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ERNDIM
Diagnostic proficiency testing
2012
Southern Europe
Lyon Centre

ANNUAL REPORT 2012

In 2012, 22 labs participated to the Proficiency Testing Scheme Southern Europe.
Organizing Centre: Dr Christine Vianey-Saban, Dr Cécile Acquaviva-Bourdain, Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, Centre de Biologie et de Pathologie Est, Lyon.

Geographical distribution of participants

Country	Number of participants
France	10
Italy	5
Spain	4
Portugal	2
Switzerland	1
TOTAL	**22**

Logistic of the scheme

- **2 surveys** 2012-1: patient A, B and C
  2012-2: patient D, E and F

Origin of patients:

- Patient A : Intermittent Maple Syrup Urine Disease – Dr Jim Bonham, Shieffield. **This sample has been sent to all labs participating to the DPT scheme in Europe**
- Patient B :Hyperammonemia, Hyperornithinemia, Homocitrullinuria (HHH) syndrome – Dr OdileRigal, Dr Jean-François Benoist, Hôpital Robert Debré, Paris
- Patient C : Mucopolysaccharidosis type I – Centre de Biologie Est, Lyon
- Patient D : “RedBulluria” – Dr Marie-Hélène Read, CHU Caen
- Patient E : CblC – Dr Encarnacio Riudor, Dr Jose Antonio Arranz, Barcelona
- Patient F : SCAD deficiency – Dr Olivier Boulat, Dr Clothilde Roux, CHUV, Lausanne. This urine sample had been distributed in 2007.

- Mailing: samples were sent by DHL at room temperature.

Timetable of the schemes

- **May 2 :** shipment of samples of Survey 1 and Survey 2 by DHL and of the clinical data by e-mail
- **May 7 :** analysis of samples of the first survey
- **May 25 :** deadline for result submission (Survey 1)
- **June 18 :** analysis of samples of the second survey
- **July 6 :** deadline for result submission (Survey 2)
- **July 30 :** report of Survey 1 by e-mail
- **August 6 :** report of Survey 2 by e-mail
- **September 4 :** meeting in Birmingham
- **December28 :** annual report with scoring sent by e-mail

Date of receipt of samples

	Survey 1 + 2
+ 24 hours	22

Date of reporting

Since 2011, the website reporting system is compulsory for all centres

Exceptionally this year, the website remained open 3 days after the deadline; therefore all labs could enter their results in time. This will not happen next year.

Recommendations = **advice for further investigation**. Advice for further investigations is scored 1 point whereas advice for treatment is not scored.

Scoring of results

The scoring system established by the International Scientific Advisory Board of ERNDIM is still the same. Three criteria are evaluated:

A	Analytical performance	Correct results of the appropriate tests	2
		Partially correct or non-standard methods	1
		Unsatisfactory of misleading	0
I	Interpretation of results	Good, diagnosis is established	2
		Helpful but incomplete	1
		Misleading / wrong diagnosis	0
R	Recommendations for further investigations	Complete	1
		Unsatisfactory of misleading	0

Since most of the laboratories in Southern Europe don't give therapeutic advices to the attending clinician, this criteria is not evaluated.

The **total score** is calculated as the sum of these 3 criteria without weighting. The maximum that can be achieved is 5 for one sample.

Meeting of participants

It took place in Birmingham on Tuesday 4September 2012 from 9.00 to 10.30, before the SSIEM Meeting.

❖ Participants

Representatives from13 labs were present: E Riudor, JA Arranz (Hospital Vall d'Hebron, Barcelona),J Garcia Villoria (Hospital Clinic, Barcelona), E Redonnet-Vernhet (Bordeaux), S Funghini (Florence),U Caruso (Genova), O Boulat, C Roux (Lausanne), B Merinero, C Perez-Cerda (Madrid), M Gastaldi (Marseille), O Rigal, A Imbard (Robert Debré, Paris), Chris Ottolenghi (Hôpital Necker, Paris), D Rodrigues (IBMC, Porto), S Bekri (Rouen), MD Boveda, D Castañeiros (Santiago de Compostella).

❖ Information from the Executive Board and the Scientific Advisory Board

- **Scoring and certificate of participation**: scoring is done by 2 scheme organizers. For the Lyon Centre, the results have been also scored by Dr Viktor Kozich from Prague Centre. Certificate of participation for 2012 will be issued for participation and it will be additionally notified whether the participant has received a performance support letter. This performance support letter is sent out if the performance is less than 60% (score < 18 / 30).
- One warning letter will be sent for 2011, and two for 2012.
- **Deputies for the Scientific Advisors**: it is one of the requirements for accreditation. The feeling of the SAB is that it would be better if each Scientific Advisor nominated a deputy from within their own organisation. Cécile Acquaviva is nominated as deputy for our scheme
- **Appeals Policy:** ERNDIM did not have a formal appeals policy or procedure. The SAB agreed that the hierarchy of appeals would be first to the scheme Scientific Advisor, then to the SAB, and finally to the Executive Committee (EC) / Board of Trustees (BoT). Sara Gartner will receive initial appeals and inform the EC that an appeal has been lodged and referred to the SAB. Sara will then inform the chair of the SAB, who will need to look at the relevant data. The chair of the SAB will then write to the chair of the EC. If the participant is not satisfied with the outcome then the BoT would need to be notified as the final responsibility for the decision lies with the BoT.
- **Reference materials** provided by SKML (mix of the four samples of the scheme) are still available, and can be ordered through the ERNDIM website. Participants are encouraged to use them as internal control, but they cannot be used as calibrants. The possibility of providing 2 levels with different concentrations is under investigation.
- **Training**: SSIEM Academy training courses.
  - A 2 days course has been organized on Tuesday and Wednesday 2 and 3O ctober in Manchester. The program for biochemists included disorders of:
    - Methionine and folic acid disorders; transulfuration and remethylation pathways (BrianFowler)
    - Techniques, pitfalls, and secondary changes in amino acid disorders (JörgenBierau)
    - Rare compounds (Christine Vianey-Saban)
  - The lectures are available on the SSIEM website
  - The next SSIEM Academy will take place in Lyon on Tuesday and Wednesday 16 and 17 April 2013 on organic acidurias and fatty acid oxidation defects. Advertising and registration are available on the SSIEM website.
- The new **ERNDIM website** is now open: www.erndim.org
- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 300 ml of urine from a patient affected with an established inborn error of metabolism or "normal" urine, together with a short clinical report. If possible, please collect 1500 ml of urine: this sample can be sent to all labs participating to one of the DPT schemes. Each urine sample must be collected from a single patient (don't send urine spiked with pathological compounds). Please don't send a pool of urines, except if urine has been collected on a short period of time from the same patient. For "normal" urine, the sample must be collected from a

symptomatic patient (don't send urine from your kids!). Annex 1 gives the list of the urine samples we already sent.

As soon as possible after collection, the urine sample must be heated at 50 ℃ for 20 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Separate 4 aliquots in 10 ml plastic tubes, add stoppers and freeze these aliquots and the rest of the urine sample in a bulk. Send the bulk and the aliquots on dry ice by rapid mail or express transport to:

Dr Christine Vianey-Saban, Dr Cécile Acquaviva,
Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, 5ième étage,
Centre de Biologie et de Pathologie Est, Groupement Hospitalier Est,
59, Boulevard Pinel, 69677 Bron cedex, France.

