



C. VIANEY-SABAN, C. ACQUAVIVA-BOURDAIN
Service Maladies Héréditaires du Métabolisme
Centre de Biologie et de Pathologie Est
59, Boulevard Pinel
69677 Bron cedex
Tel 33 4 72 12 96 94
e-mail christine.saban@chu-lyon.fr
cecile.acquaviva-bourdain@chu-lyon.fr

ERNDIM
Diagnostic proficiency testing
2008
Southern Europe
Lyon Centre

ANNUAL REPORT 2008

In 2008, 20 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	9
Italy	4
Spain	4
Portugal	2
Switzerland	1
TOTAL	20

Logistic of the scheme

- **2 surveys** 2008-1 : patient P1, P2 and P3
2008-2 : patient P4, P5 and P6
- **Origin of patients:** 5 out the 6 urine samples have been kindly provided by participants. They will get a 20% discount on the DPT fee for 2009.
- Patient P1 : Methylmalonic aciduria and homocystinuria : CblC/D – Dr L Contini, Dr F Lilliu, Servizio Malattie Metaboliche del Bambino, Cagliari
- Patient P2 : MPS III – Dr V Kozich, Dr P Chrastina, Institute of Inherited Metabolic Disorders, Prague. This sample has been sent to all labs participating to the DPT scheme in Europe.
- Patient P3 : L-2hydroxyglutaric aciduria – Dr O Boulat, CHUV, Lausanne
- Patient P4 : Glycerol kinase deficiency – Dr ML Cardoso, Dr D Quelhas, Dr MR Rodrigues, Instituto de Genetica, Porto
- Patient P5 : α -mannosidosis – Centre de Biologie Est, Lyon
- Patient P6 : 3-methylcrotonylglycinuria – Dr D Rabier, Hôpital Necker, Paris

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

Timetable of the schemes

- May 5 : shipment of samples of Survey 1 and Survey 2 by DHL and of the forms by e-mail
- May 26 : deadline for result submission (Survey 1)
- June 23 : analysis of samples of the second survey
- July 2 : report of Survey 1 by e-mail
- July 14 : deadline for result submission (Survey 2)
- July 28 : report of Survey 2 by e-mail
- September 2 : meeting in Lisbon
- April 2 : annual report with scoring sent by e-mail

Date of receipt of samples

Once again, DHL has been very efficient.

	Survey 1 + 2
+ 24 hours	19
+ 72 hours	1

Date of reporting

All labs sent reports, but with some delay for the second survey, despite a reminder from organizers. This will no be possible, when submission will be on the website.

	Survey 1	Survey 2
	(3 weeks)	(3 weeks)
Receipt of results:	20 labs	20 labs
Before deadline	20	15
+ 1 day		2
+ 2 days		2
+ 1 week		1
No answer	0	0

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 or 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 or 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 Lisbon on 2 September 2008 from 9.00 to 10.30, during the SSIEM Meeting.

❖ Participants

Representatives from 11 labs were present: JA Arranz, E. Riudor (Vall d'Hebron, Barcelona), F Lilliu (Cagliari), S Funghini, E Pasquini (Florence), U Caruso, MC Schiaffino (Genova), O. Boulat, C. Roux (Lausanne), G. Briand (Lille), B. Merinero (Madrid), O. Rigal (Robert Debré, Paris), L. Lacerda, D. Quelhas, L. Vilarinho (Porto), C Rizzo (Roma), MD Boveda, JA Cocho, M Rebollido Fernandez (Santiago de Compostella).

❖ Informations from the Executive Board and the Scientific Advisory Board

- Certificate of participation for 2008 will be issued for participation and it will be additionally notified whether the participant has received an assistance letter. This assistance letter is sent out if the performance is less than 50%. Good performers are those whose performance is more than 75%.
- No assistance letter has been sent in 2007.
- 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 a "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. 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 °C for 20 minutes. Make sure that this temperature is achieved in the entire urine sample, not only in the water bath. Then aliquot the sample in 10 ml plastic tubes (minimum 48 tubes), add stoppers and freeze. Be

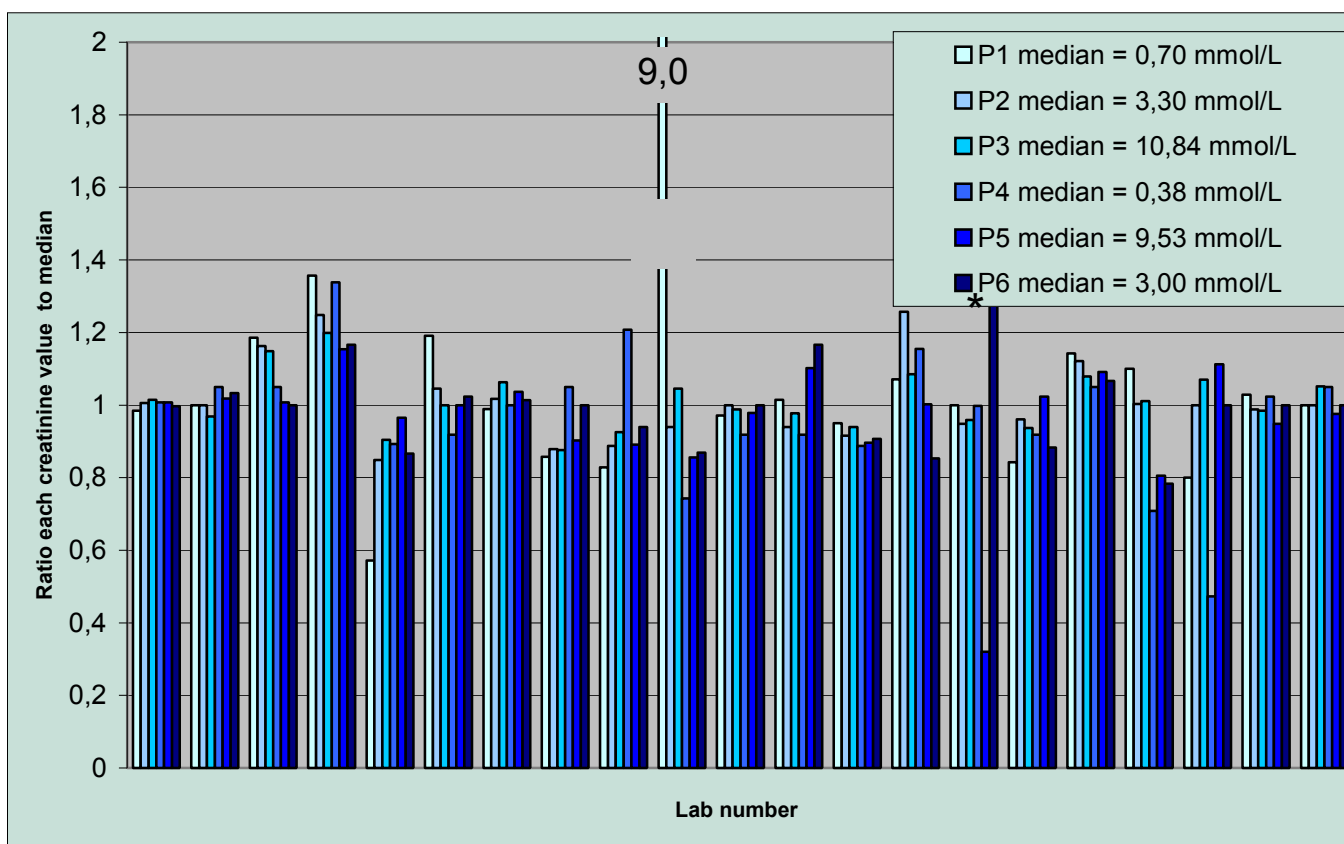
careful to constantly homogenize the urine while aliquoting the sample. Send the aliquots on dry ice by rapid mail or express transport to: Dr Christine Vianey-Saban, Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, 5ième étage, Centre de Biologie et de Pathologie Est, Groupement Hospitalier Est, 59, Boulevard Pinel, 69677 Bron cedex, France. Please send me 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.

