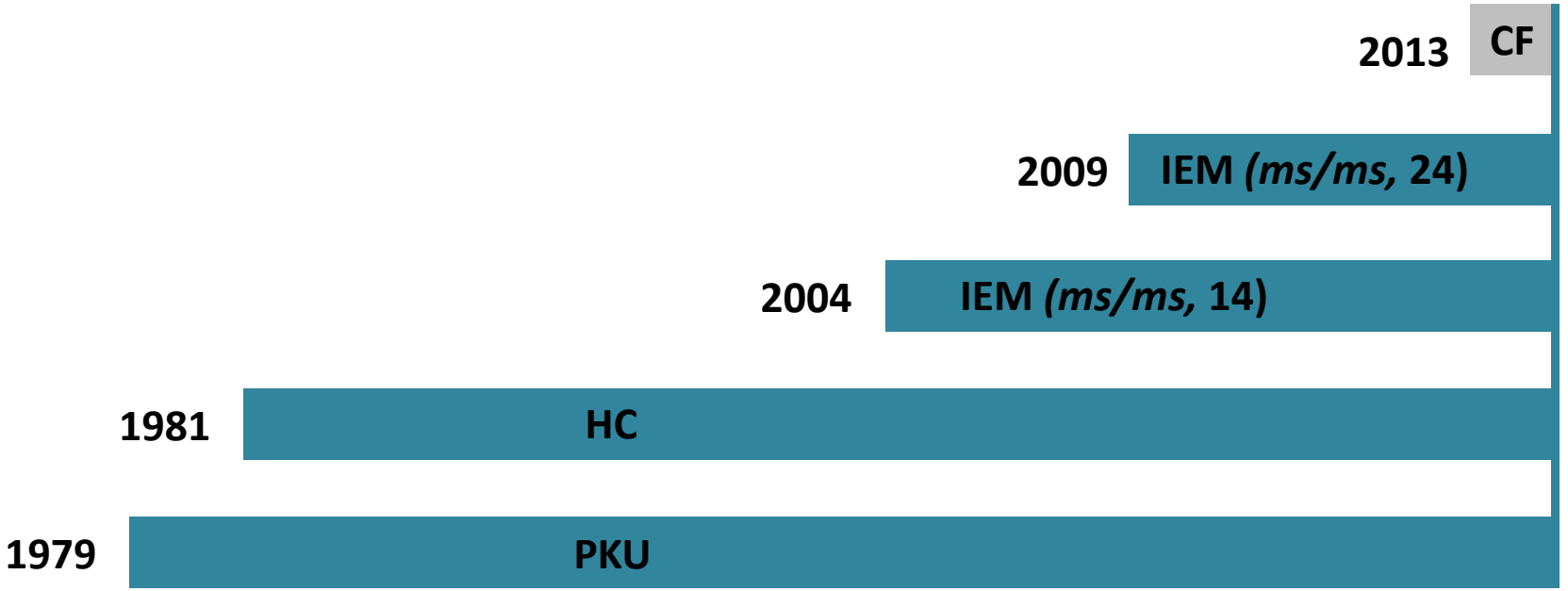
Negative population growth rate: negative natural and migration growth rates.

Portuguese National Program for Newborn Screening

2014

- Congenital hypothyroidism (HC)
- *ms/ms* (24 IEM, PKU included)
- Cystic Fibrosis (CF, pilot study)

Jacinto de Magalhães (1938-1987)



Portuguese National Program for Newborn Screening

- 1 national laboratory (400 daily samples)
- Not mandatory program with 99.8% coverage
- Sample collection at hospital or health family centers (3rd-6th day)
- Average age for treatment beginning: 10.1 days
- Results communication:
  - Normal cases – www.diagnosticoprecoce.org
  - 2nd sample request (mail)
  - Immediate sending to specialized medical center (8 centers distributed along the country, including islands)

Portuguese National Program for Newborn Screening

Total birth prevalence for all screened disorders: 1: 1.659

Newborns	Disorders	Birth prevalence
3.375.266	PKU / HPA	1: 10.459
3.343.098	HC	1: 2.986
820.433	IEM	1: 2.399

NBS by *ms/ms*: 2004 - 2014

Method

Analysis of amino acids and acylcarnitines as butyl esters (Rashed *et al.*, 1995)

(2 API 2000 triple quadrupole tandem mass spectrometers – Applied Biosystems)

2nd tier test

Succinylacetone (Tyr>210 µM, modified from *Allard et al.*, 2004)