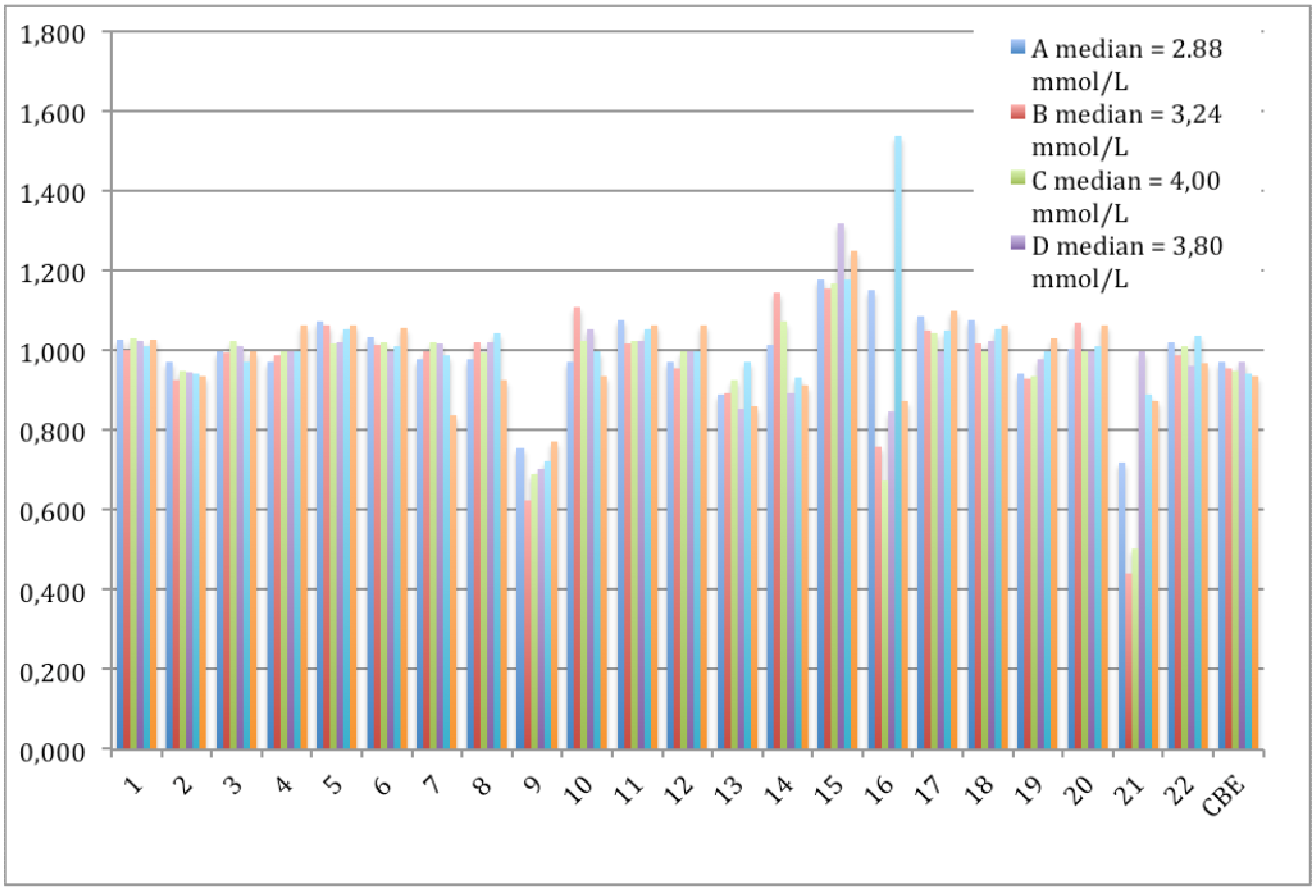
Please send us an e-mail on the day you send the samples.

- **Lab identification**: since 2007, it has been accepted that the ERNDIM number is used for "in centre" communication but anonymous identification is used for the Annual Report on the website or other purposes.

❖ Discussion of results

- **Creatinine measurement**

Creatinine determination was satisfying for most labs, except for lab 9, 13 and 21 who have systematically low values (lab 21 had the same problem last year). Lab 15 has systematically high values, whereas lab 16 has a high distribution of values.Creatinine values are expressed in the figure as the ratio of each measurement over the median from all labs.
CV is < 9.2 % for all samples (5 –9.2 %), excluding lab 9, 16 and 21, and this is higher than the interlab CV 2011 for Special Assay in urine (6.9 %, n = 113), and the interlab CV 2011 for Quantitative organic acids (6.2 %, n = 70).



Patient A – Intermittent Maple Syrup Urine disease (MSUD)

This sample has been provided by Dr Bonham from Sheffield and has been distributed to all labs in Europe.
The clinical data were "6 year old male, recurrent unexplained ataxia".
This case has been presented by Jim Bonham in the joint ERNDIM session. Details concerning this patient are available on ERNDIM website.

Diagnosis

Most likely diagnosis

Eighteen labs concluded to maple syrup urine disease (mild form, intermittent form, branched-chain 2-keto-acid dehydrogenase), one lab to dihydrolipoyl dehydrogenase (E3) deficiency. Three labs gave a wrong diagnosis (no metabolic disorder, normal profile, oligosaccharidosis).

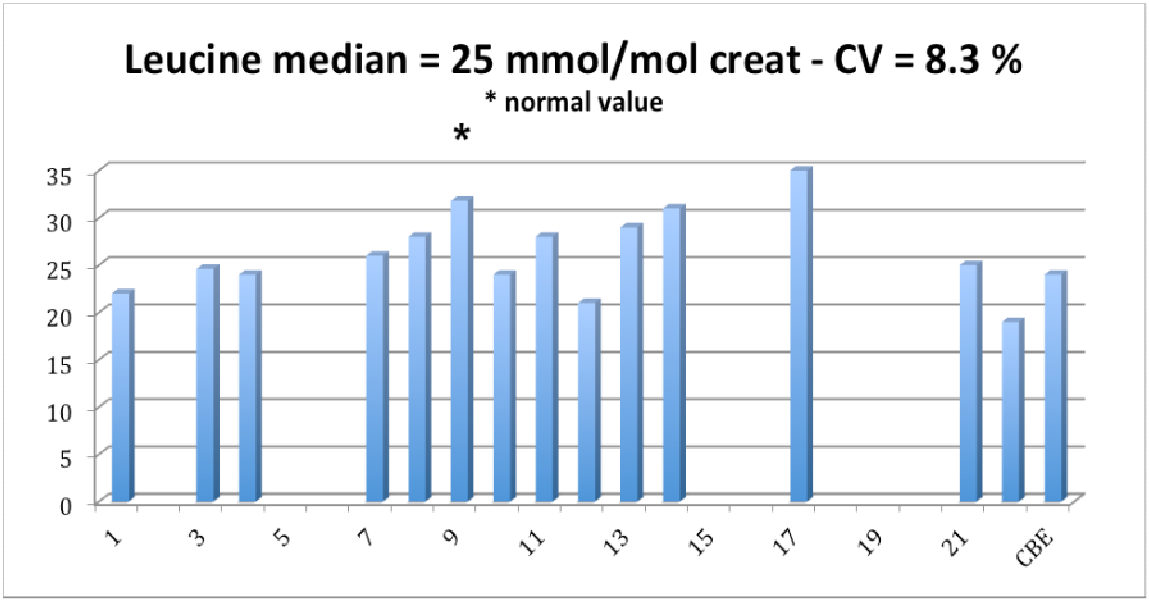
Other possible diagnosis

Dihydrolipoyl dehydrogenase (E3) deficiency was considered by 6 labs, maple syrup urine disease by one lab and thiamine deficiency by another one.

Diagnostic reliability

Score	Significance	Number of labs
1	Certain	11
2	Fairly certain	11
3	Tentative	0
-1	To be entered	0
-2	Not performed	0

All labs performed **aminoacid** analysis, and all labs but one reported an increase of branched-chain amino acids: increase of leucine (13 labs), abnormal presence of alloisoleucine (12 labs), increase of isoleucine (9 labs), and increase of valine (4 labs).



All labs also performed **organic acid** analysis, and all of them reported an increase of branched-chain 2-hydroxy or 2-ketoacids: 2-hydroxyisovaleric acid (21 labs), 2-hydroxy-3-methylvaleric acid (10 labs), 2-hydroxyisocaproic acid (9 labs), 2-ketoisocaproic acid (5 labs), and 2-keto-3-methylvaleric (3 labs). An increase of 4-hydroxyphenyllactic acid (liver disease?) was also reported (11 labs). But lactic acid excretion was normal excluding *a priori* E3 deficiency.