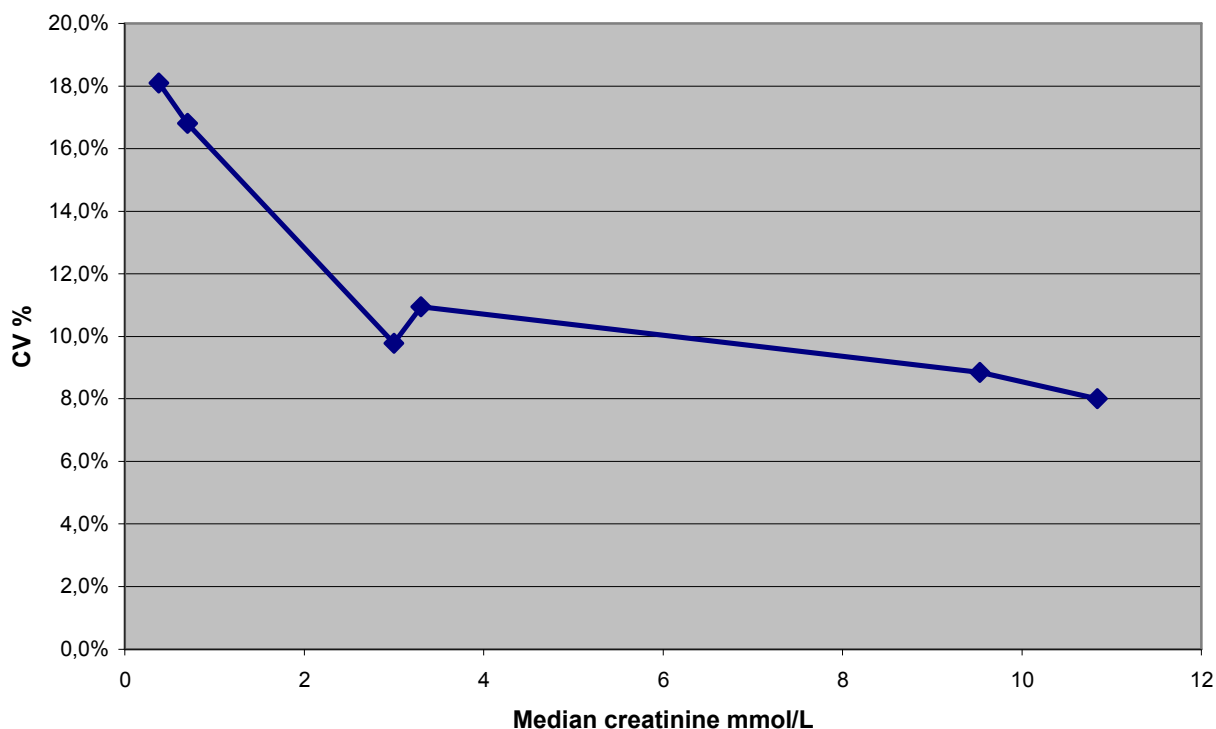
❖ **Discussion of results**

- **Creatinine measurement**

Results improved compared to last year.



If we exclude 3 wrong values (obviously errors of dilution or inversion), coefficient of variation is inversely related to creatinine value.



- **Patient P1 – Methylmalonic and homocystinuria, intracellular defect of cobalamin metabolism : CblC/D patient**

The urine sample was collected from a 2 months old infant, who presented respiratory distress, hepatomegaly, splenomegaly, anemia, thrombocytopenia, hypotonia, feeding difficulties, and neurological damage. Plasma amino acids, at the time of the diagnosis, was:

Methionine = 6 mmol/L

Homocystine = 73 mmol/L

Total homocysteine = 95 mmol/L

Confirmation of diagnosis in fibroblasts was performed by Dr B. Fowler (Basel):

✓ Deficient methionine and serine formation when cells are grown in normal medium:

Methionine = 0.03 nmol/16h/mg prot - controls: 1.0 - 4.0

Serine = 0.05 nmol/16h/mg prot - controls: 0.38 - 3.7

✓ Increase of synthesis of methionine in cells grown in medium supplemented with varying concentrations of hydroxo-cobalamin (Fowler et al. *Pediat Res* 1997;41:145).

✓ [⁵⁷Co] Cobalamin uptake = 14.0 pg/mg prot - controls: 40 - 156

✓ [1-¹⁴C] propionate incorporation in intact fibroblasts = 0.75 nmol/mg prot/16h - controls: 3.5 - 24.4

Increase of propionate incorporation in medium supplemented with a high concentration of hydroxocobalamin.

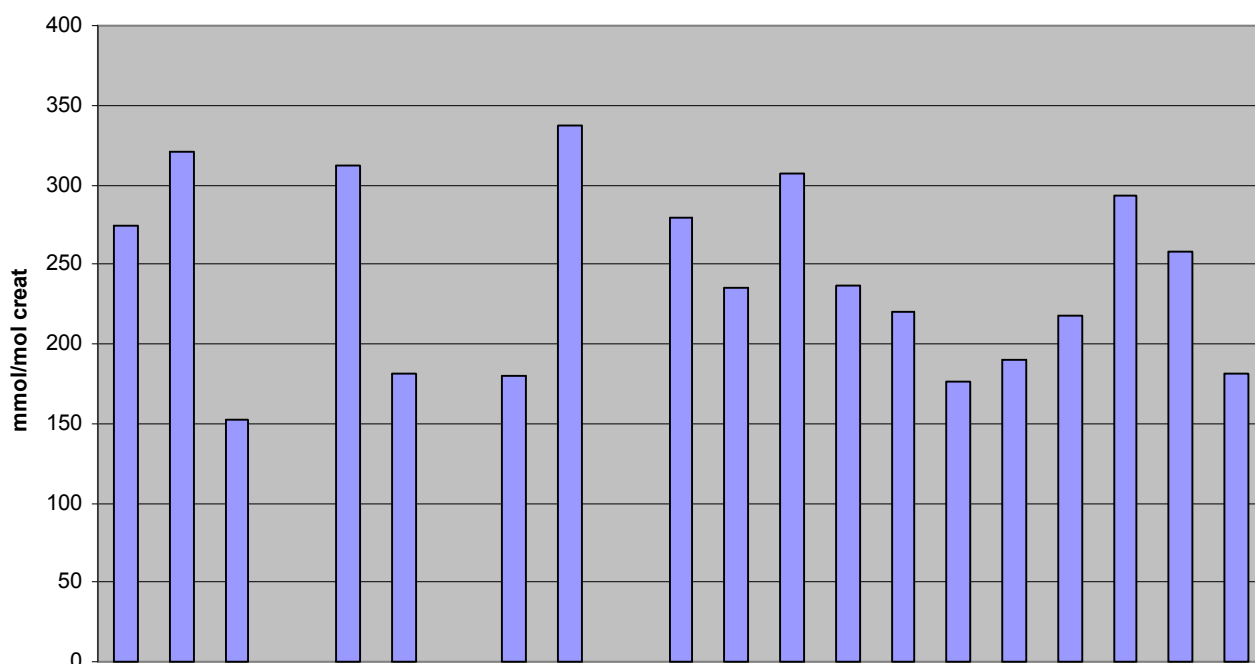
Results indicate CblC/D, and *in vivo* response to cobalamin would be expected, but complementation analysis and mutation analysis were not yet performed.

Diagnosis

- 19 labs gave a correct diagnosis
- 1 lab concluded to methylmalonic aciduria

All labs performed **aminoacids**, and all, except one, reported an increase of homocystine.

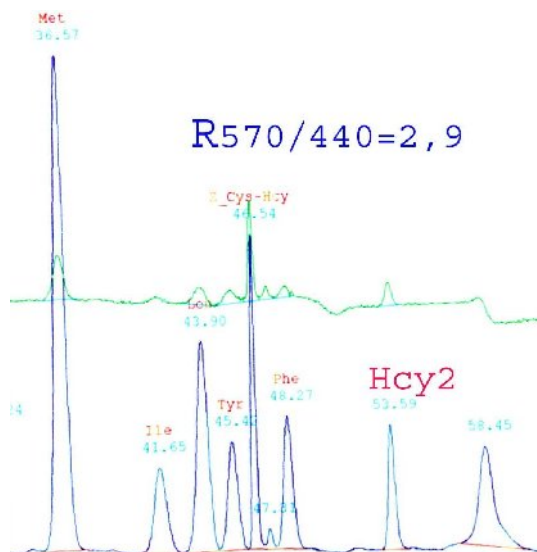
Homocystine - median = 237 mmol/mol creat - CV = 25%



The CV observed for homocystine was better than the interlab CV observed for the ERNDIM amino acids 2007 QAP control (67.7%).

