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 France 2017

ANNUAL REPORT 2017

In 2017, 25 labs participated to the DPT France scheme.
Scientific Advisor: Dr Christine Vianey-Saban
Deputy Scientific Advisor: Dr Cécile Acquaviva-Bourdain
Maladies Héréditaires du Métabolisme, Centre de Biologie et de Pathologie Est, CHU Lyon, France.
Scheme Organiser: Dr Xavier Albe, CSCQ (Centre Suisse de Contrôle de Qualité), Chemin du Petit-Bel-Air 2, 1225 Chêne-Bourg, Switzerland.

Note: This annual report is intended for participants of the ERNDIM DPT France scheme. The contents should not be used for any publication without permission of the Scientific Advisor.

Geographical distribution of participants

Country	Number of labs
France	11
Italy	5
Spain	5
Portugal	2
Czech Republic	1
Switzerland	1
Total	**25**

Logistic of the scheme

- **2 surveys** 2017-1: patient A, B and C
  2017-2: patient D, E and F

Samples

- Patient A : **Citrullinaemia type I**. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient B : **MNGIE**

- Patient C : **Formiminoglutamic aciduria**
- Patient D : **GM1 gangliosidosis**
- Patient E : **No IEM**
- Patient F : **Imerslund-Gräsbeck**

Samples have been kindly provided by Johanne Croft, Sheffield, UK, and Christelle Corne, Grenoble, France.

Mailing

Samples were prepared and sent by CSCQ (Centre Suisse de Contrôle de Qualité) at room temperature. One mailing for the 2 surveys.

Timetable of the schemes

– February 6	Shipment of samples of Survey 1 and Survey 2 by CSCQ
– February 20	Clinical data available on CSCQ website and start analysis of samples (Survey 1)
– March 6	Reminder for website submission
– March13	Deadline for result submission (Survey 1)
– April 7	Interim report of Survey 1 available on CSCQ website
– May 22	Clinical data available on the CSCQ website and start analysis of samples (Survey 2)
– June 5	Reminder for website submission
– June 12	Deadline for result submission (Survey 2)
– July 17	Interim report of Survey 2 available on CSCQ website
– November 21	Meeting in Manchester
– November 23	SAB meeting: definition of critical errors
– December 21	Annual Report with definitive scoring sent by e-mail

CSCQ Website reporting

Since 2011, the website reporting system is compulsory for all centres. Please read carefully the following advices:

- Selection of tests: **don't select a test if you will not perform it**, otherwise the evaluation program includes it in the report.
- Results
  - Give quantitative data as much as possible.
  - Enter the key metabolites with the evaluation **in the tables** even if you don't give quantitative data.
  - If the profile is normal: enter "Normal profile" in "Key metabolites".
  - **Do not enter results in the "comments" window**, **otherwise your results will not be included in the evaluation program.**

Analyte	Method	Key Metabolite	Quant. result	Unit	Evaluation	Qual. result
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	
Amino acid quantitative	LC-MS/MS	Please specify key metabolite	******	mmol/mol creat	To be entered	

Comments:

- Recommendations = **advice for further investigation**.
  - Scored together with the interpretative score.
  - Advices for treatment are not scored.
  - **Don't give advice for further investigation in "Comments on diagnosis"**: it will not be included in the evaluation program.

	Survey 1	Survey 2
	(3 weeks)	(3 weeks)
Receipt of results	24 labs	25 labs
No answer	**1**	**0**

Scoring of results

The scoring system established by the Scientific Advisory Board (SAB) of ERNDIM has changed in 2013. Two 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 and recommendations	Good, diagnosis is established	2
		Helpful but incomplete	1
		Misleading / wrong diagnosis	0

The **total score** is calculated as the sum of these 2 criteria without weighting. The maximum that can be achieved is 4 for one sample.

Meeting of participants

It took place in Manchester on Tuesday 21 November 2017 from 18.00 to 19.30, during the ERNDIM participant meeting.

❖ Participants

Representatives from 10 labs were present: J Garcia Villoria, A Ribes (Hospital Clinic, Barcelona), JA Arrantz, C Carnicer-Caceres (Vall d'Hebron, Barcelona), MH Read (Caen), PA Binz, O Braissant, C Roux-Petronelli (Lausanne), M Mordas (Lisbon), B Merinero, P Ruiz-Sala (Madrid), M Gastaldi (Marseille), G Polo (Padova), C Ottolenghi, C Pontoizeau (Hôpital Necker, Paris), S Boenzi, C Rizzo (Rome).

❖ Information from the Executive Committee and the Scientific Advisory Board (SAB)

- **Scoring**
  - Scoring is done by 2 scheme organizers, who change every year. The results of DPT France 2017 have also been scored by Dr Johanne Croft, from DPT UK. At the SAB meeting on November 23, the definitive scores have been finalized.
  - The concept of **critical error** has been introduced in 2014. A critical error is defined as an error resulting from seriously misleading analytical findings and / or interpretations with serious clinical consequences for the patient. For 2017, the SAB decided that non-identification of an increase of citrulline for sample A is a critical error, as well as non-identification of an increase of methylmalonic acid for sample F. For sample D, since the clinical details were indicative of a lysosomal storage disease, and the overall proficiency was quite satisfying, the SAB decided that it was a critical error to conclude to HHH syndrome or to a urea cycle disorder, without mentioning the possibility of a lysosomal storage disease.
  - **Score for satisfactory performance is ≥ 62%** (at least 15 points for a maximum of 24 points).
  - Due to the poor overall proficiency, sample C has been considered as **educational**. Samples may be classed as educational in exceptional cases, e.g. when the metabolite pattern in a sample is particularly challenging and diagnosis is hard to reach or when non-standard methods are required. The SAB decides whether a sample is classed as educational. When a sample, that has been classed educational in an earlier survey, is circulated again it will be scored routinely and cannot be educational for a second time. Therefore the maximum scoring for 2017 is 20 points.
- **Certificate of participation** for 2017 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 65% (score < 13 / 20) or if a critical error has been noticed. One performance support letter will be sent by the Scheme Advisor for 2017 because of a critical error and low score (sample F). The partial submitter will receive a letter from the ERNDIM Executive Administrator, Sara Gardner.
- **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. 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 56 °C for 1 hour. 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,
UF Maladies Héréditaires du Métabolisme, 5ème 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 mail the samples.