Recommendations was OK for the labs who reached a correct diagnosis

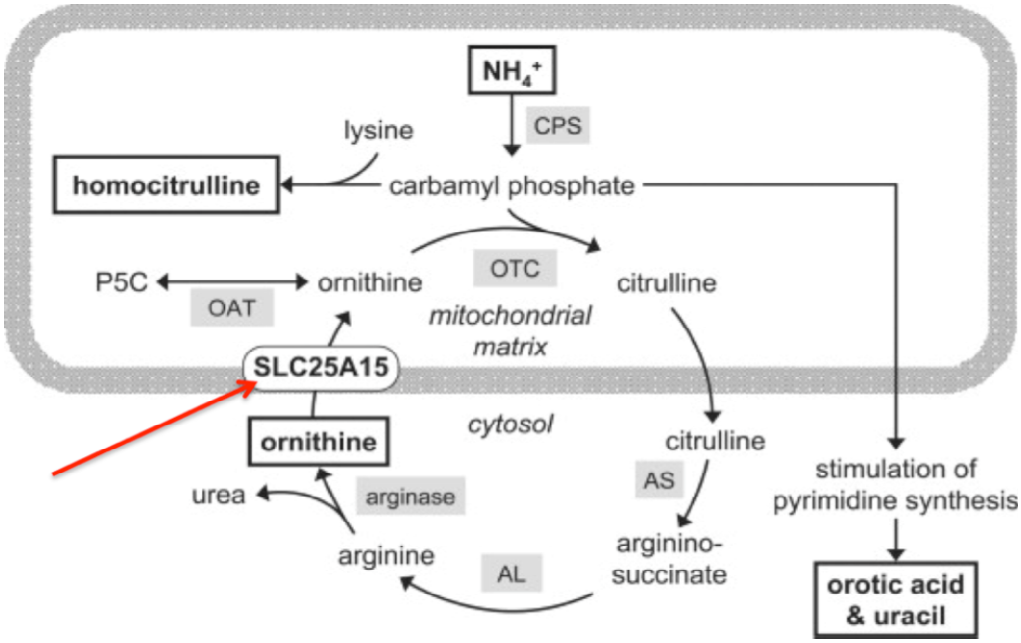
Scoring

- Analytical: increase of leucine and/or presence of alloisoleucine (score 1), increase of branched-chain 2-hydroxy and/or 2-ketoacids (score 1)
- Interpretation of results: MSUD (score 2), E3 deficiency (score 1)
- Recommendations: plasma amino acids or mutation analysis of BCKDH genes or enzyme assay of BCKDH complex (score 1).

- **Patient B – HyperammonemiaHyperornithinemiaHomocitrullinuria (HHH) syndrome**

The patient, a 12.5 years old girl, is born from consanguineous parents (first cousins), after a normal twin pregnancy (monochorial, bi-amniotic). She was hospitalised at 5 days of life because of drowsiness, axial hypotonia with peripheral hypertonicity and feeding difficulties, leading to coma with poor reactivity. Ammonia was 697 µmol/L, pH = 7.43, and lactate = 3.2 mmol/L. She was transferred to neonatal intensive care unit. She was intubated, received assisted ventilation, and hemofiltration during 16 hours. Phenylbutyrate *per os* 250 mg/kg/d was added. Metabolic investigation performed at that time was in agreement with HHH syndrome, with high plasma glutamine and ornithine levels (2832 µmol/L and 509 µmol/L, respectively), contrasting with low citrulline and arginine levels (10 µmol/L and 44 µmol/L, respectively). In urine, an increase of glutamine, ornithine, homocitrulline and orotic acid was observed. Ammonaemia normalized within 24h (142 µmol/L) but the child remained comatous and presented seizures. A protein restricted diet, supplemented with arginine was started. Axial hypotonia persisted but she had a good contact. She was discharged at 17 days of life with treatment. Since she was born from a twin pregnancy, her twin sister has been investigated and she was found to be also affected with HHH syndrome… At 9 years of age, this patient cannot walk without help; she has pyramidal syndrome, and psychomotor retardation. At 12.5 years of age, she had orthopaedic surgery, which ameliorates her walk. The urine sample has been collected at that time, under treatment.

HHH is due to the defect in the transporter SLC25A15, which allows the transport of ornithine through the mitochondrial membrane (the figure is from Korman et al. J Neurol Sci 2004;15:53).



Diagnosis

Most likely diagnosis

Only 10 labs concluded to HHH syndrome, all other concluding to either OTC deficiency or urea cycle disorder. Only one lab obtained no conclusive results.

Other possible diagnosis

One lab evoked a possible HHH syndrome, while 4 others suggested a urea cycle disorder, most likely OTC deficiency.

Diagnostic reliability

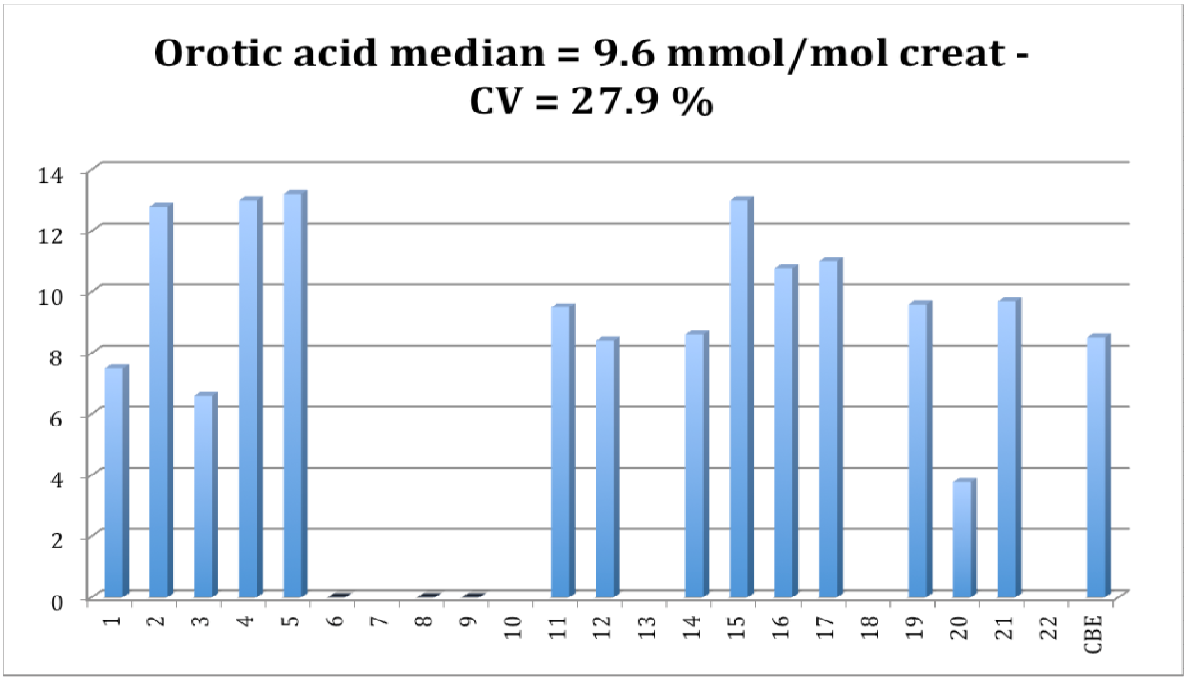
Score	Significance	Number of labs
1	Certain	12
2	Fairly certain	8
3	Tentative	2
-1	To be entered	0
-2	Not performed	0

All labs but one performed **amino acid** analysis: an increase of homocitrulline was reported by only 10 of them, although the median concentration was 35 mmol/mol creatinine. Because of the age of the patient (12.5 years), an exogenous origin for homocitrulline from milk consumption could be excluded. A slight increase of ornithine was reported by 8 labs (median = 9 mmol/mol creatine).

A questionnaire has been sent to all labs to investigate where homocitrulline is eluted in the different systems. Results from participants where:

- Ion exchange chromatography with ninhydrine detection
    - Jeol analyzer: Hcit is eluted just before (n=3) or with methionine (search for an abnormal 570/440 ratio, n=2)
    - Biochrom: almost same retention time than Met (n=4), possibility to use a special program, and using this technique Hcit is eluted before Met (http://www.biochrom.co.uk/content/1/57/application-notes.htlm, application Note 30.3)
    - Hitachi: eluted approximately 2 minutes after Met (n=1)
- UPLC MassTrak amino acid analysis (Waters): can co-elute with GABA (n=1)
- Tandem mass spectrometry: specific transition (m/z = 190>173)(n=1 and the scheme organizer)

All labs performed **organic acids**, and all but 2 reported an increase of hippuric, 17 an increase of uracile, and 13 an increase of orotic acid. Fourteen labs also performed measurement of orotic acid by another method (HPLC, tandem MS, …).