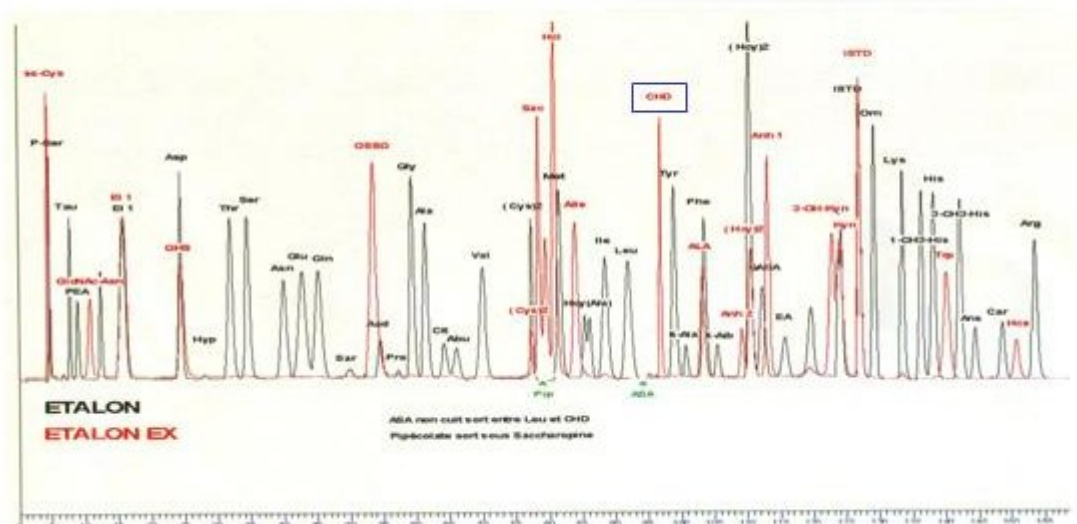
Only seven labs also reported an increase of cysteine-homocysteine mixt disulfide (CHD). Information from users is that the mixt disulfide is eluted:

- Jeol analyzer: RT = 46.5 min between Tyr (RT = 45.4 min) and Phe (RT = 48.3 min), ratio 570/440 nm = 2.9



- Hitachi analyser: RT = 82.5 min between argininosuccinic acid (RT = 81 min) and β-alanine (RT = 84.2 min)

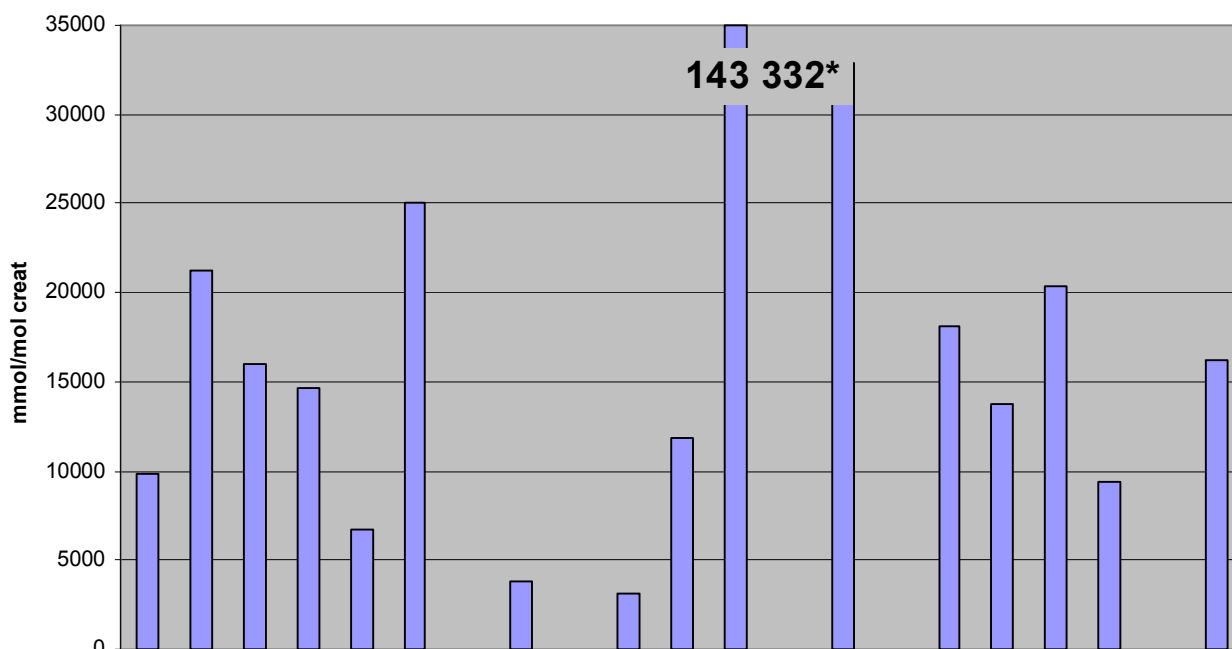
- Biochrom: RT = 96 min between argininosuccinic acid (RT = 93 min) and Tyr (RT = 98 min), ratio 570/440 nm = 2.2



- LC/MS-MS: monitored transition m/z 255>134 with deuterated cystine as internal standard.

All labs performed **organic acids** and all of them reported an increase of methylmalonic acid.

Methylmalonic - median = 14 679 mmol/mol creat - CV = 54,7%*



The CV calculated after exclusion of an obviously wrong value (error of dilution?) was 54.7%, which is better than the interlab CV for ERNDIM quantitative organic acid QAP 2008 (95.3%). However, stable isotopes for methylmalonic are available from several companies, and they have to be used for an accurate measurement of methylmalonic acid for the follow up of patients (especially if they are

followed by different centres). Nineteen labs reported an increase of methylcitric acid: median was 98 mmol/mol creat, but CV was 503%! A standard for methylcitrate and its stable isotope are available from Interchim: www.interchim.com, ref X-4176 and D-4162. Median for 3-hydroxypropionic acid, identified by 15 labs, was 378 mmol/mol creat, with a CV of 125%. A standard is available from Pr Brunet in Madrid: ernesto.brunet@uam.es

One lab performed acylcarnitine profiling and a high level for C4DC, C3 and C4 was noticed. A total homocysteine value of 860 mmol/mol creat was obtained by another one.

Advice for further investigations: according to Dr Brian Fowler, the recommended strategy is:

- perform first plasma total homocysteine together with plasma amino acids
- then rule out nutritional B12 deficiency (homocysteine and methylmalonic acid normalise after injections of hydroxocobalamin in nutritional B12 deficiency)
- then perform fibroblast studies: 1) propionate fixation, 2) synthesis of methionine 3) cobalamin uptake and coenzyme synthesis
- finally, perform complementation analysis and mutation analysis.

Advices from participants were correct.

Scoring

- Analytical performance: increase of homocystine (score 1), increase of methylmalonic acid (score 1)
- Interpretation of results: CblC, CblD or CblF, disorder of cobalamin metabolism, exclude absorption or transport defect (score 2), methylmalonic aciduria (score 1)
- Advice for further investigations: Plasma amino acids and/or plasma total homocysteine and/or Cbl complementation group assay in fibroblasts (score 1)

• Patient P2 – Mucopolysaccharidosis type IIIA (Sanfilippo disease)

This seven- year old boy had an abnormal perinatal history of umbilical strangulation. He presented a slowly progressing expressive dysphasia. CT scan revealed enlarged cisterna magna and possibly cerebellum hypoplasia, and EEG no specific epileptic graphoelements. The sample was obtained at the age of eight years. Medication at the time of sample collection is unknown.