Quality Control Programs

CDC (Quality Control and Proficiency)

ERNDIM

NEQAS UK

SIMMESN

NBS by *ms/ms*: 2004 - 2014

Confirmation of positive cases

- Plasmatic aminoacids analysis (ionic change chromatography)
- Urinary organic acids (GC-MS)
- Acylcarnitines (dry blood spots, *ms/ms*)
- Molecular analysis
- Urinary orotic acid (HPLC)
- Urinary succinylacetone (GC-MS)

NBS by *ms/ms*: 2004 - 2014

Amino acid disorders

*Phenylketonuria (PKU) / HPA
*Maple syrup urine disease (MSUD)
Tyrosinemia type I
Tyrosinemia type II
Homocystinuria (CBS deficiency)
MAT I/III deficiency

Urea cycle disorders

*Citrullinemia type I
*Argininosuccinate lyase deficiency (ASA)
Arginase deficiency (ARG 1)

Organic acid disorders

3-Methylcrotonyl-CoA carboxylase def. (3MCC)
*Isovaleric aciduria (IVA)
*Propionic aciduria (PA)
*Methylmalonic aciduria ($mut^{-/0}$ and Cbl C, D)
*Glutaric aciduria type I (GA1)
*3-Hydroxy-3-methylglutaryl CoA lyase def. (3HMG)
Malonic aciduria

Fatty acid oxidation disorders

SCHAD deficiency
*MCAD deficiency
*LCHAD deficiency
*VLCAD deficiency
MADD deficiency
*CPT I deficiency
*CPT II deficiency
Carnitine transport defect (CUD)

*Started in 2004

NBS by *ms/ms*: 2004 - 2014

Disorders	Positive screening criteria
Phenylketonuria (PKU) / Hyperphenylalaninaemia	Phe >150µM and Phe/Try >1,5
Maple syrup urine disease (MSUD)	XLeu >342µM and Val >350µM
Tyrosinemia type I	Tyr >210µM and succinylacetone (+)
Tyrosinemia type II	Tyr >850µM and succinylacetone (-)
Homocystinuria (CBS deficiency)	Met >57µM
Methionine adenosyltransferase deficiency (MAT I/III deficiency)	Met >57µM
Citrullinemia type I	Cit >200µM
Argininosuccinate lyase deficiency (argininosuccinic aciduria)	ASA >1µM
Arginase deficiency	Arg >50µM
3-Methyl crotonyl-CoA carboxylase deficiency	C5OH >1µM
Isovaleric aciduria	C5 >2µM
Propionic aciduria	C3 >5,2µM and C3/C2 >0,3
Methylmalonic aciduria (mut $^{-}$/^{0})	C3 >5,2µM and C3/C2 >0,3
Glutaric acidemia type I	C5DC (>0.2µM)
Methylmalonic aciduria (Cbl C, D)	C3 >5,2µM, C3/C2 >0,3 and C3/Met >0,4
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	C5OH >1µM and C6DC >0,07µM
Malonic aciduria	C3DC >0,4µM
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	C8 >0,3µM and C8/C10 > 2,5
Long-chain 3-OH acyl-CoA dehydrogenase deficiency (LCHAD)	C16OH >0,10µM, C18:1OH >0,07µM, C18OH >0,06µM and C16OH/C16 >0,04
Multiple acyl-CoA dehydrogenase deficiency (MADD)	Multiple elevations from C4 to C18
Carnitine transport defect (CUD)	C0 <7µM
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	C14:1 >0,46µM and C14:2 >0,17µM
Carnitine palmitoyl-transferase I deficiency (CPT I)	C0/(C16+C18) >30
Carnitine palmitoyl-transferase II deficiency (CPT II)	C0/(C16+C18)<3 and (C16+C18:1)/C2>0.5
Short-chain 3-OH acyl-CoA dehydrogenase deficiency (SCHAD)	C4OH >1,0µM

NBS by *ms/ms* – Performance metrics

Number of samples	820.433
Sensitivity	99.42%
Specificity	99.81%
Positive Predictive Value (PPV)	18%
Positive detection rate	1: 2.399
False positive rate	0.19%

- Cut-off adjustments
- New markers (metabolic ratios)

NBS by *ms/ms*: 2004 - 2014

False negative results

Methylmalonic aciduria (Cbl D)

C3= 6.1 µM (N<6.2)
Met= 15.2 µM (N>6)
C3/met=0.40 (N<0.4)
C3/C2=0.35 (N< 0.3)