* lab 20: reported as a normal excretion

Recommendations were OK for those who reached a correct diagnosis.

Scoring

- Analytical performance: increase of homocitrulline and/or ornithine (score 1), **i**ncrease of orotic acid (score 1)
- Interpretation of results: HHH syndrome (score 2), urea cycle disorder (score 1)
- Recommendations: plasma amino acids or mutation analysis of *SLC25A15*gene or ornithine incorporation in vitro (score 1)

- **Patient C – Mucopolysaccharidosis type I (Hurler/Scheie disease)**

The patient, an 8-year old male, is born from consanguineous parents (first cousins), living in Marocco. He was investigated for the first time at 7 years of age thanks to a charitable organization. At examination, a large umbilicus hernia (present from 1 year of age), joint stiffness, facial dysmorphia evocative of mucopolysaccharidosis and failure to thrive (size – 4 SD, weight -3 SD) were noticed. Radiography of bones revealed abnormal vertebra and ribs. But he had a normal intellectual development. At diagnosis, DMB was 69.6 mg/mmol creat (controls: 3.0-11.6) and harminewas 31.7 mg/mmolcreat (controls: 3.0-16.0). Electrophoresis of mucopolysaccharides showed a very high increase of dermatan sulfate, and to a lower extent of heparin sulfate. Alpha-L-iduronidaseactivity in leukocytes was undetectable (0 µkat/Kg; controls: 3.4-10.6, simultaneous control = 9.0), as well as in serum, contrasting with a normal total hexosaminidase activity (698 µkat/Kg in leukocytes ; controls: 240-780 - 742 µkat/Kg in serum ; controls: 148-337). Mutation analysis of *IDUA* gene showed homozygocity for the p.Pro533Arg mutation (c.1598C>G) already described in MPS I patients. The patient underwent surgical treatment of his hernia, adenoidectomy, tonsillectomy, and transtympanics drain pipes, with a good outcome. Enzyme replacement therapy was also started.

Diagnosis

Most likely diagnosis

Nineteen labs concluded to mucopolysaccharidosis type I or II, and 3 labs to mucopolysaccharidosis or to another type of mucopolysaccharidosis.

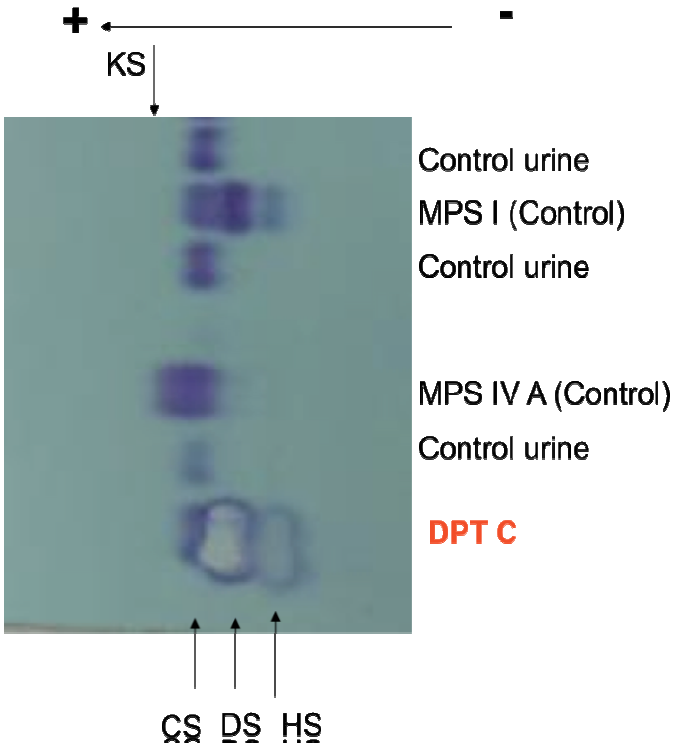
Other possible diagnosis

Height labs gave mucopolysaccharidosis type II (or VII), one lab mucopolysaccharidosis type I (or at least VII), and one lab mucopolysaccharidosis type IV as an alternative diagnosis.

Diagnostic reliability

Score	Significance	Number of labs
1	Certain	9
2	Fairly certain	10
3	Tentative	3
-1	To be entered	0
-2	Not performed	0

All labs but two performed quantification of mucopolysaccharides and reported a gross increase of GAGs. The eighteen labs who performed GAG identification reported an increase of dermatan sulfate and heparin sulfate. The figure below illustrates the abnormal electrophoretic pattern with an increase of dermatan sulfate and heparan sulfate, compared to controls and to other types of mucopolysaccharidosis.



Twelve labs performed **oligosaccharides** analysis and 9 of them reported an abnormal profile, frequent in mucopolysaccharidoses, and 3 of them a normal profile.

Recommendations were OK for those who reached a correct diagnosis.

Scoring

- Analytical performance: increase of glycosaminoglycans (score 1), increase of dermatan and heparin sulfate(score 1).
- Interpretation of results: mucopolysaccharidosis type I or II (score 2), mucopolysaccharidosis (score 1), wrong mucopolysaccharidosis (score 0).
- Recommendations: enzyme assay of α-L-iduronidase and iduronate-2-sulfatase or mutation analysis *IDUA* or *IDS* gene, or repeat MPS on a new urine sample (score 1)

- **Patient D – « RedBulluria »**

The clinical data were: “Young adult patient. He presented late in the evening a malaise. The urine sample has been taken at admission to the emergency ward”. In fact, the urine sample was obtained from a young student (« interne ») of Dr Read in Caen who accepted to drink 2 cans of RedBull. His urine has been collected few hours after the intake of RedBull. Fortunately, he did not present any adverse symptoms but such event has been reported. The content of one can of RedBull is 1000 mg taurine, 600 mg D-glucuronolactone, and 70 mg caffeine. The intake of taurine from consumption of these taurine-containing "energy" drinks is several times higher than that from the rest of the diet. There is a lack of scientific evidence to support the safety of taurine present in beverages at such concentrations.

Diagnosis

Most likely diagnosis

Twenty labs concluded that the patient does not have a metabolic disorder, and 12 of them speculated that the increase of taurine is probably of exogenous origin. One lab did not conclude and only one lab gave a wrong diagnosis.

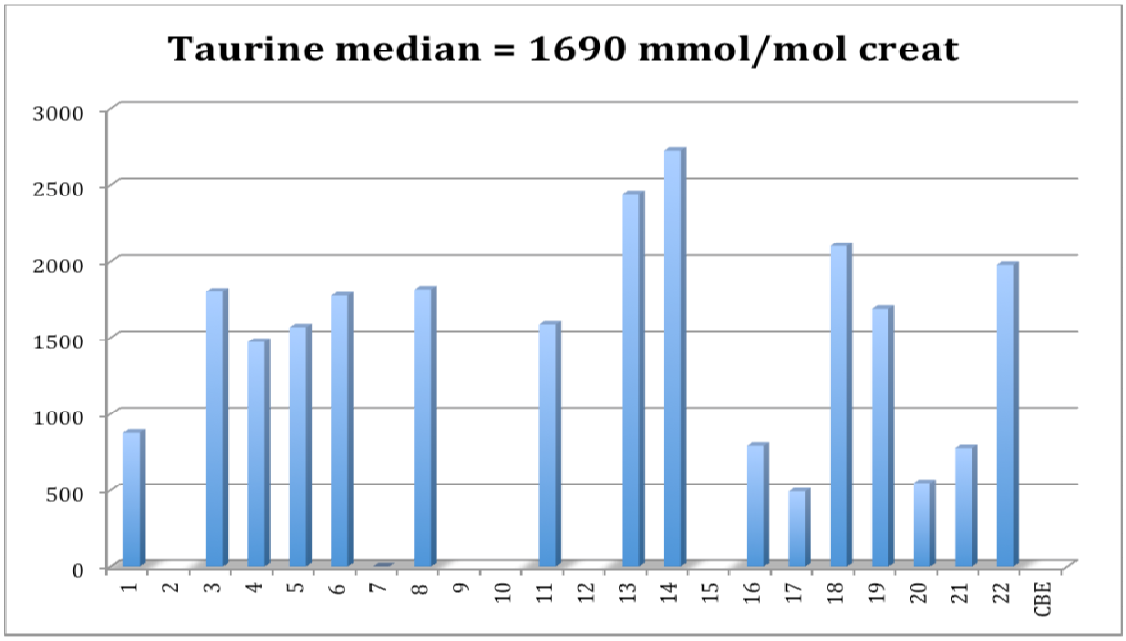
Other possible diagnosis

A mild form of molybdenum cofactor deficiency or of sulphite oxidase deficiency was proposed as an alternative diagnosis by two labs, and a most probable exogenous origin for increased taurine excretion was proposed by another one.