The sample originates from a patient with enzymatically confirmed mucopolysaccharidosis IIIA. GAG concentration at the time of diagnosis was 24.7 g/mol creat (aged matched controls < 10) and GAG electrophoresis showed elevated fractions of heparane sulphate and dermatane sulphate in one fraction, typical for MPS III.

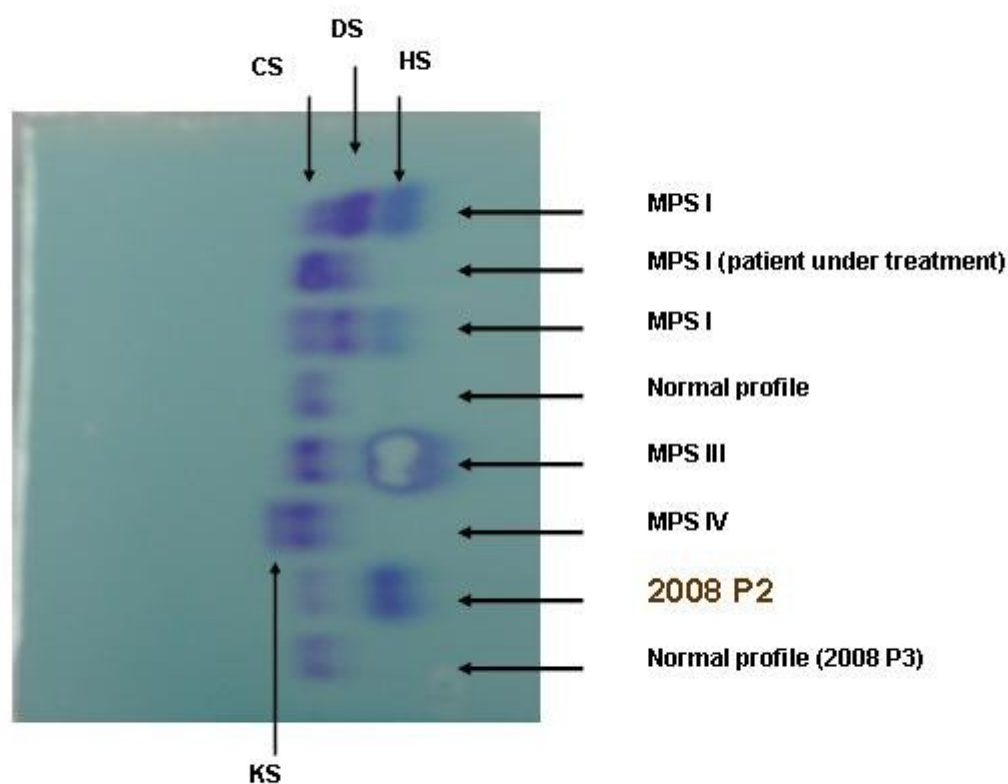
To our opinion, clinical data were not informative but it was the original clinical description which came with the sample at the time the diagnosis was established. Some additional information was found out by ambulatory investigation, but the patient did not have pronounced facial dysmorphism, fidgety, eretic, rhinolalia.

This sample has been sent to all DPT centers

Diagnosis

Only 13 labs reached a good diagnosis. One lab concluded to MPS II or III and another to mucopolysaccharidosis, whereas 4 labs gave no or a wrong diagnosis.

Only 16 labs over 20 performed **mucopolysaccharide** quantification: 15 labs reported an increase of GAG, whereas one lab reported a normal value (?). The 13 labs who performed identification of GAG reported an increase of heparane sulphate and concluded to MPS III.



C: chondroitine sulphate, D: dermatane sulphate, HS: heparane sulphate, K: keratane sulphate

Among the 14 labs who performed **oligosaccharide** profile, 12 reported a normal profile, one an abnormal profile, probably related to the mucopolysaccharidosis, and one smears at deposit.

Advice for further investigations was OK for those who reached a correct diagnosis

Scoring

- Analytical: Increase of GAG and heparane sulphate (score 2), increase of GAG (score 1)
- Interpretation of results: MPS III (score 2), mucopolysaccharidosis (score 1).
- Recommendations: activity of the 4 enzymes possibly deficient in MPS III and/or mutation analysis of the corresponding genes and/or MPS analysis on a new urine sample (score 1).

- **Patient P3 - L-2-hydroxyglutaric aciduria**

This 10 year-old girl had a moderate retardation of psychomotor development, cerebellar ataxia, behaviour problems, and learning difficulties. She did not present epilepsy. She is homozygote for a

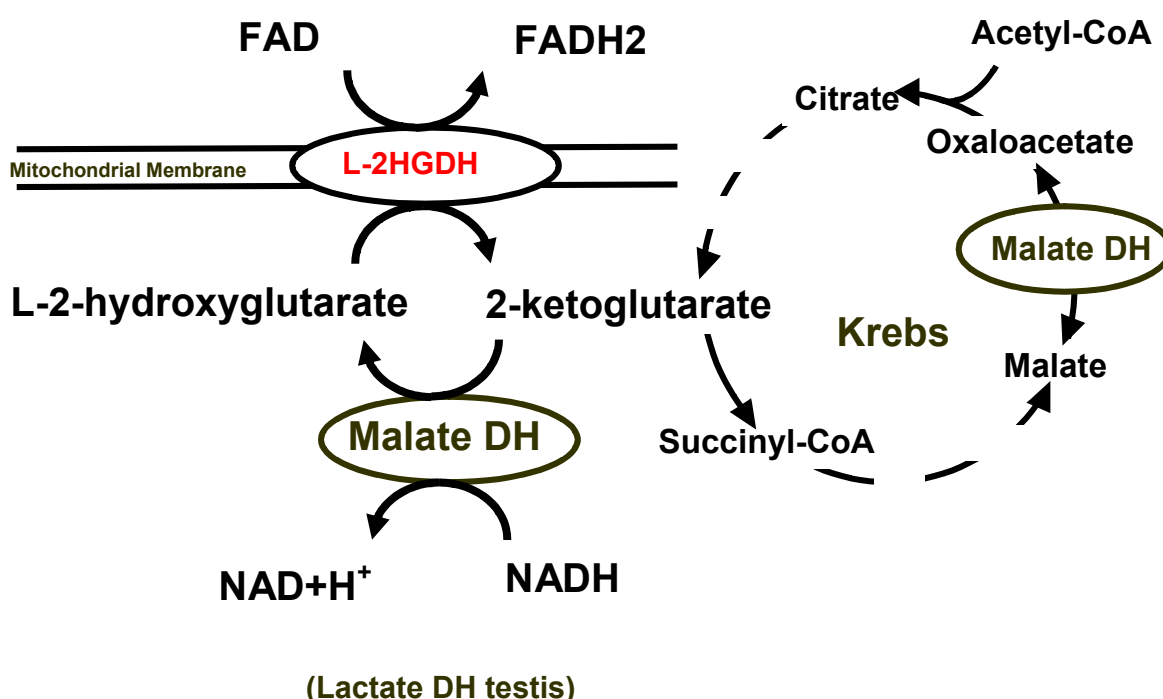
splice site mutation in the *L2HGDH* gene (IVS6+1G>A) and her sister, who died at the age of 11 months after a first episode of gastroenteritis, had the same genotype.

L-2-hydroxyglutaric aciduria is a rare inborn error of metabolism (> 100 patients described). The biochemical diagnosis relies on urinary organic acid profile. 2-hydroxyglutaric acid excretion varies from 226 to 4294 mmol/mol creat (controls <30). Differentiation of L- and D-2-hydroxyglutaric isomers is necessary and is achieved by the use of chiral derivatives (O-acetyl-di-(D)-2-butyl esters) or of chiral columns (GC, HPLC). 2-hydroxyglutaric acid is increased in CSF: 23 – 474 µmol/L (controls <2). Increase of other hydroxydicarboxylic acids (glycolic, glyceric, 2,4-dihydroxybutyric, citric, and isocitric acids) has been reported as well as an increase of lysine in plasma and CSF.