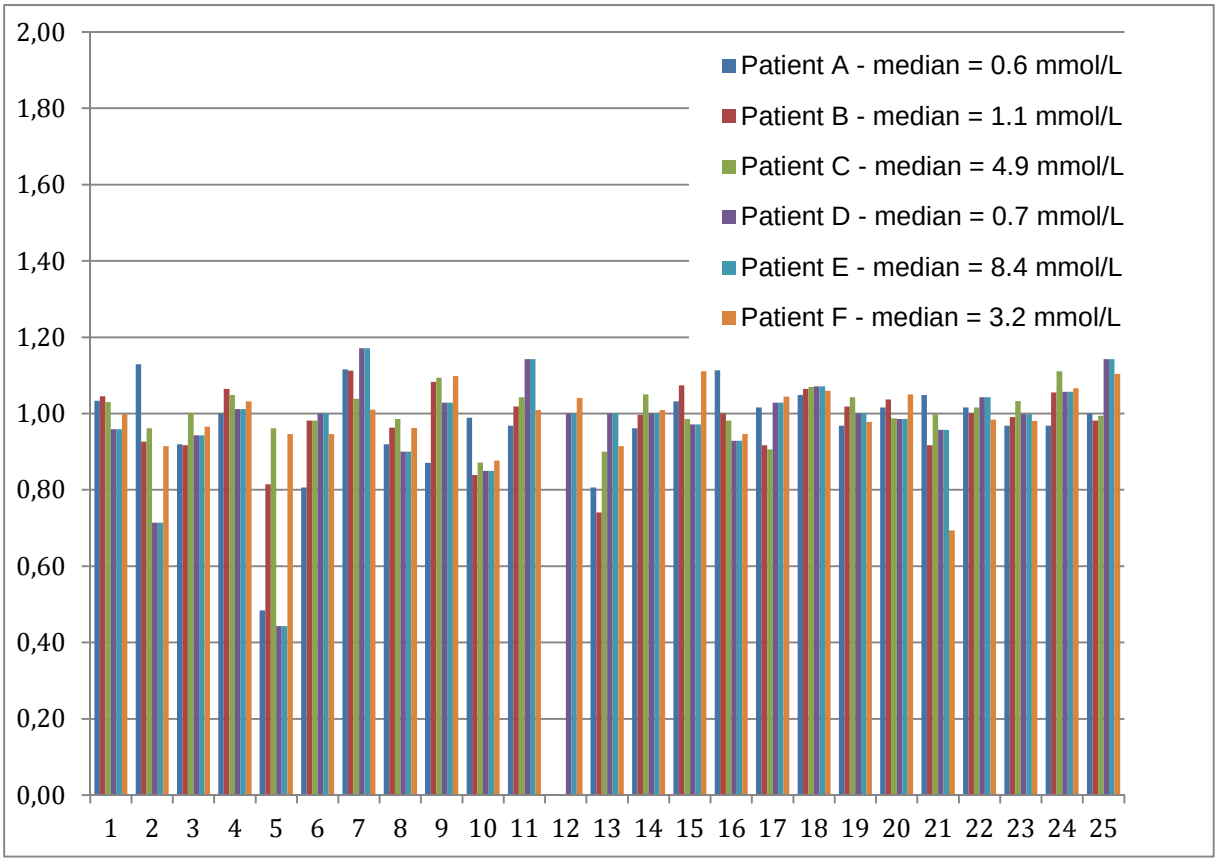
- **Lab identification**: since 2007, it has been accepted that the ERNDIM number is used for "in centre" communication but anonymous identification is used for the Annual Report on the website or other purposes.

❖ Discussion of results

- **Creatinine measurement**

Creatinine determination was mostly satisfying. Three labs had systematic low values (2, 5 and 10). There were no outlier values. Creatinine values are expressed in the figure as the ratio of each measurement over the median of all labs.
The interlab CV is < 8.8 % for all samples (4.4 % – 8.8 % for low creatinine values): this is satisfying but higher than the interlab CV 2016 for Special Assay in urine (3.8 %, n = 175).

Creatinine: ratio to median



- **Patient A – Citrullinaemia type I (gene *ASS1* – OMIM *643470)**

Provided information: "Infant presented at Emergency Department. Febrile, query infection. Sample collected after commencing therapy."

Detailed information: The patient presented, at 3 days of life, with febrile-query infection. Ammonaemia was 542 µmol/L. On the first urine sample, orotic acid was 1800 mmol/mol creatinine, and citrulline 1400 mmol/mol creatinine. This urine sample was collected after commencing therapy. The results for this patient have been presented during the general meeting and are available on the ERNDIM website (www.erndim.org).

Diagnosis

Most likely diagnosis

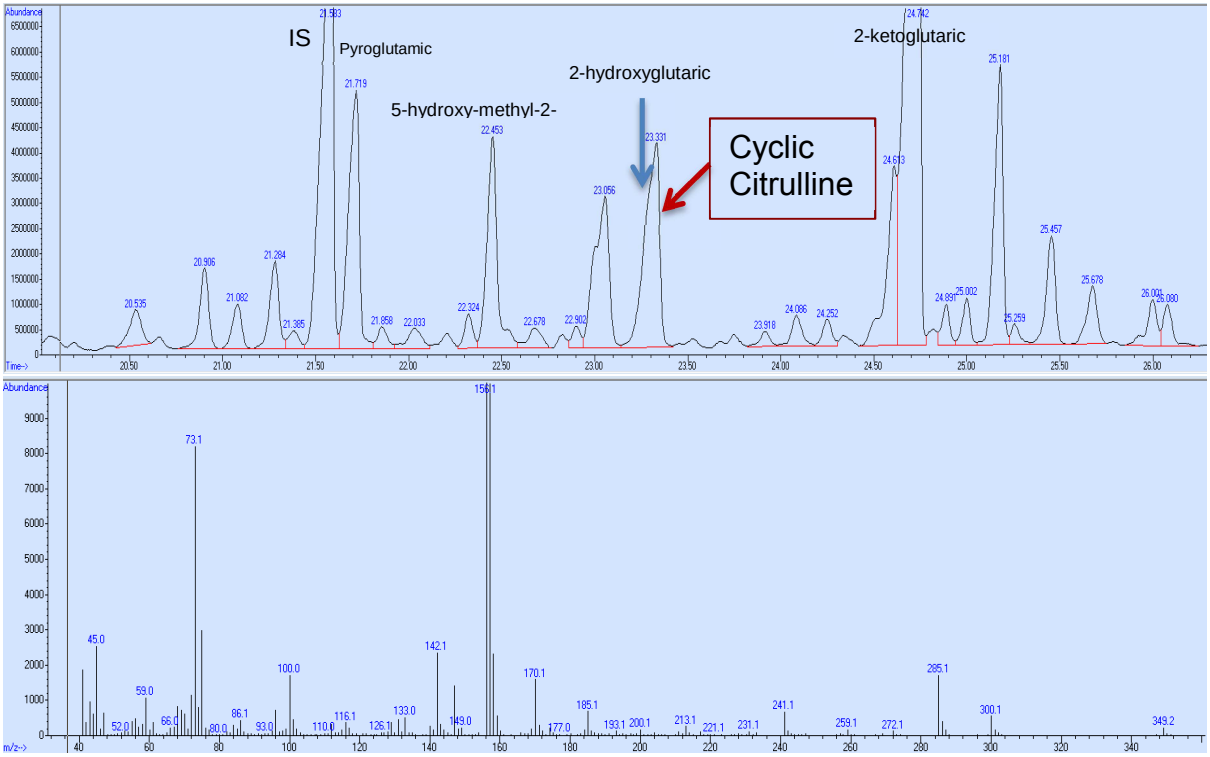
– Citrullinaemia type I	24

Alternative diagnosis

– Citrullinaemia type II	2
– Other urea cycle disorder	2

All 24 participants performed amino acids and all of them reported an increase of citrulline (median 15 109 mml/mol creat – range: 37 – 26 000; n = 24). Nine of them also reported an increase of arginine (median 75 mmol/mol creat – range: 67 – 151; n = 9).

Only 17 participants performed organic acids: all but one reported an increase of orotic acid (median 70.3 mmol/mol creat – range: 28 – 108.7; n = 8), and 6 reported an increase of cyclic citrulline, which is eluted few seconds later than 2-hydroxyglutaric acid (see spectrum below).



A specific measurement of orotic acid has been performed by 20 participants and reported as increased by 16 of them (median 71.0 mml/mol creat – range: 18.6 – 289; n = 16).

Scoring

- Analytical performance: increase of citrulline (score 1), increase of orotic acid (score 1).
- Interpretation of results and recommendations: citrullinaemia type I (score 2), another urea cycle disorder (score 1)

The SAB of ERNDIM stated that to miss the increase of citrulline would have been a critical error.

A similar urine sample has been distributed in 2011: the overall performance has improved.