Diagnostic reliability

Score	Significance	Number of labs
1	Certain	8
2	Fairly certain	7
3	Tentative	6
-1	To be entered	1
-2	Not performed	0

All labs performed **amino acid** analysis and 17 of them reported an increase of taurine (figure below: median for taurine = 1690 mmol/mol of creatinine – CV = 39%). It has to be noticed that measurement of amino acids by tandem mass spectrometry does not allow a reliable measurement of taurine in positive mode. Four labs specified that sulphocystein was undetectable.



Interestingly, one lab reported an increase of β-alanine, β-aminoisobutyric acid, and γ-aminobutyric acid (30, 20, and 11mmol/mol creat, respectively). By tandem MS, the scheme organizer also found

an increase of β-alanine (19 mmol/mol creatinine), but not of β-aminoisobutyric acid and GABA (19 and <5 mmol/mol creat, respectively). This is probably due to the high taurine excretion, and the competitive inhibition of a renal transport system for β-aminoacids, probably due to their structural analogy, leading to increased excretion ofβ-alanine, and β-aminoisobutyric acid (and γ-aminobutyric acid?): Scriver et al.,N Engl J Med 1966;274:635-643.

Taurine (2-aminoethanesulfonic acid)

$H_2N\text{-}CH_2\text{-}CH_2\text{-}SO_3H$

β-alanine (3-aminopropionic acid)

$H_2N\text{-}CH_2\text{-}CH_2\text{-}COOH$

BAIBA (D-3-aminoisobutyric acid)

$H_2N\text{-}CH_2\text{-}CH_2\text{-}COOH$

CH_3

GABA (4-aminobutyric acid)

$H_2N\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}COOH$

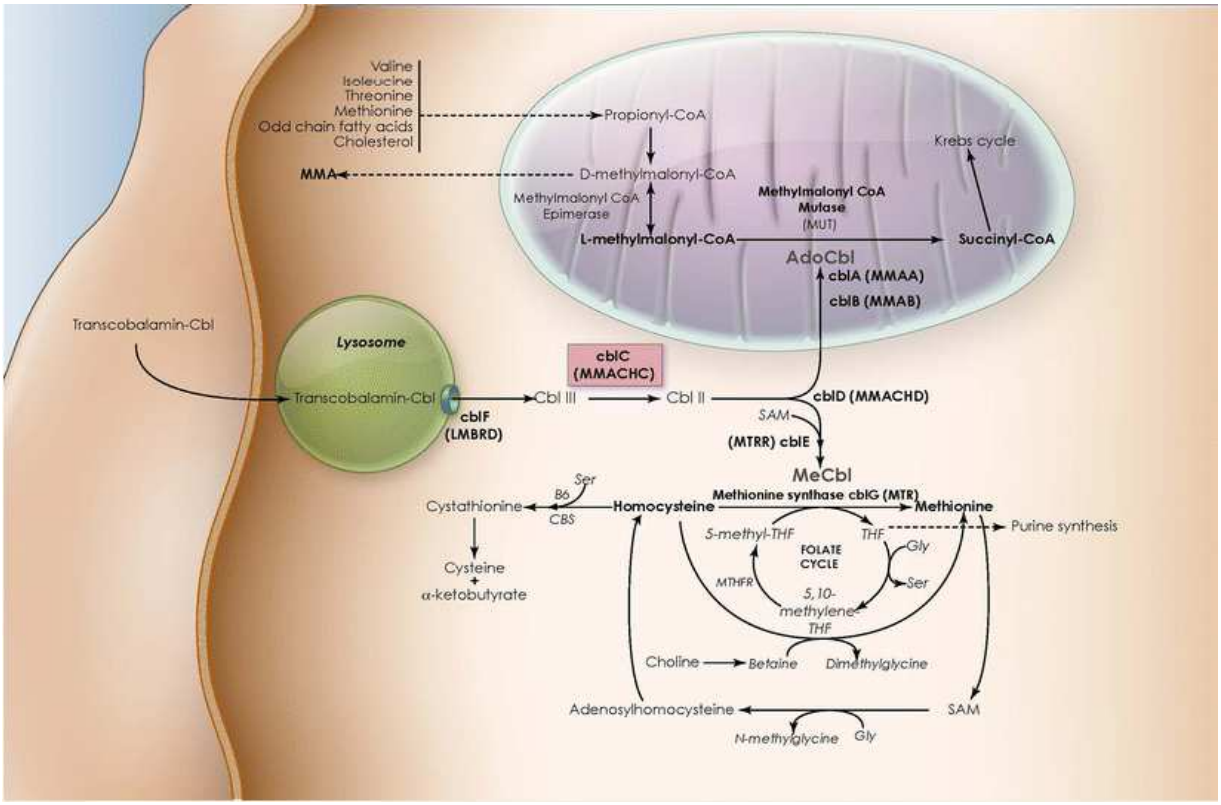
All labs also performed **organic acid** analysis, and did not report any significant abnormality, except an increase of furoyl derivatives (2 labs) and caffeine (1 lab), probably due to RedBull consumption. Two labs also specified that there was no marker of 4-hydroxybutyrate (GBH) abuse.

Scoring

- Analytical: increase of taurine (score 1), no abnormality with performed investigation (score 1)
- Interpretation of results: no metabolic disorder (score 2),
- Recommendations: avoid drinks with taurine, or plasma amino acids or re-analyze a new urine sample, or pharmacotoxical tests, … (score 1)

- **Patient E – CblC defect**

The patient, a girl, is born from non consanguineous parents. At 16 days of life, abnormal ocular movements, nystagmus, retinitis, cerebral atrophy and failure to thrive were noticed. At 5 months, she presented a haemolytic uraemic syndrome, and she was hospitalized at that time. Metabolic studies showed an increase of methylmalonate in urine (740 mmol/mol creat) and plasma (203.5 µmol/L), a high total homocysteine (172 µmol/L) and glycine level in plasma (572 µmol/L). Methylcitrate excretion was 11.3 mmol/mol creatinine. Genetic studies confirmed a CblC form with a homozygous mutation. The urine sample has been collected at 26 months of age, under treatment.



According to Carrillo-Carrasco et al, J InherMetab Dis 2012;35:91

The MMACHC protein, which is defective in CblC, acts as a "trafficking chaperone" forcobalamins. MMACHC may interact with the lysosomal efflux transporter of cobalamin, *LMBRD1*, to accept a variety of cobalamin derivatives for passage into the cytoplasm. Depending on the type of cobalamin derivative encountered, MMACHC catalyzes a series of versatile reactions to produce suitable intermediates for coenzyme synthesis; it can catalyze a reductive decyanation reaction when encountering CNCbl to yield cob(II)alamin and a dealkylation reaction when encountering AdoCbl or MeCbl using glutathione as an electron donor. MMACHC is proposed to interact with MMADHC, a protein that sorts the cobalamin intermediates to the cytosol or the mitochondria for production of AdoCbl and MeCbl. Additional roles in cobalamin transport and metabolism are suggested by the recent demonstration that MMACHC can be found in the mitochondria (Carrillo-Carrasco et al, 2012).