L-2-hydroxyglutaric aciduria is due to L-2-hydroglutarate dehydrogenase deficiency, a membrane bound FAD dependent enzyme of the inner mitochondrial membrane which converts L-2-hydroxyglutarate to 2-ketoglutarate. *L-2-HGDH* gene has been identified in 2004 (Topcu et al, Hum Mol Genet 2004;13:2803; Rzem et al, Proc Natl Acad Sci USA 2004;101:16849) but the role of this metabolic pathway remained unknown for a long time. It had been showed that urinary excretion of L-2-hydroxyglutaric acid (L2OHGA) was independent of feeding, but relied exclusively on endogenous production: approximately 25% from liver and 75 % from muscle.

The toxicity of L-2-hydroxyglutaric is due to its excitotoxic effect (increases uptake of glutamate). It oxidizes lipids and proteins (mainly in cerebellum) and reduces the brain capacity to regulate the production of free radicals. However it has no inhibitory effect on mitochondrial respiratory chain. The blood brain barrier (BBB) has a low permeability to this compound (dicarboxylic acid).

Very recently, it has been demonstrated that the production of L-2-hydroglutarate has no metabolic function, but is due to the lack of specificity of malate dehydrogenase which dehydrogenates 2-ketoglutarate. Therefore, the degradation of L-2-hydroglutarate by L-2-hydroxyglutarate dehydrogenase is a “metabolite repair” mechanism and this enzyme belongs to the group of « house-cleaning » enzymes (Rzem et al, J Inher Metab Dis 2007;30:681). Lactate dehydrogenase exhibits also the same lack of specificity, but its metabolic role is probably non significant since L-2-hydroglutarate excretion is similar in males and females. The following figure summarizes this pathway:



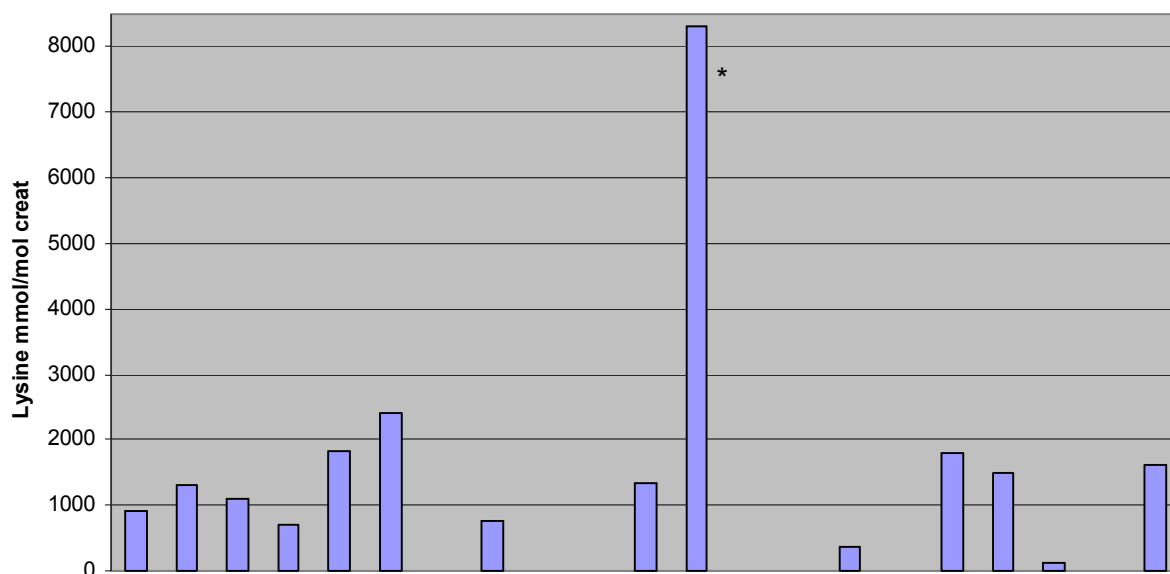
Diagnosis

All labs concluded to 2-hydroxyglutaric aciduria.

All labs except one performed amino acids: 7 reported an increase of lysine (62 - 84 mmol/mol creat).

All labs performed **organic acids** and reported an increase of 2-hydroxyglutaric (122 - 8324 mmol/mol creat). One of them performed analysis of enantiomers and identified the L-form.

2-hydroxyglutaric - median = 1332 mmol/mol creat - CV = 48,5 %*



(an obviously wrong value * has been eliminated for the calculation of CV)

2-hydroxyglutaric is included in the “quantitative organic acids” scheme. The interlab CV in 2008 was 107.4% (n=63).

Advice for further investigations was OK for all labs.

Scoring

- Analytical performance: increase of 2-hydroxyglutarate (score 2)
- Interpretation of results: 2-hydroxyglutaric aciduria or L-2-hydroxyglutaric aciduria (score 2)
- Advice for further investigations: separation of D- and L-2-hydroxyglutaric (score 1)

• Patient P4 – glycerol kinase deficiency

This four months old boy is the first child of non consanguineous parents, born after a normal pregnancy and delivery. Arthrogryposis and abnormal insertions of fingers were noticed. He had a normal growth and development until the age of 2 months when he presented a cardiac-respiratory arrest following an episode of vomit aspiration. Echocardiogram revealed hypertrophic cardiomyopathy. At MRI, corpus callosum was thin and he had hypoplastic left cerebellum lobe. He developed a severe hypoxic-ischemic encephalopathy. Biochemical investigation revealed hypertriglyceridemia and hyperglycerolemia.

Mutation analysis of *GK* gene on chromosome X (Dr van Amstel, Utrecht) identified c.883+1G>A a splice site mutation in intron 10 which disrupts the donor splice site (AAAAATACGTGAGTTT) and is deleterious for correct splicing of the mRNA. Complex form was also excluded by normal endocrinological studies (ACTH, cortisol, aldosterone and renine), CK and muscular biopsy. At 10 months of age, he has macrocephaly, hydrocephalus, dysautonomic responses to stress or postural changes.

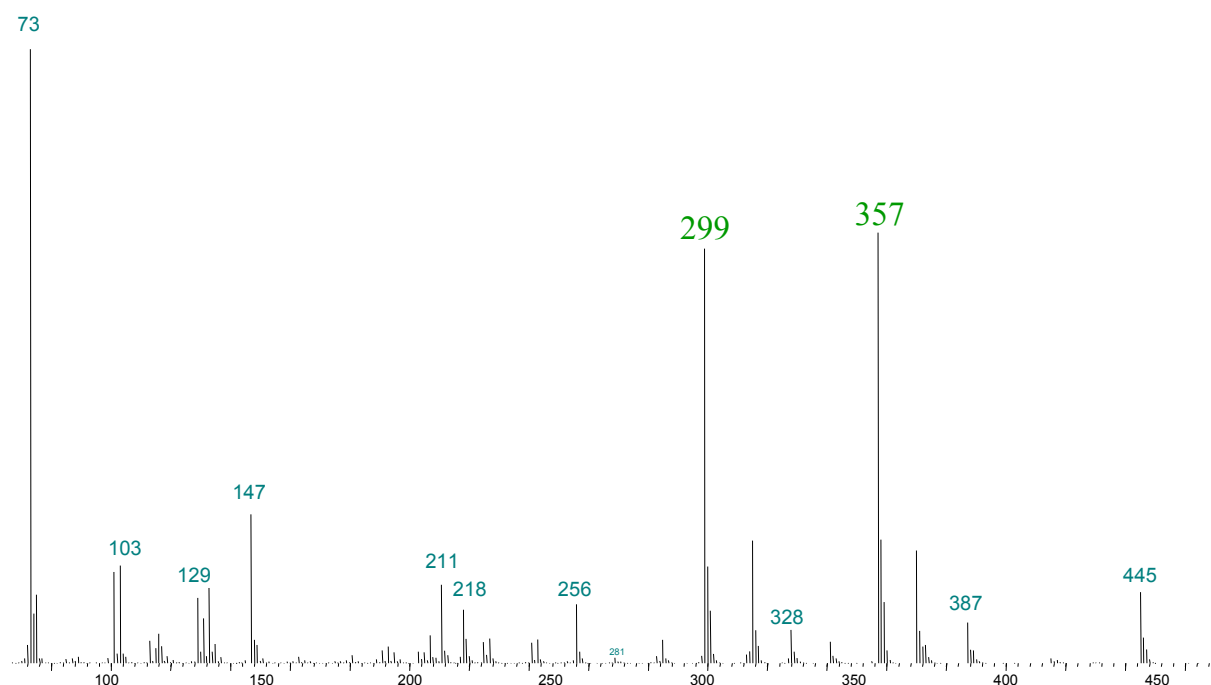
This is an unusual presentation of glycerol kinase, and the association to another disorder is discussed. The case report of this patient has been presented as a poster at the SSIEM Lisbon meeting (Rodrigues et al. Glycerol kinase deficiency - First Portuguese patient, unusual presentation, poster n°156).

