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ERNDIM Diagnostic Proficiency Testing France 2016

ANNUAL REPORT 2016

In 2016, 24 labs participated to the DPT France scheme.
Scheme Advisor: Dr Christine Vianey-Saban
Deputy Scheme Advisor : Dr Cécile Acquaviva-Bourdain
Service Maladies Héréditaires du Métabolisme et Dépistage Néonatal, Centre de Biologie et de Pathologie Est, Lyon, France.
Scheme Organizer: Dr Xavier Albe, CSCQ (Centre Suisse de Contrôle de Qualité), Chemin du Petit-Bel-Air 2, 1225 Chêne-Bourg, Switzerland.

Geographical distribution of participants

Country	Number of labs
France	10
Italy	5
Spain	5
Portugal	2
Netherlands	1
Switzerland	1
Total	**24**

Logistic of the scheme

- **2 surveys** 2016-1: patient A, B and C
  2016-2: patient D, E and F

Origin of patients:

- Patient A: **Primary hyperoxaluria type II (*GRHPR* gene)**. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient B: **Hypermethioninemia due to methionine S-adenosyltransférase (MAT) deficiency**
- Patient C: **Glycerol kinase deficiency**
- Patient D: **Ethylmalonic encephalopathy (*ETHE1* gene)**

- Patient E: **Mucopolysaccharidosis type IVA (Morquio A disease)**
- Patient F: **Argininosuccinic aciduria**

Samples have been kindly provided by: Christelle Corne (Grenoble, France), Brian Fowler (Basel, Switzerland), and George Ruijter (Rotterdam, The Netherlands).

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

1 February 2016	Shipment of samples of Survey 1 and Survey 2 by CSCQ
22 February 2016	Clinical data available on CSCQ website and start analysis of samples (Survey 1)
7 March 2016	Reminder for website submission
14 March 2016	Deadline for result submission (Survey 1)
13 April 2016	Interim report of Survey 1 by e-mail
23 May 2016	Clinical data available on CSCQ website and start analysis of samples (Survey 2)
6 June 2016	Reminder for website submission
13 June 2016	Deadline for result submission (Survey 2)
30 June 2016	Interim report of Survey 2 by e-mail
6 September 2016	Meeting in Rome
30 November 2016	Scientific Advisory Board meeting: decision on critical errors and final scoring
21 December 2016	Annual report with definitive scoring sent by e-mail

Date of receipt of samples

	Survey 1 + 2
+ 24 hours	24

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	24 labs
No answer	**0**	**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 Rome on Tuesday 6 September 2016 from 9.00 to 10.30, before the SSIEM Symposium.

❖ Participants

Representatives from 17 labs were present: J Garcia Villoria, A Ribes (Hospital Clinic, Barcelona), JA Arrantz (Vall d'Hebron, Barcelona), S Mesli, I Redonnet-Vernhet (Bordeaux), S Funghini (Florence), U Caruso (Genova), C Corne, S Vergnaud (Grenoble), PA Binz, O Boulat, O Braissant, C Roux-Petronelli (Lausanne), I Tavares de Almeida (Lisbon), M Gastaldi (Marseille), G Polo, A Zacchettin, M Zampieri (Padova), F Habarou (Hôpital Necker, Paris), MC Freitas, D Quelhas (Porto), C Rizzo (Rome), W Onkenhout (Rotterdam), S Bekri, A Tebani (Rouen), JA Cocho (Santiago de Compostella), M Unceta Suarez (Vizcaya).

❖ Information from the Executive Board and the Scientific Advisory Board

- **Scoring and certificate of participation**: scoring is done by 2 scheme organizers, who change every year. The results of DPT France 2016 have been also scored by Dr Petr Chrastina, from DPT Czech Republic. At the SAB meeting on November 30, 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 2016, the SAB decided that non-identification of an increase of glyceric acid and oxalic acid for sample A is a critical error, as well as non-identification of argininosuccinic for sample F.
- Certificate of participation for 2016 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 62% (score < 15 / 24) or if a critical error has been noticed. Two performance support letter will be sent by the Scheme Advisor for 2016 because of a critical error (sample F). The partial submitters 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,
  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.