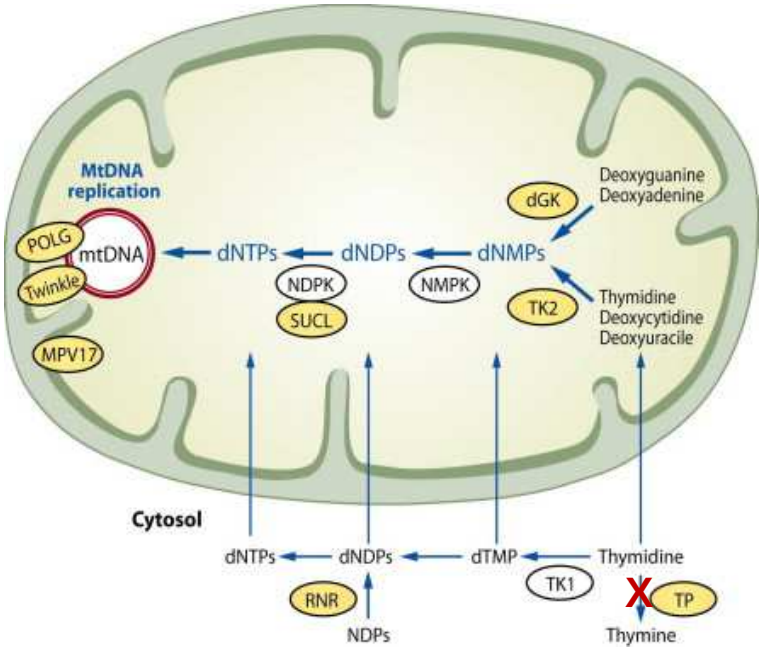
	2011	2017
Analytical performance	95 %	98 %
Interpretative performance	92 %	100 %
Overall performance	94 %	99 %

- **Patient B – MNGIE – Thymidine phosphorylase deficiency (*TYMP* gene, OMIM *131222)**

Provided information: "48-year old patient. Investigated because of diabetes and hypothyroidism from the age of 42, and diarrhoea with anorexia and denutrition from the age of 44."

Detailed information: This 48-year old patient was investigated because of diabetes and hypothyroidism starting from the age of 42, diarrhea with anorexia from the age of 44, with subsequent severe denutrition. She is receiving parenteral nutrition. Diagnosis was confirmed by measurement of thymidine phosphorylase activity in leukocytes and by mutation analysis of *TYMP* (also called *ECGF1*) gene.

Thymidine phosphorylase (TP) is a cytosolic enzyme required for nucleoside homeostasis. In MNGIE, TP activity is severely reduced and consequently levels of thymidine and deoxyuridine in plasma are dramatically elevated. The increased levels of intracellular thymidine and deoxyuridine cause imbalances of mitochondrial nucleotide pools that, in turn, lead to the mtDNA deletions or mtDNA depletion.



From Suomalainen & Isohanni. Neuromuscul Disord. 2010;20(7):429-37

Diagnosis

Most likely diagnosis

- MNGIE (mitochondrial DNA depletion syndrome 1) 18
- Respiratory chain disorder 2
- Mellitus decompensation 2
- GLUT2 1
- Glycogen storage disorder type I 1

Other possible diagnosis

- Respiratory chain disorder 5
- Dihydropyrimidine dehydrogenase deficiency 2
- MNGIE like disease as *POLG*, *RRM2B*, … 1
- 3-hydroxyisobutyrate dehydrogenase deficiency 1

All 24 participants performed organic acid analysis, and reported:

- **Increase of thymine** **16**
  (median = 43.7 mmol/mol creat, range : 14 – 69 ; n=4)
- **Increase of uracil** **12**
  (median = 49.5 mmol/mol creat, range : 16 – 103 ; n=5)
- Increase of lactic acid 22
  (median = 3 560 mmol/mol creat, range : 1 063 – 18 600 ; n=10)
- Increase of 3-hydroxybutyric acid 16
  (median = 372 mmol/mol creat, range : 221– 1 273 ; n=6)

Only 9 participants performed purines & pyrimidines analysis and reported:

- **Increase of thymidine** **9**
  (median = 263 mmol/mol creat, range : 115 – 338 ; n=7)
- **Increase of deoxyuridine** **9**
  (median = 329 mmol/mol creat, range : 51 – 373 ; n=7)
- **Increase of thymine** **8**
  (median = 45 mmol/mol creat, range : 37 – 392 ; n=7)
- **Increase of uracil** **7**
  (median = 102 mmol/mol creat, range : 62 – 113 ; n=5)

Scoring

- Analytical performance: Identification of at least 3 metabolites: thymine or uracil or thymidine or deoxyuridine (score 2), identification of only one or two metabolites (score 1).
- Interpretation of results and recommendations: Thymidine phosphorylase deficiency (MNGIE) or mitochondrial DNA depletion syndrome (score 2), respiratory chain disorder (score 1) as first or alternative diagnosis.

A similar urine sample has been distributed in 2013 but was not scored due to the poor proficiency and considered as educational.

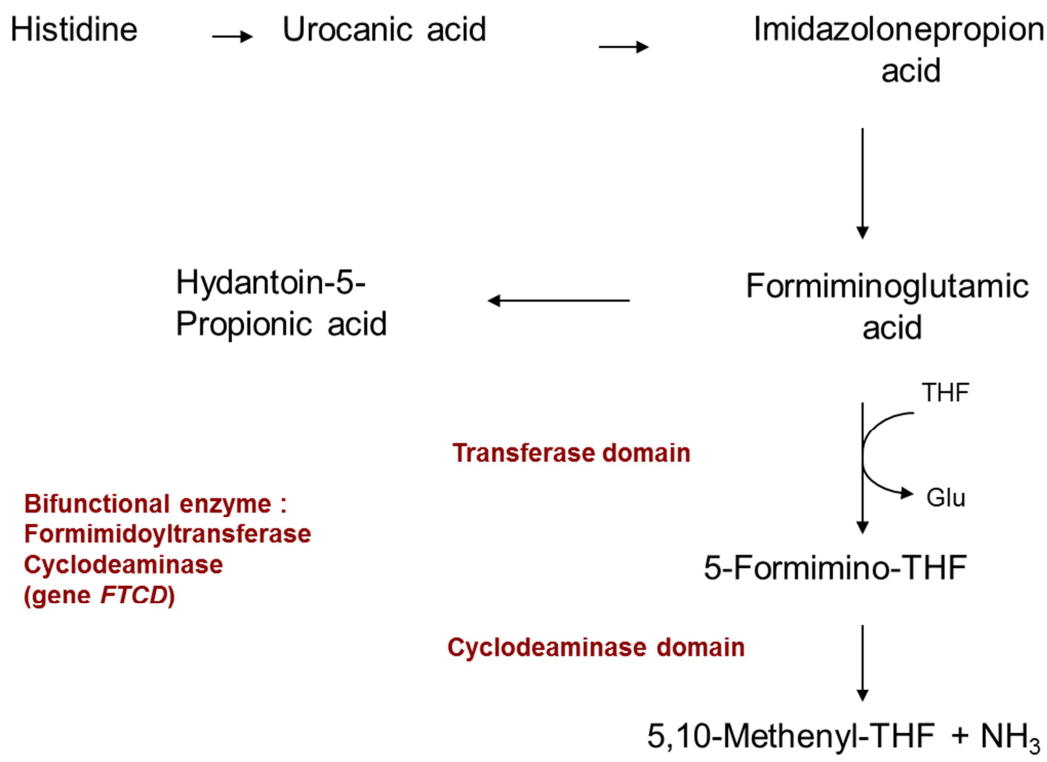
- **Patient C – Formiminoglutamic aciduria - Formimidoyltransferase cyclodeaminase deficiency (*FTCD* gene, OMIM #229100)**

Provided information: “5-year old girl. Normal development until 1 year. Delayed speech, psychomotor restlessness. Diagnosis of autism.”