Present-day cut-off: C3<5.2 µM

(p.R250X/ p.R250X)

Cut-off adjustment

CPT II deficiency

C0= 15.1µM (N: 9.1-68)
C2 = 17.2µM (N: 7.0-49)
C16= 2.26µM(N: 0.83-7.99)
C18= 1.0µM(N: 0.23-2.28)
C18:1=1.51µM(N: 0.34-3.4)
C18:2= 0.13µM(N: 0-0.8)
C0/(C16 + C18)= 4.6(N: 3-30)
(C16+C18:1)/C2= 0.22(N <0.5)

(p.S113L/ p.S113L)

Argininosuccinic aciduria

Patient 1
Cit=23µM (N<50µM)
ASA=0.90µM (N<1.1µM)

Patient 2
Cit= 24 µM (N<50)
ASA= 0.26 µM (N<1.1)

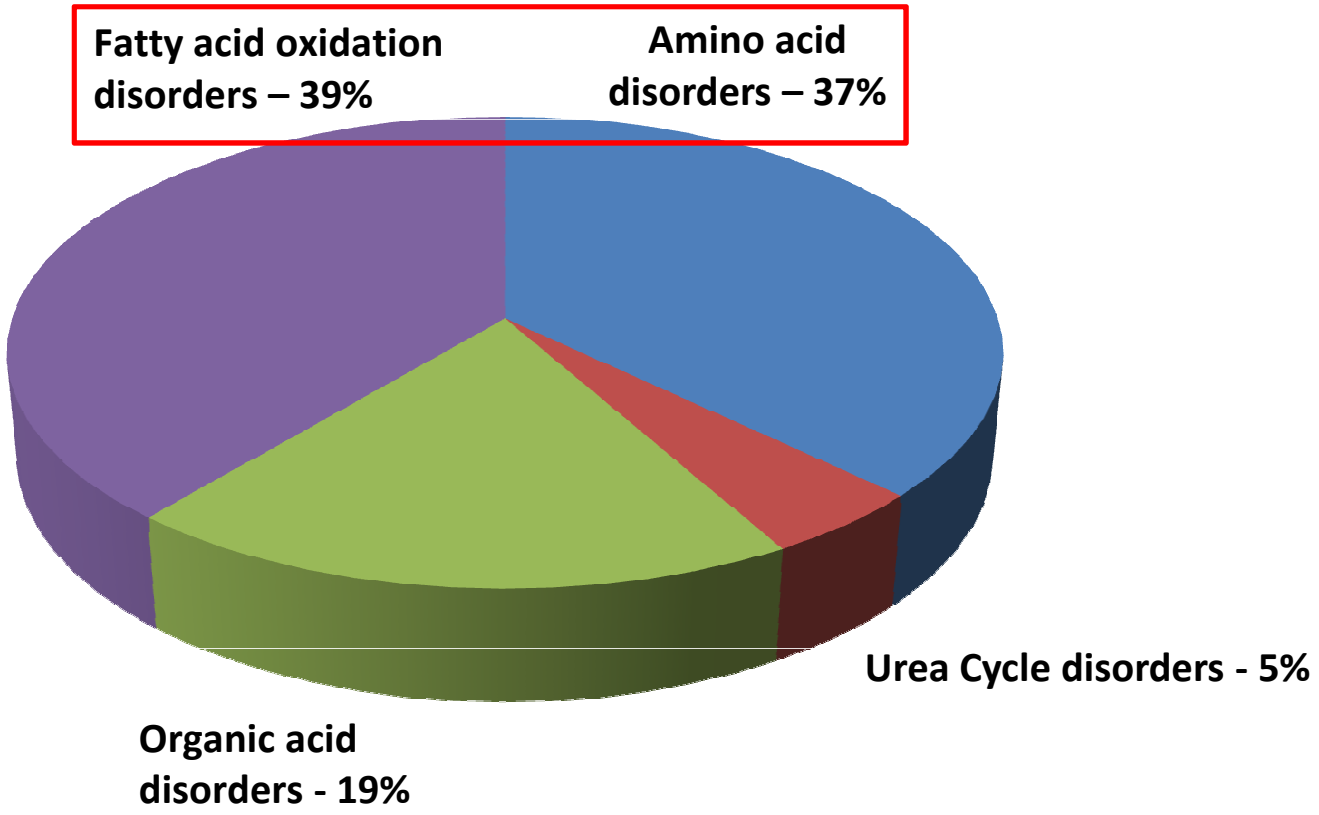
(p.R12Q/ p.R12Q)