Diagnosis

Most likely diagnosis

Eighteen labs concluded to CblC defect (or eventually CblD or CblF defect, or methylmalonic aciduria with homocystinuria), whereas 4 labs concluded to isolated methylmalonic aciduria.

Other possible diagnosis

CblD or CblF defect was proposed by 4 labs, all forms of methylmalonic aciduria by 3 others, cobalamin deficiency by 2 labs, and a disorder of absorption or transport of cobalamin by one lab.

Diagnostic reliability

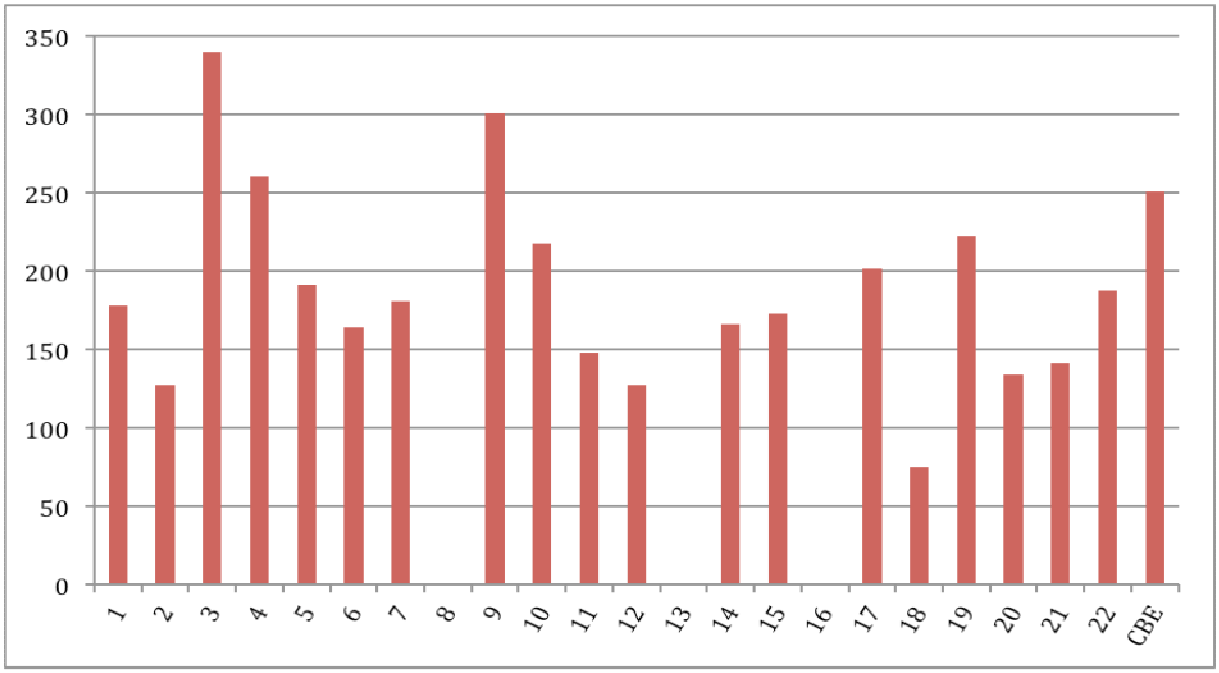
Score	Significance	Number of labs
1	Certain	11
2	Fairly certain	10
3	Tentative	1
-1	To be entered	0
-2	Not performed	0

All labs performed **amino acids**, but only 10 reported an increase of homocystine (0.6 – 4 mmol/mol creatinine), and 9 an increase of glycine. Interestingly, 2 labs reported an increase of cysteine-homocysteine mixt disulfide. It is a helpful marker in treated patients or when a sample has been poorly stored, and levels of homocystine low. We sent a questionnaire to all participants to ask information about cysteine-homocysteine mixt disulfide elution in the different systems:

- Using ion exchange chromatography, with nihydrine detection cysteine-homocysteine mixt disulfide is eluted:
  - Jeol: just after but very close to tyrosine (n=3) or with Tyr (n=1)
  - Biochrom: same retention time than cystathionine (n=1), just before Tyr (n=2)
  - Hitachi: between argininosuccinate and beta-alanine (n=1)
- UPLC Mass Trak amino acid analysis (Waters): elutes as 2 peaks, near Norvaline the internal standard (n=1)
- Liquid chromatography-tandem mass spectrometry: specific transition (*m/z* 255>134) (scheme organizer)

Also very interestingly, 3 labs reported an increase of cystathionine (7.8; 9; 15 mmol/mol creat), which is a helpful marker for remethylation defects.
Six labs specified that homocystine excretion was normal, and one that cysteine-homocysteine mixt disulfide excretion was normal. Five labs performed measurement of total homocysteine in urine (which is an unusual test) and identified an increased excretion: 6.3 – 26.9 mmol/mol creatinine – controls:?).

All labs also performed **organic acid analysis** and all reported an increase of methylmalonic acid (figure below: median = 179.5 mmol/mol creatinine – CV = 35%).



Sixteen labs reported an increase of methylcitrate (12 ; 38 ; 109 ; 136.7 ; 143 mmol/mol creat). By stable isotope dilution the scheme organizer obtained a value of 62 mmol/mol creat. A standard for methylcitrate and its stable isotope are available from C/D/N Isotopes ref X-4176 and D-4162, respectively (Interchimwww.interchim.com). Two labs also reported an increase of tiglylglycine, and 2 labs an increase of 3-hydroxypropionic acid. A significant increase of N-acetyltyrosine was also present (without 4-OH-phenyl derivatives), probably related to parenteral formulae (Pedro Ruiz, Begoña Merinero: personal communication, and Chapter C in Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases, Blau, Duran et al, 2nd edition).

Urinary acylcarnitine analysis was performed by 7 labs: an increase of propionylcarnitine (C3 = 5.9 – 20 mmol/mol creat), methylmalonylcarnitine (C4DC = 5.5 – 28.3 mmol/mol), and free carnitine was observed by 6, 4 and 2 labs, respectively.

Recommendations were most often satisfying.

Scoring

- Analytical: increase of homocyst(e)ine or cysteine-homocysteine mixt disulfide (score 1), increase of methylmalonic acid (score 1)
- Interpretation of results: Cbl C (CbD, or CblF) defect, methylmalonic aciduria with homocystinuria(score 2), methylmalonic aciduria (score 1)
- Recommendations: plasma amino acids, total homocysteine, or acylcarnitines, or complementation studies, or propionate incorporation in fibroblasts, or mutation analysis *MMACHC* gene (score 1)

- **Patient F – SCAD (short-chain acyl-CoA dehydrogenase) deficiency**

Patient F is a 7-year-old girl. No information about her family was available: she was an adopted girl originating from India. She was already followed for severe dilated cardiomyopathy of unknown origin. She was hospitalized in intensive care unit, with severe cardiac decompensation in the course of a viral infection. Plasma acylcarnitine profile revealed a high increase of butyrylcarnitine (C4) = 4.2 µmol/L (controls <0.8). Mutation analysis of *ACADS* gene showed she was homozygous for both variations c.625G>A and c.730G>A. However a large deletion of the gene cannot be excluded. The question of a cardiac transplantation was raised but could not be performed because of worsening of the cardiac function, despite symptomatic treatment. The child died from cardiac failure with secondary severe liver dysfunction. It is not clear whether cardiomyopathy was due to SCAD deficiency, but biochemical and molecular investigation were indicative of SCAD deficiency. This urine sample has already been distributed in 2007 (patient 6).

Diagnosis

Most likely diagnosis

Fifteen labs (sixteen in 2007) concluded to SCAD deficiency as first diagnosis, 6 labs (4 in 2007) concluded to multiple acyl-CoA dehydrogenase (MAD) deficiency or ethylmalonic aciduria, and one lab to mitochondrial respiratory chain deficiency as first diagnosis.