Detailed information: This 5-year old girl was investigated for suspicion of autism. Her development was normal during her first year of life, but then she developed speech delay and psychomotor restlessness.

Metabolic investigation revealed on urinary organic acid profile a high increase of hydantoin-5-propionic acid, and an increase of formiminoglutamic acid in plasma amino acids by MS/MS (FIGLU = 30 µmol/L – reference values <1) and urine amino acids (FIGLU = 610 mmol/mol creatinine – reference values <5).
Folate supplementation did not modify the metabolic profiles: persistent high increase of hydantoin-5-propionic acid, and high FIGLU (= 664 mmol/mol creatinine – reference values <5) in urine.
Mutation analysis of *FTCD* gene is under investigation.

Formimidoyltransferase cyclodeaminase is a bifunctional enzyme with a transferase domain and a cylclodeaminase domain and has tetrahydrofolate (THF) as cofactor (see figure below). That is why patients with folate deficiency present with a slight increase of hydantoin-5-propionic acid and FIGLU.



Diagnosis

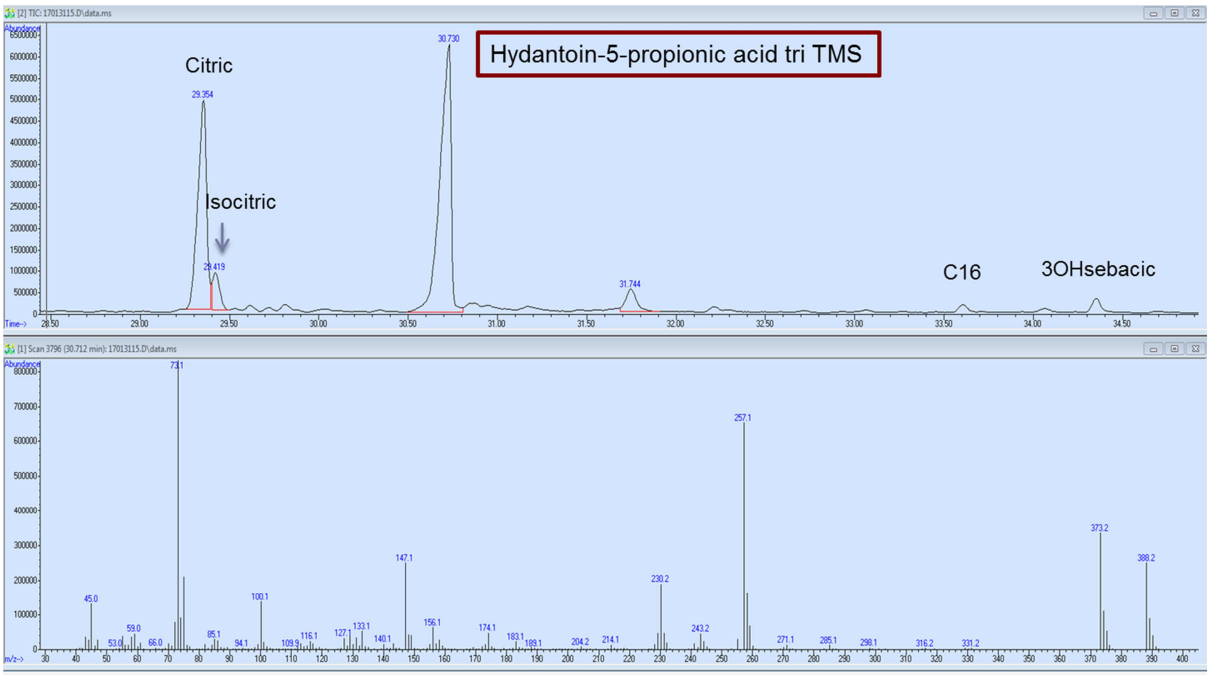
Most likely diagnosis

- **Formiminoglutamic acidura** 9
  (glutamate formiminotransferase deficiency, formimidoyltransferase cyclodeaminase deficiency)
- No significant abnormality, no diagnosis 12
- Creatine transporter defect 2
- Homocarnosinase deficiency 1

Other possible diagnosis

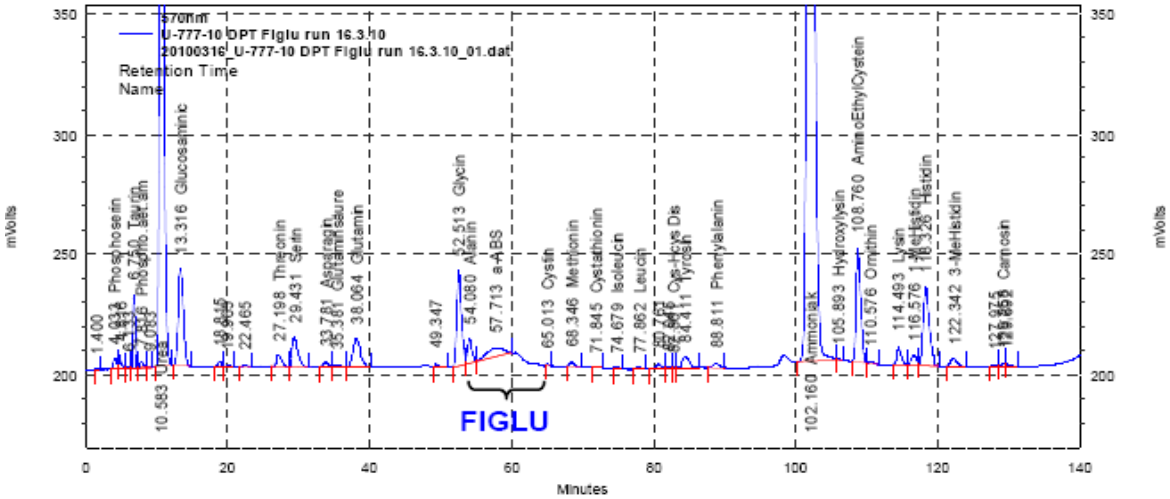
- Folate deficiency, folate metabolism deficiency 1
- Mitochondrial disorder 1

All participants performed **organic acid analysis**: but only 9 of them reported an **increase of hydantoin-5-propionic acid** (139 ; 400 ; 2369 mmol/mol creat – n=3). Hydantoin-5-propionic acid (see spectrum below) is eluted between vanilmandelic and 4-hydroxyphenyllactic acids.



Among the 23 participants who performed amino acids analysis, only 5 reported an increase of FIGLU (416 mmol/mol creat). Using ion exchange chromatography, FIGLU give a large peak overlapping citrulline (see figure below).