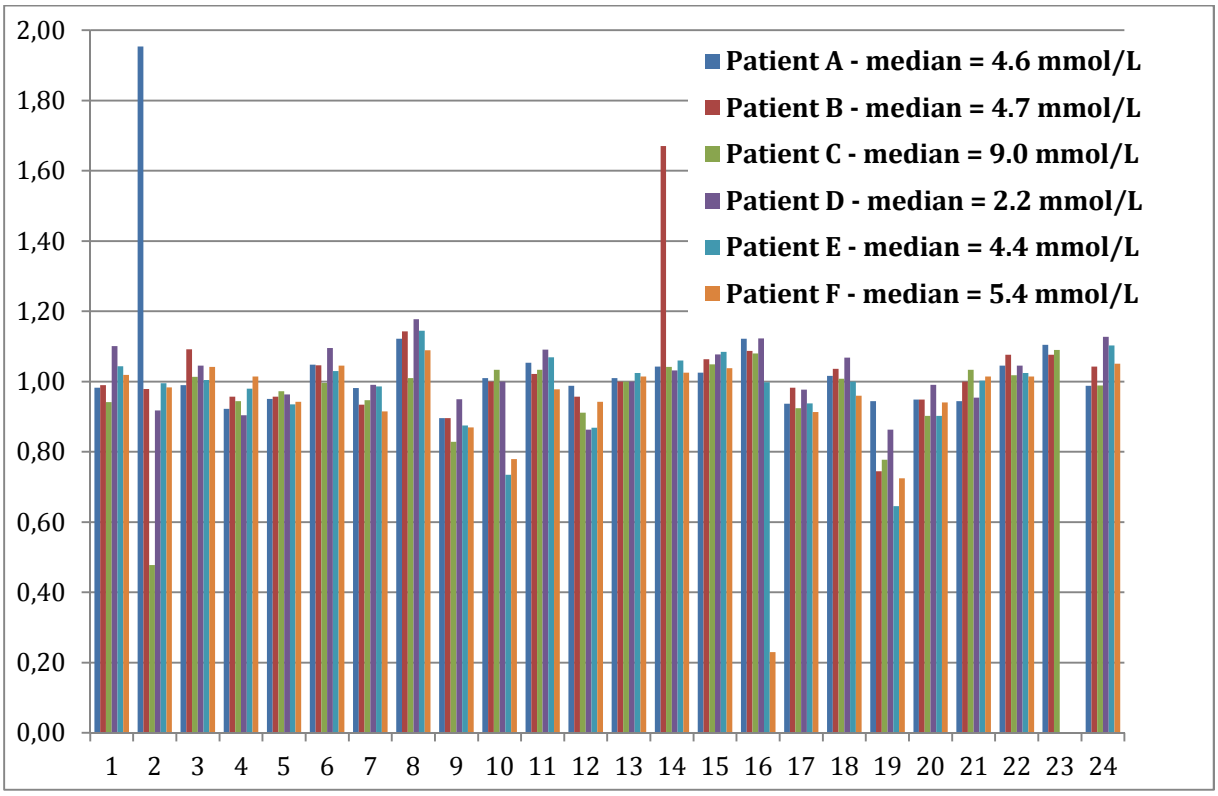
  Please send us an e-mail on the day you send the samples.
- **Lab identification**: since 2007, it has been accepted that the ERNDIM number is used for "in centre" communication but anonymous identification is used for the Annual Report on the website or other purposes.

❖ Discussion of results

- **Creatinine measurement**

Creatinine determination was mostly satisfying. One lab had systematic low values (lab 19), and there were 4 wrong values (lab 2, 14 and 16). Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.
CV is < 7 % (5.0 – 7.0 %) for all samples after exclusion of wrong values and this is rather similar to the interlab CV 2015 for Special Assay in urine (6.1 %, n = 188), and the interlab CV 2015 for Quantitative organic acids (6.9 %, n = 70).

Creatinine: ratio to median



- **Patient A – Primary hyperoxaluria type II (*GRHPR* gene – OMIM #*604296)**

At the age of 5 years this boy was referred for the first time to a paediatric nephrologist, because of urolithiasis. At ages 7 and 10, again renal stones were found. At the time of the urine collection, he was 10 y old and in good health. He used no medication, had a normal diet and adequate renal function. Diagnosis of hyperoxaluria type II was confirmed by mutation analysis.
The following concentrations were determined in the lab who referred this sample after preparation by the CSCQ:

- Oxalate = 230 mmol/mol creat (controls <108)
- Glycolate = 74 mmol/mol creat (controls <96)
- Glycerate = 937 mmol/mol creat 937 (controls <29)

The results for this patient have be presented during the general meeting and are available on the ERNDIM website (www.erndim.org).

Diagnosis

Most likely diagnosis

– **Hyperoxaluria type II**	**23**
– Hyperoxaluria type I	1

Alternative diagnosis

- D-glyceric aciduria 2

All participants performed organic acids and all but one reported an increase of glyceric acid (median 1215 mml/mol creat – range: 70 – 7843; n = 15), and 7 an increase of oxalic acid (median 216 mml/mol creat – range: 59 – 220; n = 6). Seven participants mentioned that glycolic acid excretion was normal but 6 reported a normal excretion of oxalic acid.

Six labs performed a specific measurement of oxalic acid and 4 of them reported an increase of this metabolite.