Late-onset forms

Positive cases and birth prevalence: 2004-2014

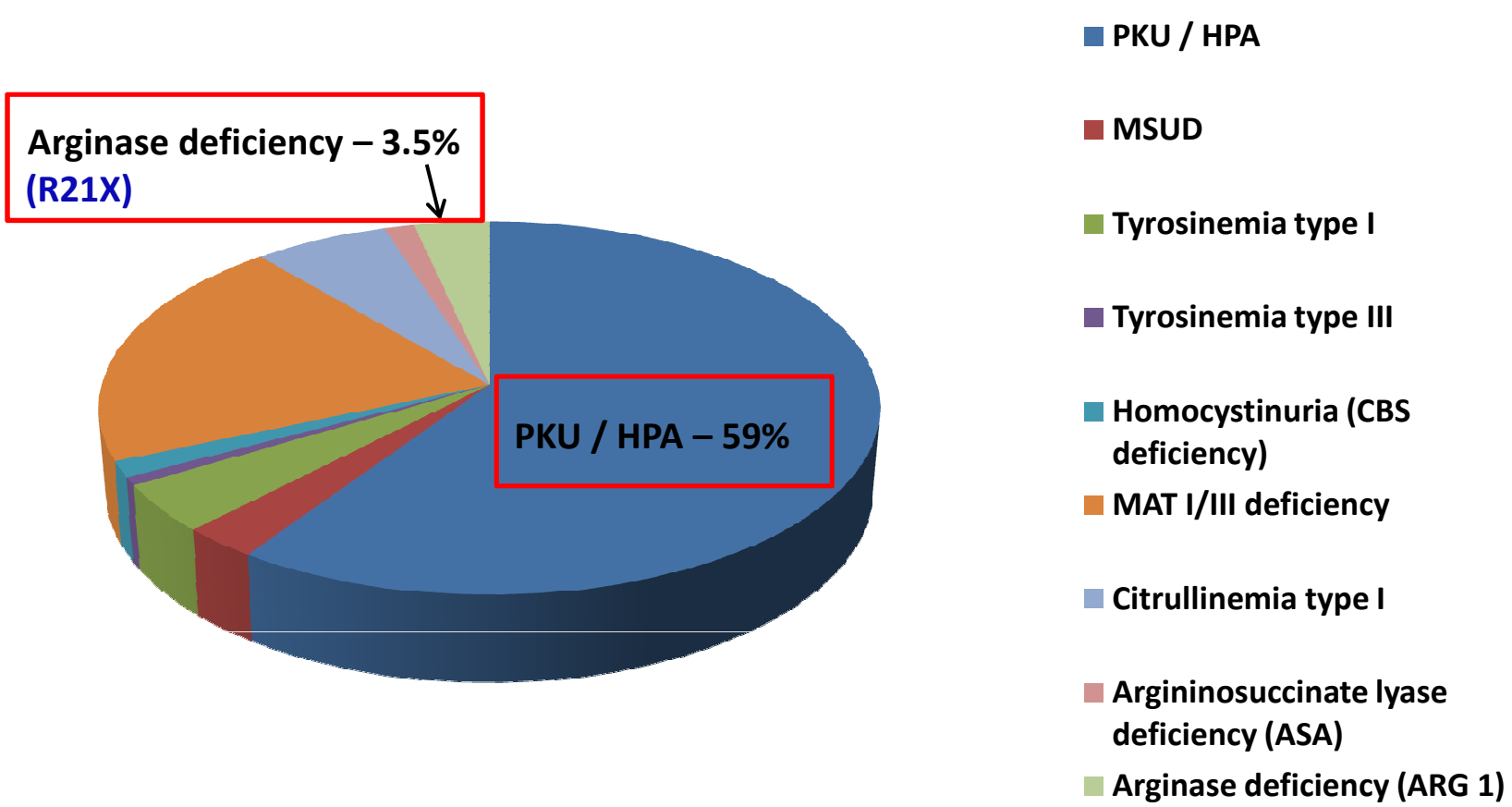
Disorder	Positive cases	Birth prevalence
Amino acid disorders	**127**	**1: 6 460**
Phenylketonuria (PKU)	65	1: 12 622
Hyperphenylalaninaemia	20	1: 41 022
Maple syrup urine disease (MSUD)	4	1: 205 108
Tyrosinemia type I	6	1: 136 739
Tyrosinemia type III	1	1: 820 433
Homocystinuria (CBS deficiency)	2	1: 410 217
Methionine adenosyltransferase deficiency (MAT I/III deficiency)	29	1: 28 291
Urea cycle disorders	**16**	**1: 51 277**
Citrullinemia type I	9	1: 91 159
Argininosuccinate lyase deficiency	2	1: 410 217
Arginase deficiency	5	1: 164 087
Organic acid disorders	**66**	**1: 12 431**
3-Methyl crotonyl-CoA carboxylase deficiency	23	1: 35 671
Isovaleric aciduria	4	1: 205 108
Holocarboxylase synthetase deficiency	2	1: 410 217
Propionic aciduria	3	1: 273 478
Methylmalonic aciduria ($mut^{-}/^{0}$)	4	1: 205 108
Glutaric aciduria type I	12	1: 68 369
Methylmalonic aciduria (Cbl C/ D)	8+1	1: 91 159
3-Hydroxy-3-methylglutaryl CoA lyase deficiency	8	1: 102 554
Malonic aciduria	1	1: 820 433
Fatty acid oxidation disorders	**133**	**1: 6 169**
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	98	1: 8 372
Long-chain 3-OH acyl-CoA dehydrogenase deficiency (LCHAD)	7	1: 117 205
Multiple acyl-CoA dehydrogenase deficiency (MADD)	5	1: 164 087
Carnitine transport defect (CUD)	8	1: 102 554
Very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD)	8	1: 102 554
Carnitine palmitoyl-transferase I deficiency (CPT I)	2	1: 410 217
Carnitine palmitoyl-transferase II deficiency (CPT II)	3	1: 273 478
Short-chain 3-OH acyl-CoA dehydrogenase deficiency (SCHAD)	2	1: 410 217
Total (screened newborns: 820 433)	**342**	**1: 2 399**