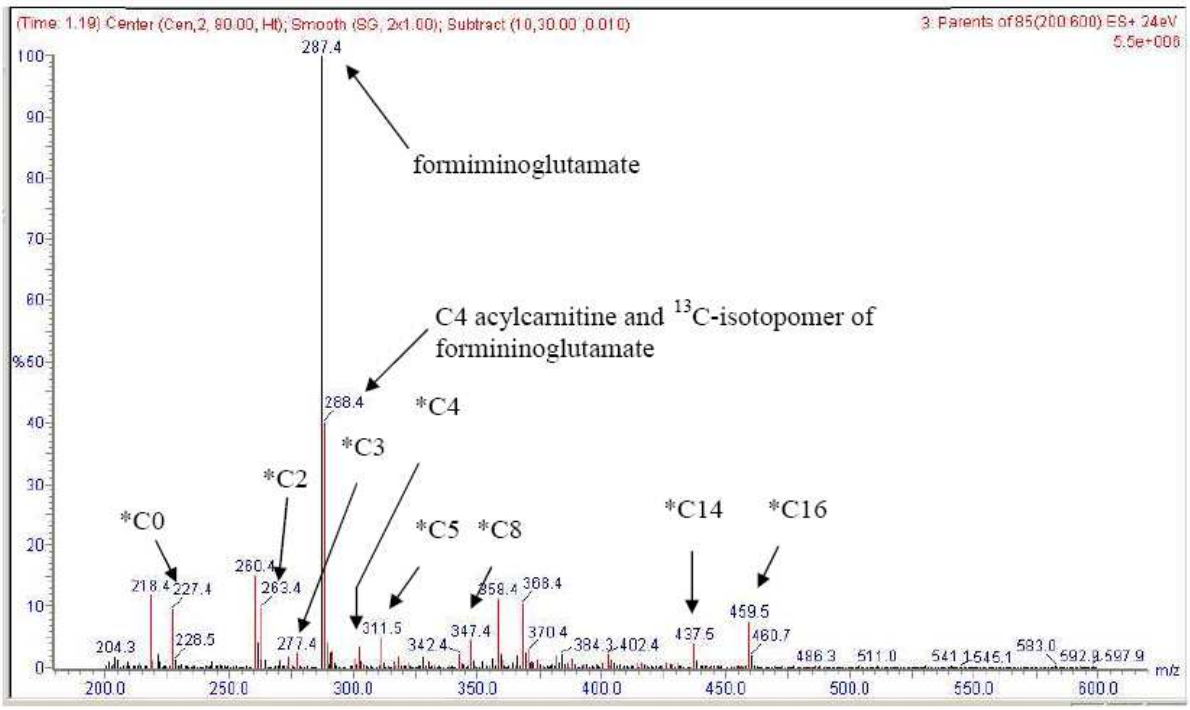
Amino acid analysis: ion exchange chromatography (Biochrom)



Courtesy Pr Brian Fowler – DPT CH

Four participants also performed acylcarnitine profile, and 3 of them reported the presence of FIGLU, while the fourth one reported a normal profile. FIGLU can be detected in the scan profile of butylated acylcarnitines, with an odd w/z of 287 (see figure below).

Glu



Courtesy Pr Brian Fowler – DPT CH

Scoring

- Analytical performance: iincrease of hydantoin-5-propionic acid (score 1), increase of FIGLU (score 1).
- Interpretation of results and recommendations: Formiminoglutamic aciduria – Formimidoyltransferase cyclodeaminase deficiency (score 2).

As the performance was very poor, the SAB considered this sample as **educational**, and will not be scored:

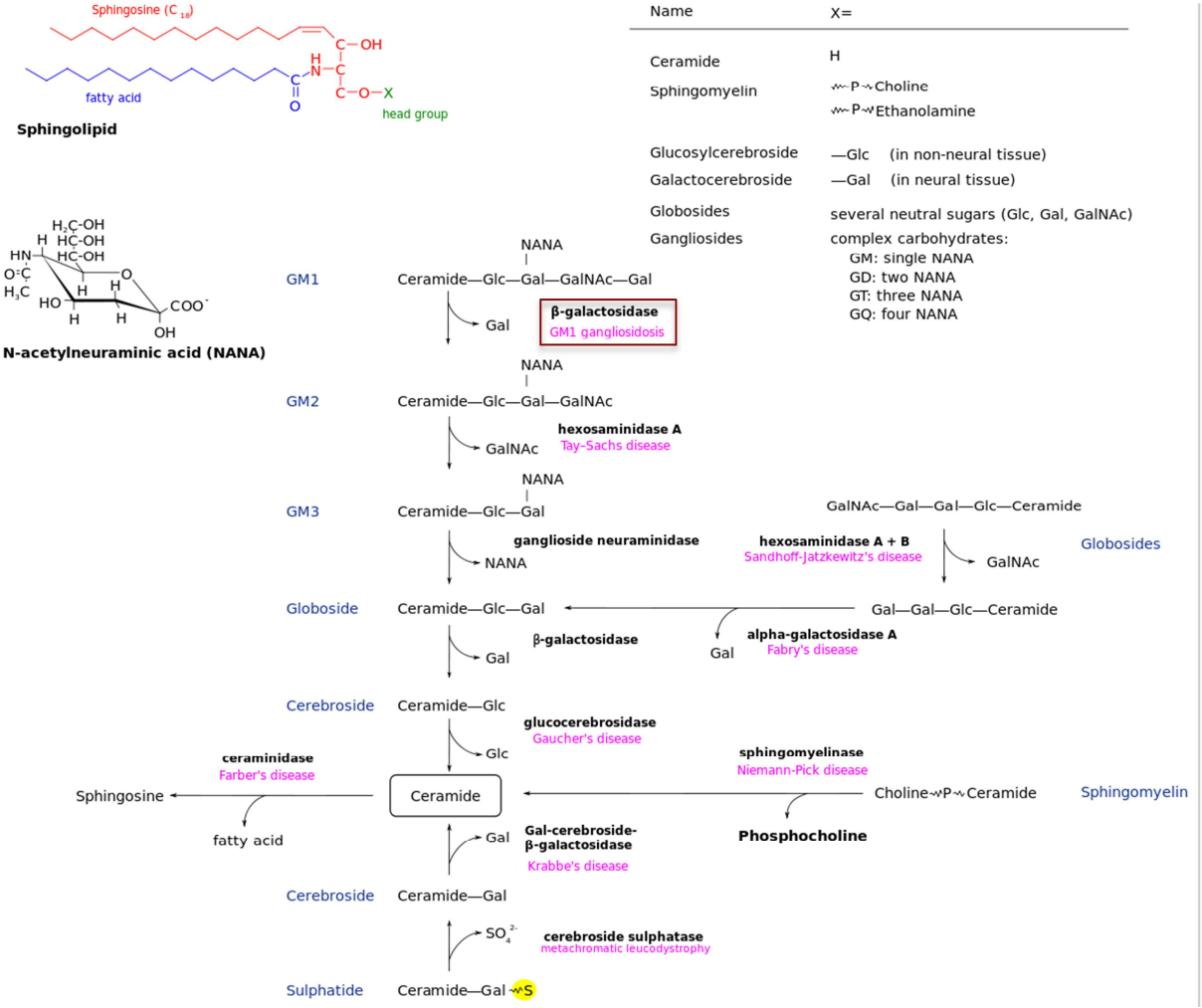
- Analytical performance : 29 %
- Interpretative performance : 38 %
- Overall performance : 33 %

- **Patient D – GM1 gangliosidosis - beta-galactosidase deficiency (*GLB1* gene - OMIM *611458)**

Provided information: "7-month-old girl. Hospitalized from birth because of ascites, dysmorphic features and hepatosplenomegaly."

Detailed information: The patient, a girl, was hospitalized from birth because of hypotonia, ascites, dysmorphic features and hepatosplenomegaly. Diagnosis of GM1 gangliosidosis was evoked by oligosaccharide analysis (LC-MS/MS): increase of galactosyloligosaccharides without sialyloligosaccharides.
Urinary glycosaminoglycan quantification (harmine) and fractionation (electrophoresis) were normal, as well as urinary free sialic acid concentration (LC-MS/MS).
Diagnosis was confirmed by measurement of beta-galactosidase activity which was deficient in leucocytes and fibroblasts.
Unfortunately, the patient died at 1 year of age.

GM1 gangliosidosis is a sphingolipidosis due to β-galactosidase deficiency, the first step in ceramide synthesis.