Diagnosis

Fifteen labs reached the write diagnosis; 4 of them advised to exclude exogenous contamination, and another to exclude fructose 1,6 diphosphatase deficiency. Because of the unusual clinical presentation for glycerol kinase deficiency, one lab proposed as diagnosis glycerol-3-phosphate acyltransferase, mutations in the *SNC5A*-encoded novel mutation in glycerol-3-phosphate dehydrogenase 1-like gene (*GPD1-L*) - Brugada syndrome. But it seems, from the literature that there is no increase of glycerol in these conditions. Four labs did not give diagnosis or concluded to a wrong diagnosis.

Aminoacid analysis was performed by 15 labs, with no significant abnormality, except for a slight hyperaminoaciduria, probably due to the low creatinine value.

All labs performed **organic acid** profile and 17 of them reported an increase of glycerol. Four labs reported an increase of glyceric acid and one lab an increase of glycerol-3-phosphate.



Mass spectrum of glycerol-3-phosphate, according to Krywawych S et al. *J Inher Metab Dis* 1986;9: 388-392.

Five labs reported abnormal values for **glycosaminoglycan** quantification using DMB test. The question of a possible interference of glycerol on DMB test was raised during the meeting. Therefore,

we performed DMB test on a 15 mM glycerol solution (approximately the glycerol concentration of the urine sample): it gave a result of 15 mg/L, thus most probably explaining the high reported values.

Interpretation and recommendations were satisfying for those who reached a correct diagnosis.

Scoring

- Analytical : increase of glycerol (score 2), increase of glyceric acid (score 1)
- Interpretation of results: glycerol kinase deficiency or possible exogenous contamination (score 2), no diagnosis or wrong diagnosis (score 0)
- Advice for further investigations: exclude contamination on a new urine sample and/or glycerol kinase activity and/or mutation analysis *GK* gene and/or plasma glycerol or triglycerides (score 1)

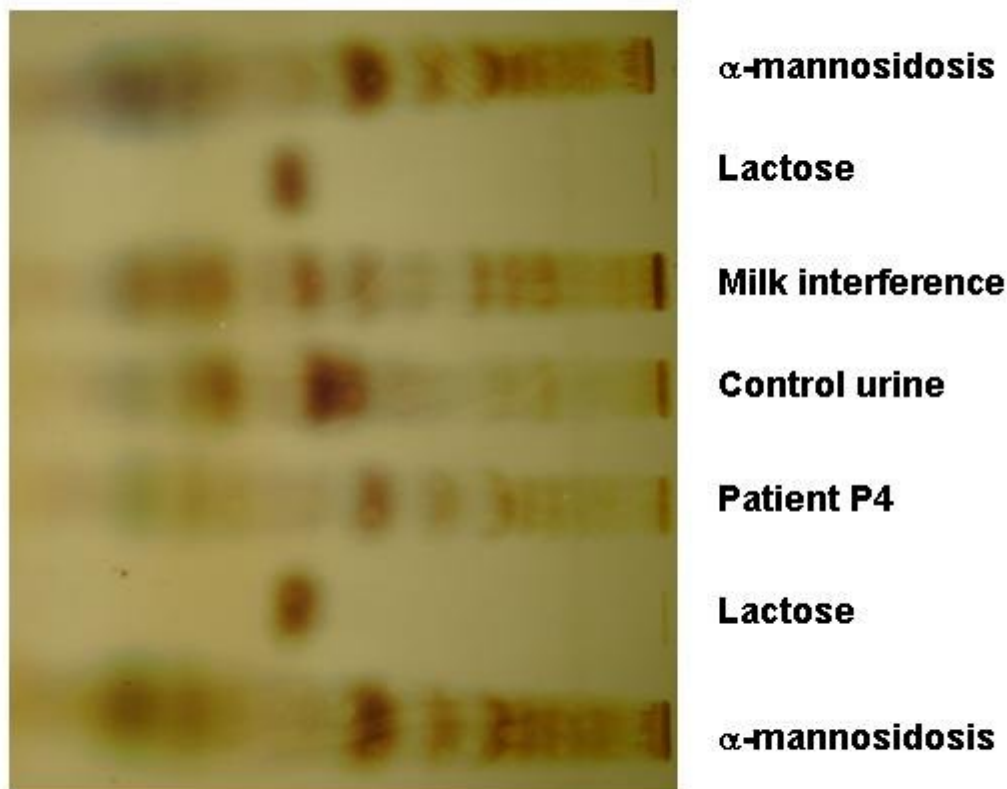
• Patient P5 – α -mannosidosis (α -mannosidase deficiency)

The urine sample was collected from a girl, 2nd child of consanguineous parents (first cousin). Birth occurred at 37 weeks of amenorrhea. Her birth weight was 2.520 kg, her height 46.5 cm. At 1 month of age, umbilical hernia was noticed. From 5 months of age, she had several episodes of bronchitis, pneumopathy and otitis. At 2.5 years, she had hepatosplenomegaly and speech delay. A metabolic work up was performed at 4 years of age because of dysmorphic features (short neck, coarse facies), thick and anteverted nostrils, hepatosplenomegaly, dysostosis (humerus, ribs, metacarpus, and vertebrae), amblyopia, and slight mental retardation. At myelogram, vacuolated lymphocytes, macrophages and plasmocytes were noticed. Oligosaccharide profile was characteristic of α -mannosidosis and the enzyme defect was confirmed in leucocytes: α -mannosidase activity = 0.2 μ kat/kg – controls: 49 – 90. At 12 years of age, when the urine sample was collected, she had hearing problems, a normal neurological examination, mental retardation (# 6 years); her weight was +2SD, with a normal height. Genu valgum, moderate dysmorphia, and slight hepatomegaly were noticed. She did not have splenomegaly nor hernia.

Diagnosis

Only 12 labs concluded to α -mannosidosis and 3 labs to mannosidosis. Four labs concluded to mucopolysaccharidosis and one lab did not give a diagnosis.

16 labs performed **oligosaccharide profile** and all, except two, reported a profile consistent with α -mannosidosis, the two other reporting an abnormal profile.



Fifteen labs performed **quantification of GAG**: 12 obtained normal results and 3 reported an increase. Among the 8 labs who performed identification of GAG's, 4 had a normal profile, 3 reported an increase of heparane sulfate, and 3 an increase of dermatane sulfate.

Advice for further investigations was OK.

Scoring

- Analytical: α -mannosidosis on oligosaccharide profile (score 2), abnormal oligosaccharide profile (score 1)
- Interpretation: α -mannosidosis (score 2), oligosaccharidosis, mannosidosis (score 1), wrong lysosomal storage disease (score 0)
- Recommendations: α -mannosidase activity (leukocytes, plasma or fibroblasts) and/or mutation analysis *MANB1* gene and/or confirm results on a second urine sample (score 1)

- **Patient P6 - 3-methylcrotonylglycinuria: isolated 3-methylcrotonyl-CoA carboxylase deficiency**

This patient has been published by Oude Luttikhuis et al, J Inher Metab Dis 2005;28:1136-1138. This 7 year-old boy is the third child of unrelated parents. At 1 year and 9 months of age, in the course of a viral infection, he developed somnolence and hypotonia, followed by general seizures. Coma

(Glasgow 8) occurred with extrapyramidal syndrome, hemiparesis, mild hepatomegaly. He was subfebrile (38°C). Laboratory investigation revealed hypoglycaemia (0.3 mmol/L), moderate acidosis (pH 7.29, bicarbonate = 15.3 µmol/L), ASAT = 61 U/L, but normal values for ALAT and ammoniaemia. Plasma carnitine was severely decreased (free = 1.5 µmol/L; total = 6.6 µmol/L). A slight elevation of Gln, Gly, Ala was observed on plasma amino acids.