NBS by *ms/ms*: 2004 - 2014



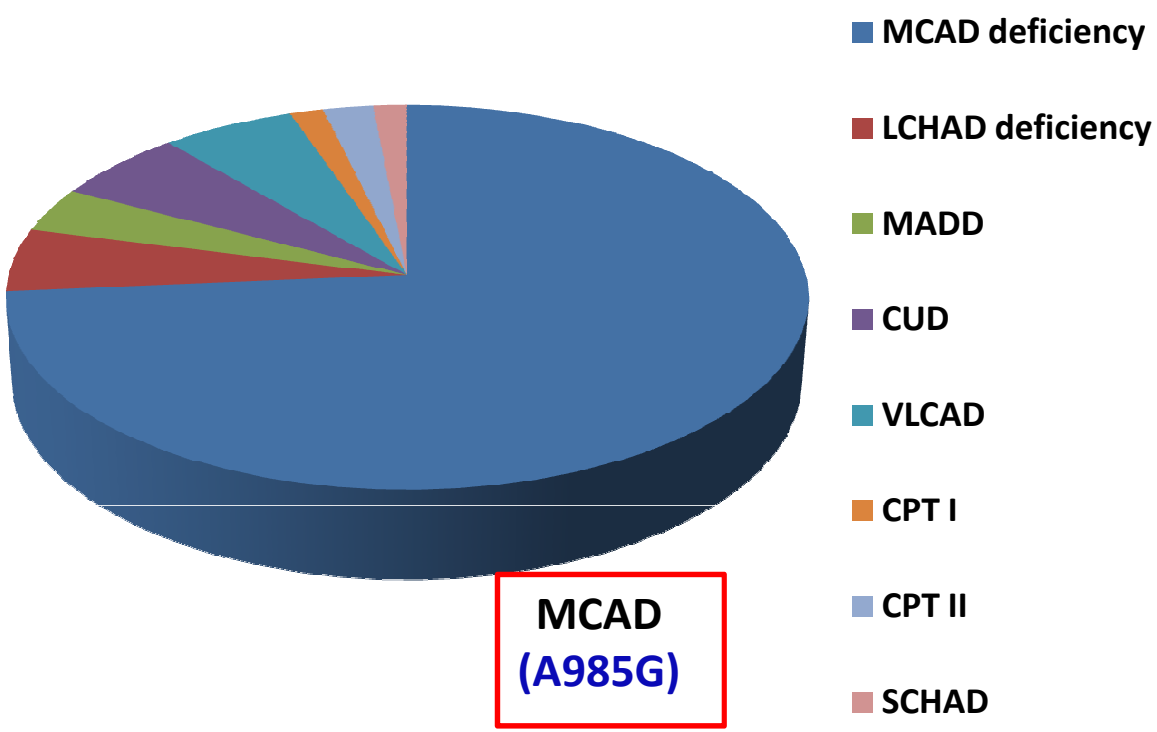
NBS by *ms/ms*: 2004 - 2014

Amino acid disorders



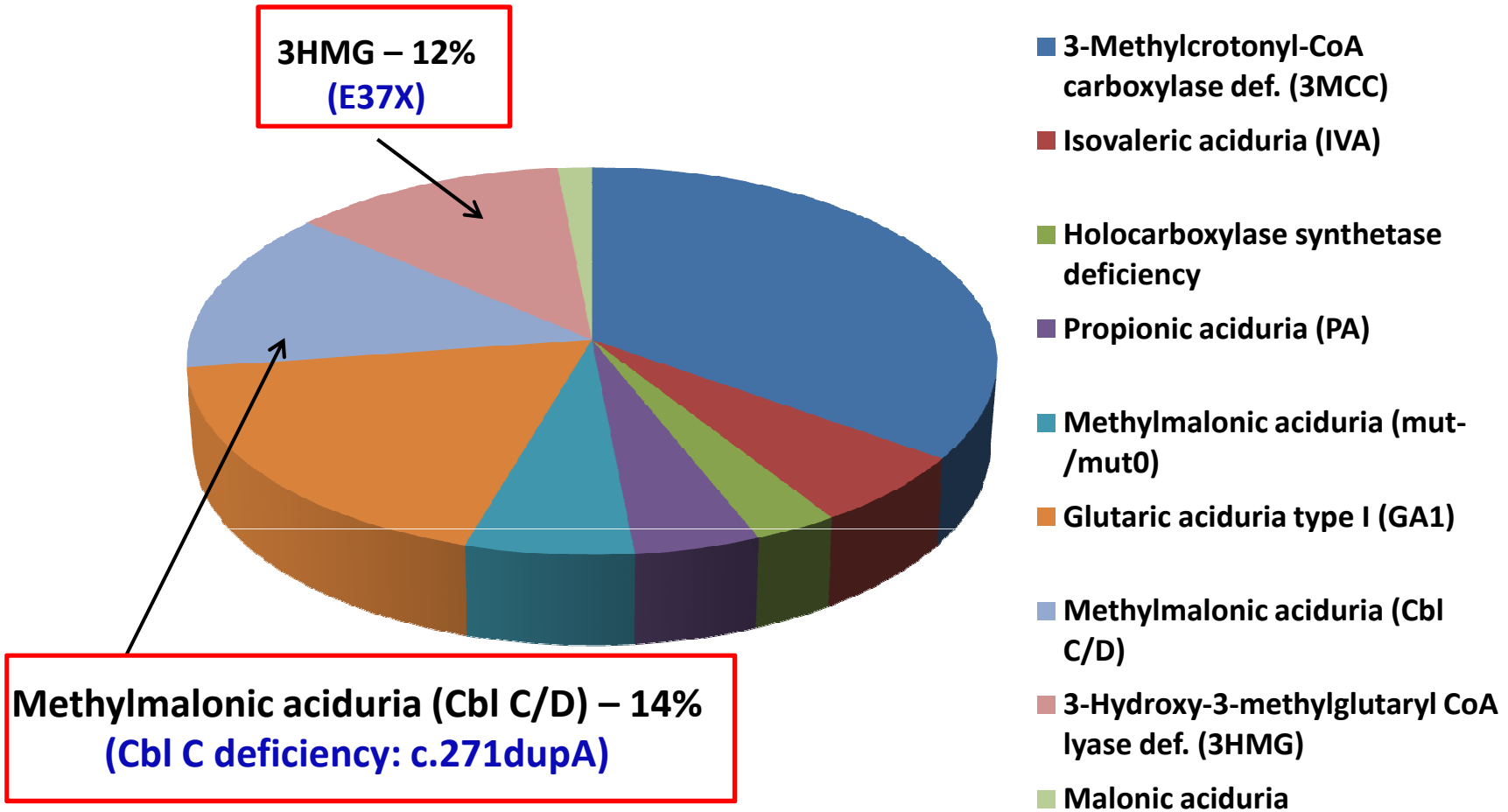
NBS by *ms/ms*: 2004 - 2014

Fatty acid oxidation disorders



NBS by *ms/ms*: 2004 - 2014

Organic acid disorders



IEM screening group (*ms/ms*)

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