Diagnosis

Most likely diagnosis

– GM1 gangliosidosis (Gangliosidosis type I, beta-galactosidase deficiency)	22
– Lysosomal storage disorder	2
– HHH syndrome	1

Alternative diagnosis

– Galactosialidosis	5
– Other oligosaccharidosis	3
– Mucopolysaccharidosis type IVB	3
– Sialidosis	2
– Mucolipidosis type II	1
– Mucopolysaccharidosis type VII	1
– Infantile sialic acid storage disease	1
– Urea cycle disorder ; CDG syndrome	1

The 20 participants who performed **oligosaccharides analysis** reported an abnormal profile consistent with GM1 gangliosidosis.

Scoring

- Analytical performance: abnormal oligosaccharide profile (score 2).
- Interpretation of results and recommendations: GM1 gangliosidosis (score 2), a lysosomal storage disorder has to be searched (score 1)

Since the overall performance was excellent (88%), the SAB considered as a critical error the participant who concluded to HHH syndrome without mentioning the possibility of a lysosomal storage disorder on the clinical presentation.

A similar urine sample has been distributed in 2015: the overall performance has improved.

	2011	2017
Analytical performance	78 %	80 %
Interpretative performance	80 %	92 %
Overall performance	79 %	86 %

- **Patient E – No inborn error of metabolism**

Provided information: “61 year-old woman. Complained of muscle pain after prolonged exercise”.

Detailed information: The urine sample was from the Scientific Advisor of DPT France after a 6 km running in Parc de la Tête d'Or in Lyon!

Diagnosis

Most likely diagnosis

- No IEM or no diagnosis or rhabdomyolysis? 16
- Fatty acid oxidation defect 7
- SCAD deficiency 1
- Isobutyryl-CoA dehydrogenase deficiency 1

Alternative diagnosis

- Fatty acid oxidation defect 10
- Glycogenosis 7
- SCAD deficiency 1
- Respiratory chain disorder 1
- LIPIN1, RYR 1

As usual in normal urine samples, many investigations have been performed.

- Amino acids (25 / 25)
  - No significant abnormality 23
  - Hyperaminoaciduria 2
- Organic acids (25 / 25)
  - No significant abnormality 25
- Acylcarnitines (10 / 25)
  - No significant abnormality 7
  - Increase of butyrylcarnitine (C4) 3
    (0.38 ; 0.64 ; 0.72 mmol/mol creatinine)

- Increase of glutarylcarnitine (C5DC) 2
  (0.22 mmol/mol creatinine)

– GAGs quantification (8 / 25)
  - No significant abnormality 8

– Oligosaccharides (6 / 25)
  - Normal profile 6

– Creatine / guanidinoacetate (5 / 25)
  - Normal results 5

– Purines / pyrimidines (5 / 25)
  - Normal results 4
  - Increase of adenine 1

– GAGs fractionation (2 / 25)
  - Normal profile 2

– Lactate, oxalate, polyols, sialic acid, pipecolic acid, …

Scoring

- Analytical performance: normal metabolic investigation with at least non-significant amino acid and organic acid profiles (score 2), wrong results for one test (e.g. hyperaminoaciduria, increase of adenosine, increase of butyrylcarnitine) limited the maximum score to 1.
- Interpretation of results and recommendations: no indication for an inborn error of metabolism as first or alternative diagnosis (score 1), the recommendation of performing CK, plasma / DBS acylcarnitines or to ask for more detailed clinical information (score 1).

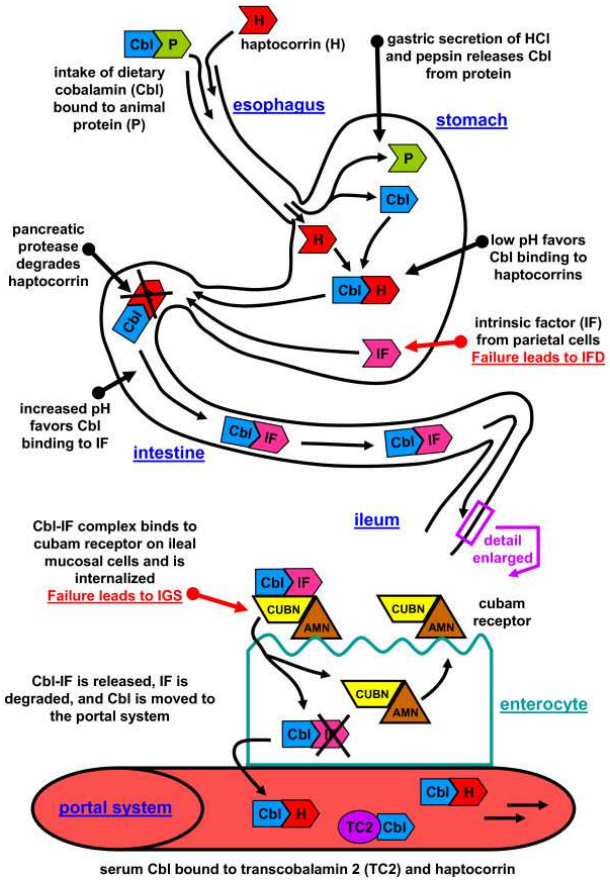
- **Patient F – Imerslund-Gräsbeck syndrome (*CUBN* or *AMN gene*, OMIM #261100)**

Provided information: “2.5 year-old boy, born from consanguineous parents. Investigated because of failure to thrive and aregenerative anemia: haemoglobin = 60 g/L.”

Detailed information: This 2.5 year-old boy is born from consanguineous parents. He was investigated because of failure to thrive and aregenerative normocytic anaemia (haemoglobin = 60 g/L). Despite a normal diet, vitamin B12 was decreased in plasma. Proteinuria was noticed.
Plasma total homocysteine was 80 µmol/L.
He received blood transfusion and was treated with intramuscular hydroxocobalamin. Anaemia was corrected but not the proteinuria. The urine sample was collected at that time.
Mutation analysis of *CUBN* and *AMN* genes is pending

Imerslund-Gräsbeck syndrome is caused by a defect of cubam, the receptor of cobalamins binded to intrinsic factor (IF-Cbl) in the endocytic apparatus of polarised epithelial cells of enterocytes.
Cubam comprises 2 components:
- Cubilin (*CUBN* gene)
- Amnionless (*AMN* gene)

Cubam is essential for endocytosis of IF-Cbl, and other molecules, including vitamin-D binding protein, albumin, transferrin and apolipoprotein A. Proteinuria is often reported.



From Tanner et al, Orph J Rare Dis 2012;7:56

Diagnosis

Most likely diagnosis

– Imerslund-Gräsbeck syndrome	4
– Methylmalonic aciduria	6
– Methylmalonyl-CoA mutase deficiency	6
– Cbl C	5
– Cbl D	4
– Cbl A	3
– Cbl F	2
– Methylmalonyl-CoA epimerase (MCEE) deficiency	2
– Methylmalonic aciduria with homocystinuria	2
– Cbl J	2
– Cbl B	2
– Cbl defect	2
– Intrinsic factor deficiency	1
– SUCLA, SUCLG1	1
– Transcobalamin deficiency	1