The organic acid profile was indicative of 3-methylcrotonylglycinuria: 3-hydroxyisovaleric acid = 3017 mmol/mol creat (controls < 20); 3-methylcrotonylglycine = 1247 mmol/mol creat (controls ND); 3-hydroxypropionate, methylcitrate: undetectable.

A low protein diet (1.15 g/kg/d), low in leucine, was initiated, together with glycine (140 mg/kg/d), carnitine (50 mg/kg/d), and biotin (1mg/kg/d) supplementation.

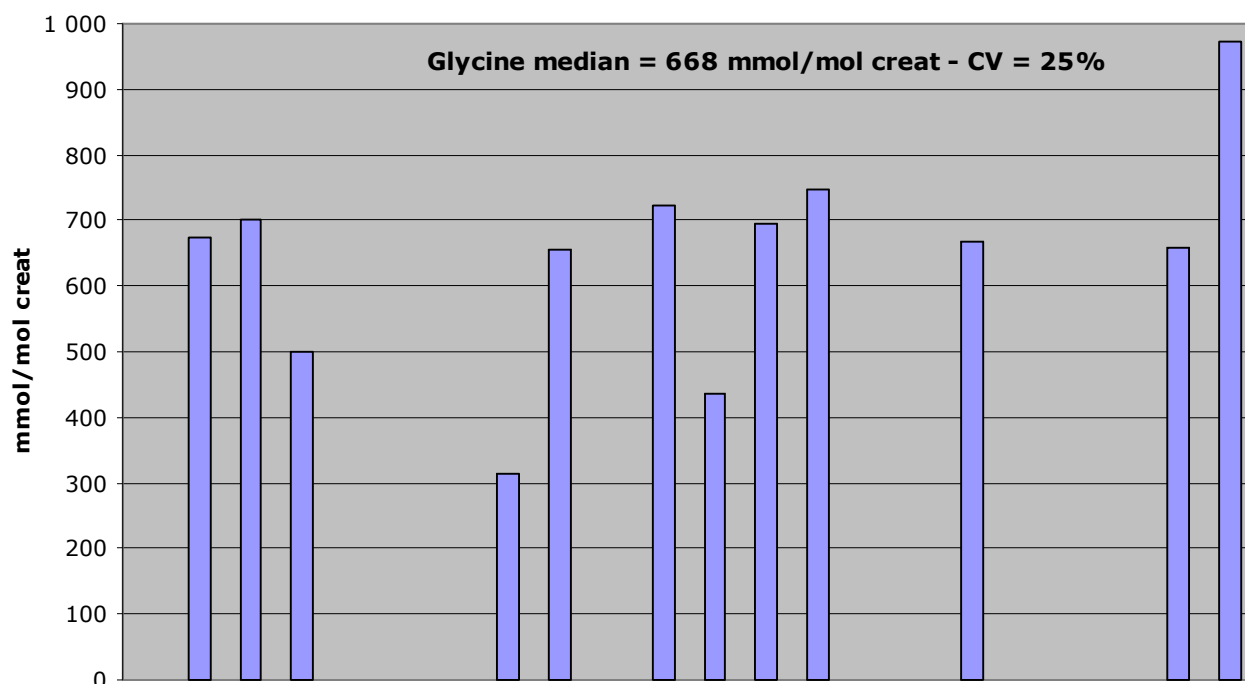
Measurement of 3-methylcrotonyl-CoA carboxylase in cultured skin fibroblasts confirmed the diagnosis (= 0.12 µkat/kg prot - controls: 4.1 ± 1.8)

This patient is now 7 year-old. He has a satisfying psychomotor development, but some learning difficulties. He is receiving: protein 18g/d, glycine (1gx3/d), carnitine (500mgx2/d).

Diagnosis

All labs concluded to 3-methylcrotonyl-CoA carboxylase deficiency.

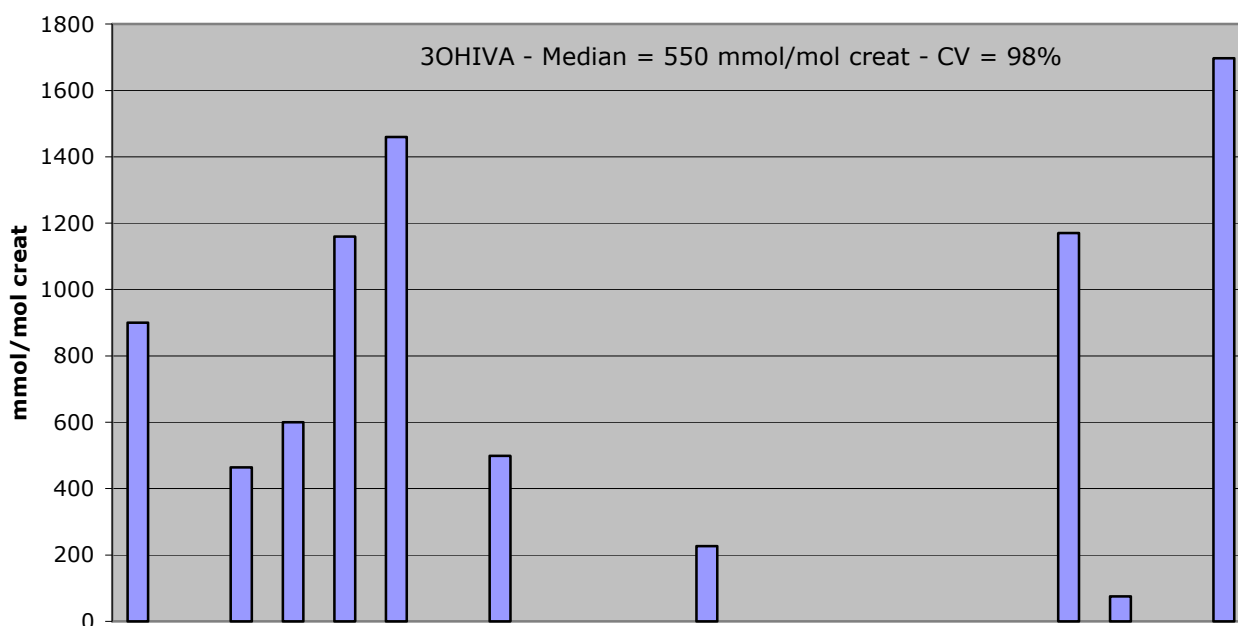
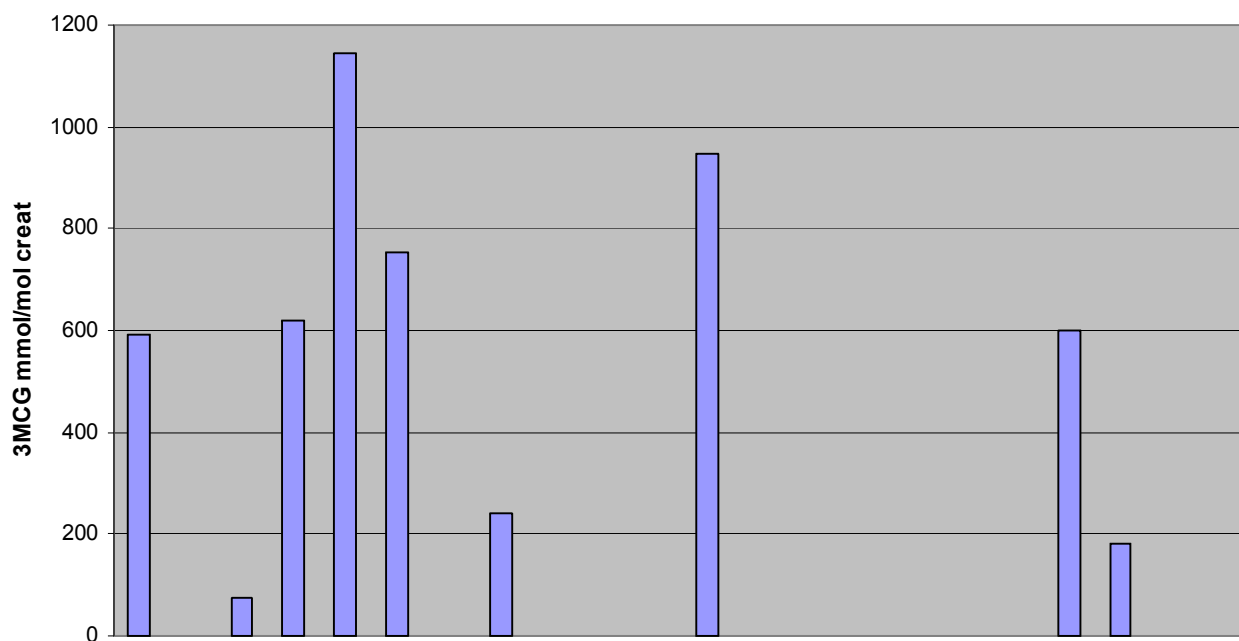
Thirteen labs reported an increase of glycine, and 6 labs a slight increase of α-amino adipic acid, on amino acid analysis.