Other possible diagnosis

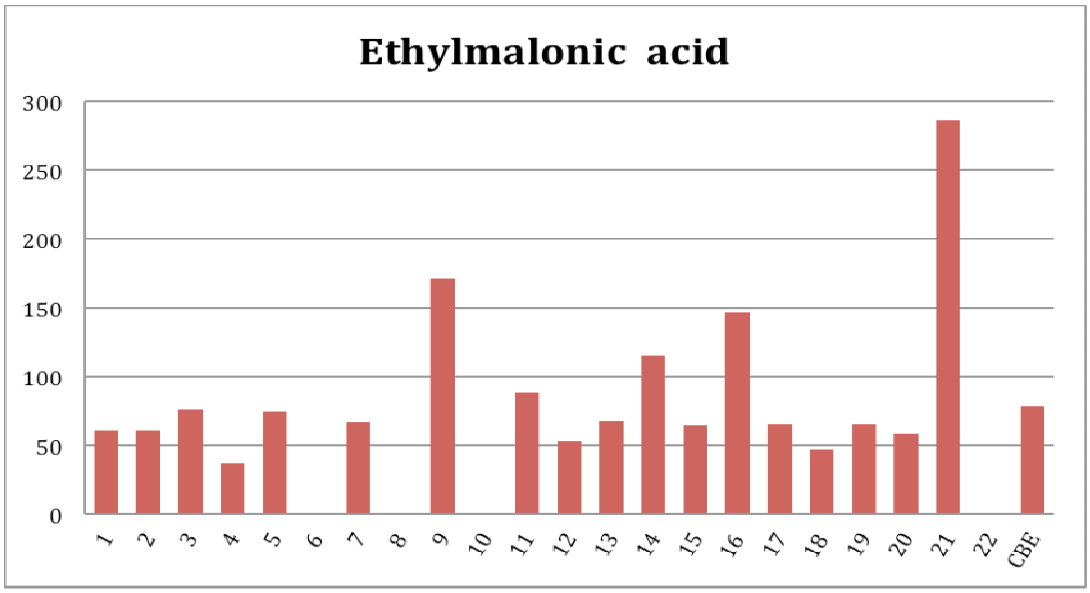
The other possible diagnoses were MAD (5 labs), SCAD deficiency (2 labs), mitochondrial respiratory chain deficiency (2 labs), riboflavin transporter defect (1lab), ethylmalonic aciduria (1 lab), ETHE (1 lab), ethylmalonyl-CoA decarboxylase deficiency (1 lab).

Diagnostic reliability

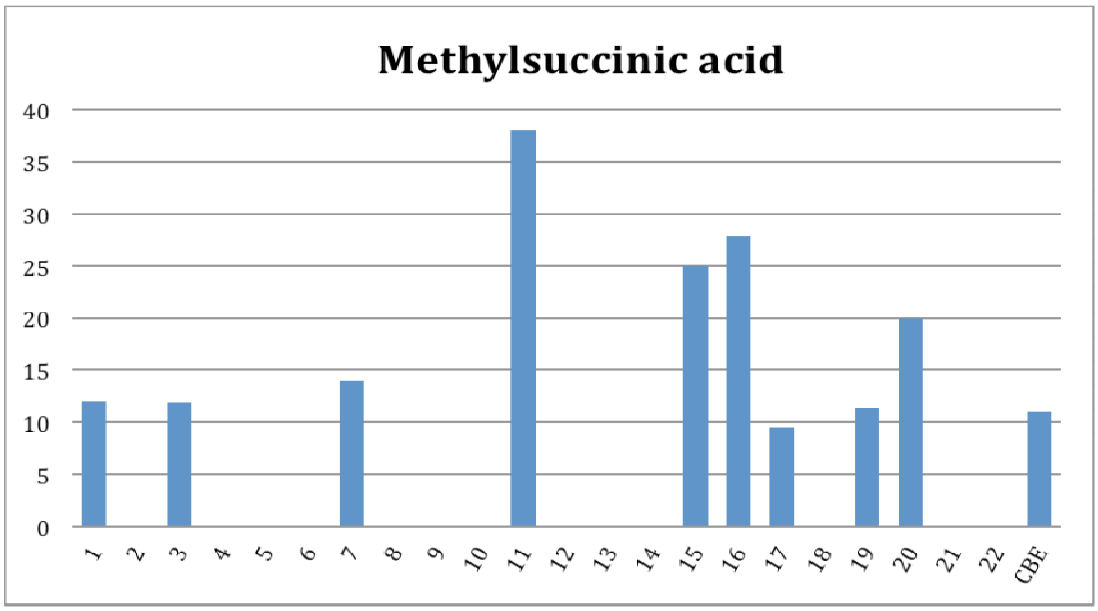
Score	Significance	Number of labs
1	Certain	6
2	Fairly certain	8
3	Tentative	8
-1	To be entered	0
-2	Not performed	0

All labs performed **organic acid analysis**, and all reported an increase of

- Ethylmalonic acid: median = 66 mmol/mol creatinine – CV = 56% (in 2007: median = 58 mmol/mol creatinine – CV = 43%)



- Methylsuccinic acid: median = 14 mmol/mol creatinine – CV = 58% (* lab 11 excluded) (in 2007: median = 15 mmol/mol creatinine – CV = 46%)



- Traces of butyrylglycine: 2 labs

Conversely some labs specified that excretion of acylglycine derivatives (4 labs), glutaric acid (4 labs), 2-hydroxyglutaric acid (3 labs) was normal or undetectable. Metabolites of aspirine were also reported (6 labs).

Nine labs performed **acylcarnitine** analysis: 7 of them reported an increase of butyrylcarnitine (C4 = 0.6 ; 3.9 ; 4 mmol/mol creat – scheme organizer : 3.9), whereas 2 labs reported it as normal (2.15 ; 3.1 mmol/mol creat).

This sample was somewhat misleading, first because the clinical presentation oriented more towards a respiratory chain defect or a fatty acid oxidation defect other than SCAD deficiency, and second because an increase of ethylmalonic acid is not specific for SCAD deficiency. However, an increase of methylsuccinic acid excretion higher than10 mmol/mol creatinine, associated to an increase of ethylmalonic acid, should orientate to SCAD deficiency and therefore a plasma/blood acylcarnitine profile should be recommended.

Recommendations were OK. In our experience, measurement of SCAD activity in fibroblasts can be unreliable, especially in patients with c.625G>A and/or c.730G>A variations. The residual enzyme activity probably depends of cell culture conditions. Measurement of SCAD activity in muscle tissue is more informative, but is too invasive. We would recommend first to perform mutation analysis of *ACADS* gene, and second eventually to measure SCAD activity.

Scoring

- Analytical: increase of ethylmalonic acid (score 1), increase of methylsuccinic acid (score 1)

- Interpretation: SCAD deficiency as first or alternative diagnosis (score 2), ethylmalonic aciduria, MAD deficiency, mitochondrial respiratory chain defect (score 1)

- Recommendations: plasma acylcarnitines, or mutation analysis *ACADS* gene or SCAD activity (fibroblasts, leucocytes) or fatty acid oxidation studies (score 1)

Scores of participants

- **Survey 2012-1**

Lab n°	Patient A MSUD				Patient B HHH syndrome				Patient C Mucopolysaccharidosis type I			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	2	2	1	5	2	2	1	5
2	2	2	1	5	2	2	1	5	2	2	1	5
3	2	2	1	5	2	2	1	5	2	2	1	5
4	2	2	1	5	1	1	1	3	2	2	1	5
5	0	0	0	0	1	1	1	3	1	0	0	1
6	1	0	0	1	0	0	0	0	1	1	1	3
7	2	2	0	4	0	1	1	2	2	2	1	5
8	2	2	1	5	2	2	1	5	2	2	1	5
9	1	0	1	2	0	1	1	2	2	2	1	5
10	2	2	0	4	2	2	1	5	2	2	1	5
11	2	2	1	5	2	2	1	5	2	2	1	5
12	2	2	1	5	2	1	1	4	2	2	1	5
13	2	2	1	5	2	2	1	5	2	2	1	5
14	2	2	1	5	2	2	0	4	0	2	1	3
15	1	2	1	4	2	2	1	5	1	0	0	1
16	1	2	1	4	1	1	1	3	2	2	1	5
17	2	2	1	5	2	2	1	5	2	2	1	5
18	2	2	1	5	2	2	1	5	2	2	1	5
19	1	2	1	4	1	1	1	3	2	2	1	5
20	1	2	1	4	0	1	1	2	2	2	1	5
21	2	2	1	5	2	1	1	4	2	2	1	5
22	2	2	0	4	2	1	0	3	1	2	1	4