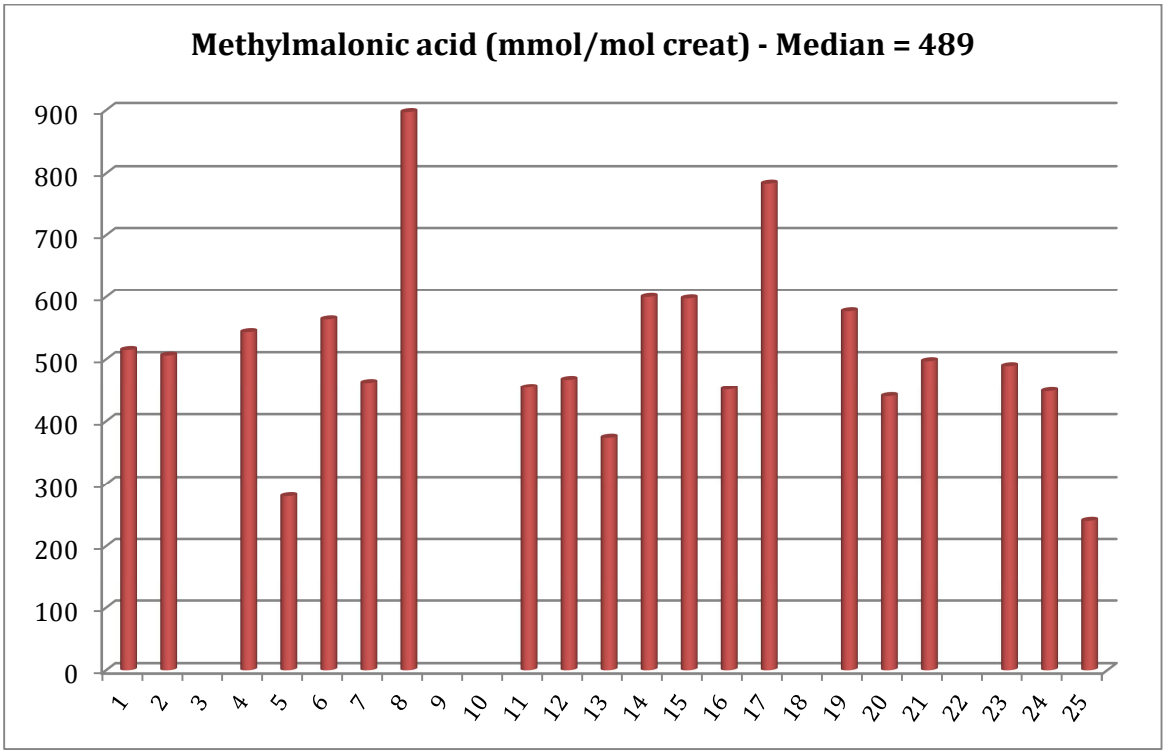
Alternative diagnosis

– Imerslund-Gräsbeck syndrome	2
– Nutritional B12 deficiency	4
– Methylmalonyl-CoA mutase deficiency	4
– Cbl D	4
– Cbl F	4
– Transcobalamin deficiency (TCII, TCI)	4
– Cbl C	3
– Cbl A	3
– Cbl J	2
– Cbl B	2
– Cbl defect	2

- Cbl X 2
- Methylmalonyl-CoA epimerase (MCEE) deficiency 1
- Methylmalonic aciduria 1
- Intrinsec factor deficiency 1
- SUCLA, SUCLG1 1

Nineteen participants reported protein determination: 11 +, 5 ++, and 3 negative.

All participants performed **organic acids**, and 22 of them reported an increase of **methylmalonic acid** (median = 489 mmol/mol creat, range: 240 – 897 ; n=21)



Methylmalonic acid excretion was not severely increased, and, according to Fowler et al (2008), was in agreement with vitamin B12 deficiency, Cbl C/D/F, Cbl A or methylmalonyl-CoA epimerase deficiency.

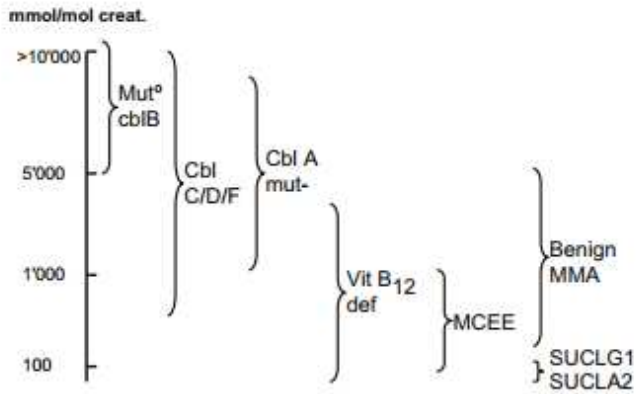


Fig. 3 Urinary MMA levels in various types of disorders. The levels shown are approximations based on ranges of values reported in the literature. Note the arbitrary nonlinear scale. Complementation groups, mut^0, mut^-, cblA, cblB, cblC, cblD, cblF. MCEE, methylmalonyl-CoA epimerase deficiency; MMA, methylmalonic acid; SUCLG1, succinate-CoA ligase, alpha subunit; SUCLA2, succinate-CoA ligase, ADP-forming beta subunit

From Fowler et al, JIMD 2008;31:350

The aetiologies of vitamin B12 deficiency are:

- Absorption defects: intrinsic factor deficiency (*GIF* gene), Imerslund-Gräsbeck syndrome (cubam receptor deficiency), or eventually haptocorrin deficiency (transcobalamin I – *TCI* gene), an unconfirmed disorder.
- Transport defects: transcobalamin deficiency (*TC2* gene), or transcobalamin receptor deficiency (*CD320* gene: mild MMA excretion).
- Nutritional deficiencies: subjects on strict vegetarian diet / vegans, breastfed babies of vegan mothers or mothers with unrecognized disturbed vitamin B12 metabolism.

Surprisingly, only 9 participants mentioned the possibility of vitamin B12 deficiency.

Seventeen participants also reported an increase of methylcitric acid (median = 41 mmol/mol creat, range: 19.4 – 140; n=9), and 11 participants an increase of 3-hydroxypropionic acid (median = 33 mmol/mol creat, range: 10 – 94; n=6)**.**

All but one participants performed **amino acid analysis,** and all reported an increase of glycine (median = 1463 mmol/mol creat, range: 686 – 15 291; n=23). Six of them reported an increase of homocystine (range: 0.9 - 10 mmol/mol creatinine; n=5) whereas 8 other reported it as undetectable. Six labs also measured total homocysteine but only half of them mentioned an increase (range: 2.5 – 6.0 mmol/mol creat; n=4).

Scoring

- Analytical performance: Increase of methylmalonic acid without a grossly elevated homocystinuria (score 2).
- Interpretation of results and recommendations: Imerslund-Gräsbeck syndrome, or a disorder of cobalamin absorption and transport, or nutritional B12 deficiency as first or alternative diagnosis (score 2), disorder of intracellular utilisation of cobalamins, or mutase deficiency, or epimerase deficiency (score 1).