The CV for glycine was worse than in the amino acid QAP (25% versus 7.9 % in 2008).

All labs performed **organic acids**, and all of them reported an increase of 3-methylcrotonylglycine and all, except one, an increase of 3-hydroxyisovaleric acid (standards for 3-methylcrotonylglycine and 3-hydroxyisovaleric acid, and their stable isotopes are available from Dr Herman Ten Brink in Amsterdam: HJ.tenBrink@vumc.nl). No metabolites of acetylsalicylate were detected which could explain the increase of α -amino adipic acid.

3MCG - median = 609 mmol/mol creat - CV = 58%



The interlab CV for 3-hydroxyisovaleric acid was 51.3 % in the ERNDIM quantitative organic scheme in 2008.

One lab performed **acylcarnitine profile** and identified an increase of C5OH (3-hydroxyisovalerylcarnitine).

Advice for further investigation was OK for all labs.

Scoring

- Analytical: Increase 3-methylcrotonylglycine (score 1), increase of 3-hydroxyisovaleric acid (score 1)
- Interpretation: Isolated 3MCC deficiency as first diagnosis (score 2),
- Recommendations: 3MCC activity (fibroblasts, leukocytes) or mutation analysis *MCCC1* and *MCCC2* genes or plasma / blood acylcarnitines (score 1)

Scores of participants

❖ Survey 2008-1

Lab n°	Patient P1 CbIC/D				Patient P2 MPS III				Patient P3 2-hydroxyglutaric aciduria			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	2	1	5	0	0	0	0	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	2	2	1	5	2	2	1	5
5	2	2	1	5	2	2	1	5	2	2	1	5
6	2	2	1	5	0	0	0	0	2	2	1	5
7	2	2	1	5	2	2	1	5	2	2	1	5
8	2	2	1	5	1	1	1	3	2	2	1	5
9	2	2	1	5	2	2	1	5	2	2	1	5
10	1	1	0	2	0	0	0	0	2	2	1	5
11	2	2	1	5	2	2	1	5	2	2	1	5
12	2	2	1	5	2	2	1	5	2	2	1	5
13	2	2	1	5	2	2	1	5	2	2	1	5
14	2	2	1	5	1	1	1	3	2	2	1	5
15	2	2	1	5	1	0	0	0	2	2	1	5
16	2	2	1	5	2	2	1	5	2	2	1	5
17	2	2	1	5	2	2	1	5	2	2	1	5
18	2	2	1	5	0	0	0	0	2	2	1	5
19	2	2	1	5	2	2	1	5	2	2	1	5
20	2	2	1	5	2	2	1	5	2	2	1	5

❖ Survey 2008-2

Lab n°	Patient P4 Glycerol kinase def.				Patient P5 α -mannosidosis				Patient P6 3MCC deficiency			
	A	I	R	Total	A	I	R	Total	A	I	R	Total
1	2	0	1	3	2	1	1	4	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	2	2	1	5	2	2	1	5
5	2	2	1	5	2	2	1	5	2	2	1	5
6	0	0	0	0	0	0	0	0	2	2	1	5
7	2	2	1	5	2	2	1	5	2	2	1	5
8	0	0	0	0	2	1	1	4	2	2	1	5
9	2	2	1	5	2	2	1	5	2	2	1	5
10	2	2	1	5	0	0	0	0	2	2	1	5
11	2	2	1	5	0	0	0	0	2	2	1	5
12	2	2	1	5	2	2	1	5	2	2	1	5
13	2	2	1	5	2	2	1	5	2	2	1	5
14	2	2	1	5	1	0	1	2	2	2	1	5
15	2	2	1	5	0	0	0	0	1	2	1	4
16	1	0	0	1	2	2	1	5	2	2	1	5
17	2	2	1	5	2	2	1	5	2	2	1	5
18	2	2	1	5	2	1	1	4	2	2	1	5
19	0	0	1	1	2	2	1	5	2	2	1	5
20	2	2	1	5	2	2	1	5	2	2	1	5

❖ Total scores

Lab number	Survey 2008-1	Survey 2008-2	Cumulative score	Cumulative score (%)
1	10	12	22	73%
2	15	15	30	100%
3	15	15	30	100%
4	15	15	30	100%
5	15	15	30	100%
6	10	5	15	50%
7	15	15	30	100%
8	13	9	22	73%
9	15	15	30	100%
10	7	10	17	57%
11	15	10	25	83%
12	15	15	30	100%
13	15	15	30	100%
14	13	12	25	83%
15	10	9	19	63%
16	15	11	26	87%
17	15	15	30	100%
18	10	14	24	80%
19	15	11	26	87 %
20	15	15	30	100%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	10	50 %
Good performers (> 75 % good responses)	15	75 %
Poor performers (< 50 % good responses)	0	0 %

❖ Summary of scores

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Recommendations (%)	Total (%)
Patient P1	CbIC/D	98	98	95	97
Patient P2	MPS III	73	70	75	71
Patient P3	2-hydroxyglutaric	100	100	100	100
Patient P4	Glycerol kinase	83	75	85	80
Patient P5	α -mannosidosis	78	68	80	74
Patient P6	3MCC def.	98	100	100	99

DPT-scheme in 2009

- Two surveys of 3 urines, including “normal” patients
- Results have to be sent within 3 weeks
- The website system developed by CSCQ (Centre Suisse de Contrôle de Qualité) should be used as pilot, adjacent to old system for all participants in round two of 2009 samples. But overall organization of schemes in 2009 seems to be premature.
- **Poor performers:** during the last Scientific Advisory Board meeting, there was much discussion as to how satisfactory performance should be defined. Previously for the DPT schemes a score of less than 15 would indicate poor performance (<50%). The main concerns with this cut off is that ERNDIM is giving false reassurance about how well a laboratory is doing even though that laboratory may be missing a significant number of important metabolites/interpretation of results. It was felt by the group that the limits for poor performance should be higher. After some discussion, it was decided that **any laboratory with a score of below 18 (<60%) is now deemed unsatisfactory.**
- Good performers: those who reached a score > 75 %

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, if possible, for organic acids.

Meeting in 2009

There is no SSIEM Symposium next year, because of the international IEM meeting in San Diego, USA. Therefore, an ERNDIM meeting will be organized in Basel **on 22 and 23 October 2009**. Further information concerning this meeting will be available soon.

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.



Service Maladies héréditaires du Métabolisme et Dépistage Néonatal
 Centre de Biologie et de Pathologie Est
 59, Bd Pinel
 69677 Bron cedex
 Tel 33 4 72 12 96 94
 Fax 33 4 72 12 97 20

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 Glutathion synthetase
- 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 Isovaleric acidemia
 - 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