❖ **Survey 2012-2**

Lab n°	Patient D "RedBulluria"				Patient E CblC				Patient F SCAD deficiency			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	2	2	1	5	2	2	1	5
2	1	2	0	3	1	1	1	3	2	2	1	5
3	2	2	1	5	2	2	1	5	2	2	1	5
4	2	2	1	5	2	2	1	5	2	2	1	5
5	2	0	0	2	1	2	1	4	1	1	0	2
6	2	2	1	5	1	1	1	3	1	1	1	3
7	2	2	1	5	1	1	1	3	2	2	1	5
8	2	2	1	5	2	2	1	5	1	2	1	4
9	1	1	0	2	2	2	1	5	2	1	1	4
10	1	2	1	4	2	2	1	5	1	2	1	4
11	2	2	1	5	2	2	1	5	2	2	1	5
12	1	2	0	3	2	2	1	5	1	2	1	4
13	2	2	1	5	1	2	1	4	2	2	1	5
14	2	2	0	4	2	2	1	5	1	1	1	3
15	1	2	1	4	2	2	1	5	2	2	1	5
16	2	2	1	5	1	2	1	4	2	2	1	5
17	2	2	1	5	1	1	1	3	2	2	1	5
18	2	2	1	5	2	2	1	5	1	2	1	4
19	2	2	1	5	2	2	1	5	2	2	1	5
20	2	2	1	5	1	2	1	4	2	2	1	5
21	2	2	1	5	2	2	1	5	2	2	1	5
22	2	2	1	5	2	2	1	5	2	2	1	5

❖ **Total scores**

Lab number	Survey 2012-1	Survey 2012-2	Cumulative score (max = 30)	Cumulative score (%)
1	15	15	30	100%
2	15	11	26	87 %
3	15	15	30	100 %
4	13	15	28	93 %
5	4	8	12	40 %
6	4	11	15	50 %
7	11	13	24	80 %
8	15	14	29	97 %
9	9	11	20	67%
10	14	13	27	90%
11	15	15	30	100%
12	14	12	26	87 %
13	15	14	29	97 %
14	12	12	24	80 %
15	10	14	24	80 %
16	12	14	26	87 %
17	15	13	28	93 %
18	15	14	29	97 %
19	12	15	27	90 %
20	11	14	25	83 %
21	14	15	29	97 %
22	11	15	26	87%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	3	14%
Poor performers (< 60 % good responses)	2	9 %

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Recommen-dations (%)	Total (%)
Patient A	MSUD	**82 %**	**86 %**	**77 %**	**83 %**
Patient B	HHH syndrome	**73 %**	**73 %**	**86 %**	**75 %**
Patient C	MPS I	**86 %**	**89 %**	**91 %**	**88 %**
Patient D	"RedBulluria"	**89 %**	**93 %**	**77 %**	**88 %**
Patient E	CblC	**82 %**	**91 %**	**100 %**	**89 %**
Patient F	SCAD deficiency	**84 %**	**91 %**	**95 %**	**89 %**

DPT-scheme in 2013

- Two surveys of 3 urines, including "normal" patients, sent by CSCQ
- Results have to be sent within 3 weeks
- **Reporting** on CSCQ (Centre Suisse de Contrôle de Qualité) website is compulsory, before the deadline.
- **Scoring**: performed by two different scheme organizers. For the Lyon centre, this will also done by Viktor Kozich from Prague.

We remind you that to participate to the DPT-scheme, you must perform at least:

- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides

If you are not performing one of these assays, you can send the samples to another lab (cluster lab) but you are responsible for the results.

Please send quantitative data for amino acids and, as much as possible, for organic acids.

Meeting in 2013

It will take place during the ICIEM meeting in Barcelona **Tuesday 3 September 2013**, at 9.00 am.

We remind you that attending this meeting is an important part of the proficiency testing. The goal of the program is to **improve** the competence of the participating laboratories, which includes the critical review of all results with a discussion about improvements.

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ANNEX 1

PROFICIENCY TESTING – SOUTHERN EUROPE, LYON CENTER

URINE SAMPLES ALREADY SENT

• **1998 : 1**	**A**	**OCT**	
	B	**Propionic**	
• **1999 : 1**	**C**	**MPS I or II**	
	E	**Cystinuria**	SKZL
• **1999 : 2**	**D**	**CblC**	
	F	**HMG-CoA lyase**	
• **2000 : 1**	**G**	**Iminodipeptiduria**	SKZL
	H	**Glutathionsynthetase**	
• **2001 : 1**	**P1**	**Mevalonate kinase**	
	P2	**L-2-OH glutaric**	
• **2001 : 2**	**P3**	**Methylmalonic**	SKZL
	P4	**MPS IIIA San Fillippo**	
• **2002 : 1**	**P1**	**LCHAD**	
	P2	**Sulphite oxidase**	
• **2002 : 2**	**P3**	**Biotinidase**	SKZL
	P4	**MPS I**	
• **2003:1**	**P1**	**Tyrosinemia type I**	

	P2	**SC-BCAD deficiency**	
	P3	**Argininosuccinic aciduria**	
• **2003:2**	**P4**	**MCC deficiency**	
	P5	**Sialidosis**	SKZL
	P6	**MSUD**	
• **2004:1**	**P1**	**Tyrosinemia type I, treated patient**	
	P2	**Propionic acidemia**	
	P3	**Non metabolic disease, septic shock**	
• **2004:2**	**P4**	**Mevalonic aciduria (common sample)**	
	P5	**Fucosidosis**	
	P6	**Alkaptonuria**	
• **2005:1**	**P1**	**Isovalericacidemia**	
	P2	**Tyrosinemia type II (common sample)**	
	P3	**Disorder of peroxysome biogenesis**	
• **2005:2**	**P4**	**Multiple acyl-CoA dehydrogenase deficiency**	
	P5	**Alpha-mannosidosis**	
	P6	**4-hydroxybutyric aciduria**	
• **2006:1**	**P1**	**Aromatic amino acid decarboxylase deficiency**	
	P2	**Hyperoxaluria type I**	
	P3	**Mucopolysaccharidosis type VI**	
• **2006:2**	**P4**	**Hypophosphatasia (common sample)**	
	P5	**Lysinuric protein intolerance**	
	P6	**MCAD deficiency**	
• **2007:1**	**P1**	**Mitochondrial acetoacetyl-CoA thiolase (MAT)**	
	P2	**Homocystinuria due to CBS deficiency**	
	P3	**Hyperlysinemia (common sample)**	
• **2007:2**	**P4**	**Aspartylglucosaminuria**	
	P5	**Phenylketonuria**	
	P6	**SCAD deficiency**	

• **2008:1**	**P1**	**Cbl C/D**
	P2	**Mucopolysaccharidosis type III (common sample)**
	P3	**2-hydroxyglutaric aciduria**
• **2008:2**	**P4**	**Glycerol kinase deficiency**
	P5	**α-mannosidosis**
	P6	**3-methylcrotonyglycinuria**
• **2009:1**	**P1**	**Mucopolysaccharidosis type III**
	P2	**Salla disease(common sample)**
	P3	**No metabolic disorder**
• **2009:2**	**P4**	**Glutaric aciduria type I**
	P5	**Iminodipetiduria**
	P6	**Multiple acyl-CoA dehydrogenase deficiency**
• **2010:1**	**P1**	**Mevalonic aciduria**
	P2	**Aminoacylase I deficiency**
	P3	**No metabolic disorder**
• **2010:2**	**P4**	**Sialidosis type I (common sample)**
	P5	**Glutaric aciduria type I**
	P6	**Aspartylglucosaminuria**
• **2011:1**	**A**	**Molybdenum cofactor deficiency**
	B	**GAMT deficiency (common sample)**
	C	**Methylmalonicsemialdehyde dehydrogenase def.**
• **2011:2**	**D**	**Mucopolysaccharidosis type IVA (Morquio)**
	E	**Phenylketonuria**
	F	**Citrullinemia type I**