- **Scores of participants**

❖ **Survey 2017-1**

Lab n°	Patient A Citrullinaemia type I			Patient B MNGIE			Patient C Formiminoglutamic ac.		
	A	I	Total	A	I	Total	A	I	Total
1	2	2	**4**	2	2	**4**	**Educational Sample Not scored**		
2	2	2	**4**	2	2	**4**			
3	2	2	**4**	1	2	**3**			
4	2	2	**4**	2	2	**4**			
5	2	2	**4**	1	2	**3**			
6	2	2	**4**	0	2	**2**			
7	2	2	**4**	0	0	**0**			
8	2	2	**4**	1	1	**2**			
9	2	2	**4**	0	1	**1**			
10	2	2	**4**	2	2	**4**			
11	2	2	**4**	1	2	**3**			
12	**Partial submitter**								
13	2	2	**4**	2	2	**4**			
14	2	2	**4**	2	2	**4**			
15	2	2	**4**	2	2	**4**			
16	2	2	**4**	1	2	**3**			
17	2	2	**4**	1	2	**3**			
18	2	2	**4**	0	1	**1**			
19	2	2	**4**	2	2	**4**			
20	2	2	**4**	2	2	**4**			
21	1	2	**3**	2	2	**4**			
22	2	2	**4**	2	2	**4**			
23	1	2	**3**	0	1	**1**			
24	2	2	**4**	0	1	**1**			
25	2	2	**4**	1	2	**3**			

❖ **Survey 2017-2**

Lab n°	Patient D GM1 gangliosidosis			Patient E No IEM			Patient F Imerslund-Gräsbeck		
	A	I	Total	A	I	Total	A	I	Total
1	2	2	**4**	2	2	**4**	2	1	**3**
2	2	2	**4**	2	2	**4**	2	2	**4**
3	0	1	**1**	1	2	**3**	2	2	**4**
4	2	2	**4**	2	2	**4**	2	1	**3**
5	2	2	**4**	1	2	**3**	2	2	**4**
6	0	1	**1**	2	1	**3**	2	1	**3**
7	**Critical error**			2	1	**3**	2	1	**3**
8	2	2	**4**	2	2	**4**	2	1	**3**
9	2	2	**4**	2	2	**4**	2	2	**4**
10	2	2	**4**	2	2	**4**	2	1	**3**
11	2	2	**4**	2	2	**4**	2	1	**3**
12	2	2	**4**	1	1	**2**	2	1	**3**
13	2	2	**4**	2	2	**4**	2	1	**3**
14	2	2	**4**	2	2	**4**	2	2	**4**
15	2	2	**4**	1	1	**2**	2	1	**3**
16	2	2	**4**	0	1	**1**	2	1	**3**
17	0	2	**2**	2	2	**4**	2	1	**3**
18	2	2	**4**	1	1	**2**	2	2	**4**
19	2	2	**4**	1	2	**3**	2	2	**4**
20	2	2	**4**	2	1	**3**	2	1	**3**
21	2	2	**4**	2	2	**4**	2	1	**3**
22	2	2	**4**	2	2	**4**	2	1	**3**
23	2	2	**4**	2	2	**4**	2	2	**4**
24	2	2	**4**	2	2	**4**	2	2	**4**
25	2	2	**4**	2	1	**3**	2	1	**3**

❖ **Total scores**

Lab n°	Survey 2017-1	Survey 2016-2	Cumulative score (max = 20)	Cumulative score (%)
1	8	11	**19**	95%
2	8	12	**20**	100%
3	7	8	**15**	75%
4	8	11	**19**	95%
5	7	11	**18**	90%
6	6	7	**13**	65%
7	4	6 **Critical error**	**10**	50% **Critical error**
8	6	11	**17**	85%
9	5	12	**17**	85%
10	8	11	**19**	95%
11	7	11	**18**	90%
12	-	9	**9**	45%
13	8	11	**19**	95%
14	8	12	**20**	100%
15	8	9	**17**	85%
16	7	8	**15**	75%
17	7	9	**16**	80%
18	5	10	**15**	75%
19	8	11	**19**	95%
20	8	10	**18**	90%
21	7	11	**18**	90%
22	8	11	**19**	95%
23	4	12	**16**	80%
24	5	12	**17**	85%
25	7	10	**17**	85%

❖ **Performance**

	Number of labs	% total labs
Excellent performers (100 % of good responses)	**2**	**8 %**
Poor performers (< 65 % good responses) or critical error	**1**	**4 %**
Partial or non-submitters	**1**	**4 %**

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
Patient A	Citrullinaemia type I	**96 %**	**100 %**	**98 %**
Patient B	MNGIE	**60 %**	**85 %**	**73 %**
Patient C	Formiminoglutamic ac.	**Not scored**		
Patient D	GM1 gangliosidosis	**84 %**	**92 %**	**88 %**
Patient E	No IEM	**84 %**	**84 %**	**84 %**
Patient F	Imerslund-Gräsbeck	**100 %**	**68 %**	**84 %**

DPT-scheme in 2018

- 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, before the deadline. Read carefully the advices on page 2.
- **Scoring**: performed by two different scheme organizers. The concept of critical error will be maintained.

We remind you that to participate to the DPT-scheme, you must perform at least:
- Amino acids
- Organic acids
- Oligosaccharides
- Mucopolysaccharides
- Purines and pyrimidines

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 2018

It will take place during the SSIEM meeting in Athenes **Tuesday 4 September 2018** from 9.00 to 10.30 am. Information concerning the venue is available on http://www.ssiem2018.org.

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.

DPT France
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

DIAGNOSTIC PROFICIENCY TESTING (DPT) FRANCE

URINE SAMPLES ALREADY SENT

• **1998 : 1**	**A**	**OCT**
	B	**Propionic acidemia**
• **1999 : 1**	**C**	**MPS I or II**
	E	**Cystinuria (common sample)**
• **1999 : 2**	**D**	**CblC**
	F	**HMG-CoA lyase deficiency**
• **2000 : 1**	**G**	**Iminodipeptiduria (common sample)**
	H	**Glutathion synthetase**
• **2001 : 1**	**P1**	**Mevalonate kinase deficiency**
	P2	**L-2-OH glutaric**
• **2001 : 2**	**P3**	**Methylmalonic (common sample)**
	P4	**MPS IIIA San Fillippo**
• **2002 : 1**	**P1**	**LCHAD deficiency**
	P2	**Sulphite oxidase deficiency**
• **2002 : 2**	**P3**	**Biotinidase deficiency (common sample)**
	P4	**MPS I**
• **2003:1**	**P1**	**Tyrosinemia type I**
	P2	**SC-BCAD deficiency**
	P3	**Argininosuccinic aciduria**
• **2003:2**	**P4**	**MCC deficiency**
	P5	**Sialidosis (common sample)**
	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**
	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	**Methylmalonic semialdehyde dehydrogenase def.**
• **2011:2**	**D**	**Mucopolysaccharidosis type IVA (Morquio)**
	E	**Phenylketonuria**
	F	**Citrullinemia type I**
• **2012:1**	**A**	**Intermittent MSUD (common sample)**
	B	**HHH syndrome**
	C	**Mucopolysaccharidosis type I**
• **2012:2**	**D**	**“RedBulluria”**
	E	**CblC**
	F	**SCAD deficiency**
• **2013:1**	**A**	**NFU1 deficiency**
	B	**MNGIE syndrome (educational)**
	C	**Lysinuric protein intolerance (common sample)**
• **2013:2**	**D**	**Mitochondrial acetoacetyl-CoA thiolase deficiency**
	E	**Morquio disease (MPS IV)**
	F	**Glycerol kinase deficiency**
• **2014:1**	**A**	**Iminodipeptiduria**
	B	**HHH syndrome (common sample)**
	C	**4-hydroxybutyric aciduria**
• **2014:2**	**D**	**Fucosidosis**
	E	**L-2-hydroxyglutaric aciduria**
	F	**SCHAD deficiency**
• **2015:1**	**A**	**Combined malonic & methylmalonic aciduria**
	B	**Homocystinuria-CBS deficiency (common sample)**
	C	**Mucopolysaccharidosis type VI**
• **2015:2**	**D**	**N-acetylaspartic aciduria**
	E	**D-2-hydroxyglutaric aciduria type II**
	F	**GM1 gangliosidosis**
• **2016:1**	**A**	**Primary hyperoxaluria type II (common sample)**
	B	**Methionine S-adenosyltransférase (MAT) def.**
	C	**Glycerol kinase deficiency**
• **2016:2**	**D**	**Ethylmalonic encephalopathy (*ETHE1* gene)**
	E	**Mucopolysaccharidosis type IVA**
	F	**Argininosuccinic aciduria**