Among the 20 participants who performed amino acids, 10 reported a slight hyperaminoaciduria and 10 a normal profile or no significant abnormality.

Scoring

- Analytical performance: increase of glyceric acid (score 1), increase of oxalic acid (score 1).
- Interpretation of results and recommendations: hyperoxaluria type II (score 2), hyperoxaluria type I (score 1)

The SAB of ERNDIM stated that to miss the increase of glyceric acid and oxalic acid would have been a critical error.

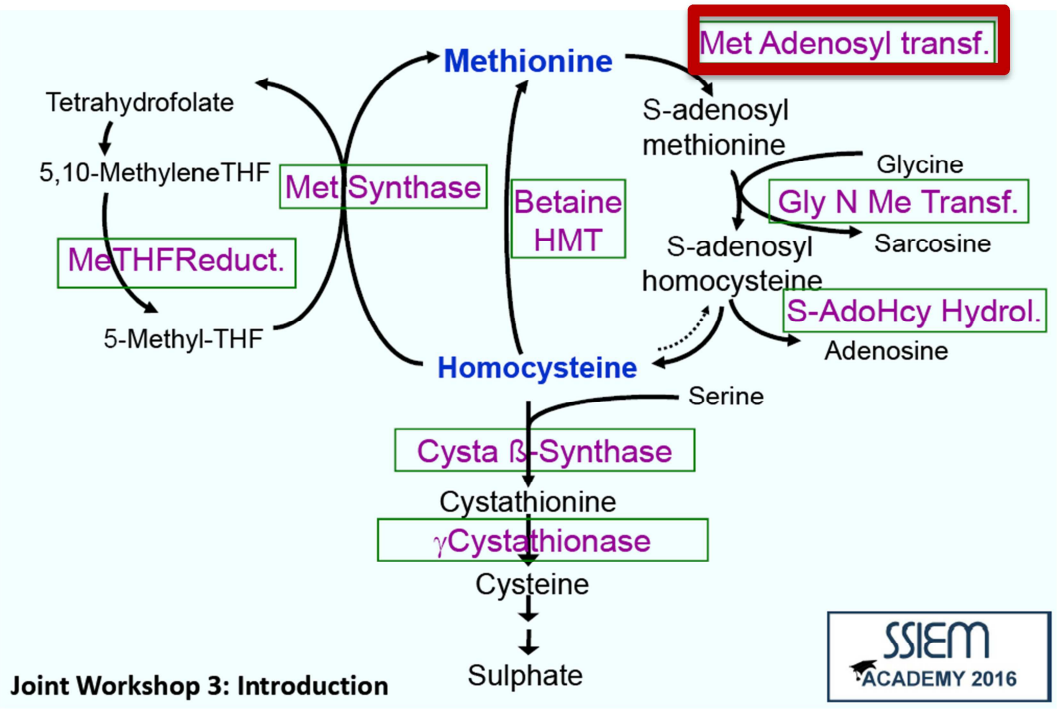
A similar urine sample has been distributed in 2006 (hyperoxaluria type I): the overall performance has slightly improved.

	2006	2016
Analytical performance	84 %	73 %
Interpretative performance	76 %	98 %
Overall performance	80 %	85 %

- **Patient B – Methionine S-adenosyltransférase (MAT) deficiency (*MAT1A* gene, OMIM #610550)**

This patient with hypermethioninemia due to methionine S-adenosyltransférase (MAT) deficiency had no clinical symptoms. She has been discovered on newborn screening that showed high methionine level. She never had clinical symptoms, and is on a mostly vegetarian “diet”. The diagnosis was confirmed by mutation analysis of the *MAT1A* gene. This urine sample has been collected at 4 years of age.

More than 60 patients with methionine S-adenosyltransferase deficiency have been described. Many of them have been detected by neonatal screening. The great majority has been so far symptom free, suggesting a benign condition. Neurological abnormalities and demyelination of the brain have been observed in a few patients. It is due to a deficiency of the hepatic form of the enzymes MAT I/III (but not the extrahepatic form, MATII). MATI & MATIII are coded by the same gene: *MAT1A.* MATI corresponds to a tetrameric form and MATIII to a dimeric form of a single α1-subunit. Treatment is generally not indicated but treatment with S-adenosylmethionine may be advisable in symptomatic patients.



Diagnosis

Most likely diagnosis

– **MAT deficiency**	**9**
– Glutamate formiminotransferase deficiency	6
– 3-methylglutaconic aciduria	3
– Mild cystathionine β-synthase deficiency	2
– No abnormality	2
– No diagnosis	2

Other possible diagnosis

– **MAT deficiency**	**2**
– Glycine-N-methyltransferase deficiency	3
– Mild cystathionine β-synthase deficiency	3
– S-adenosylhomocysteine hydrolase deficiency	2
– Folate deficiency	2
– Other	1

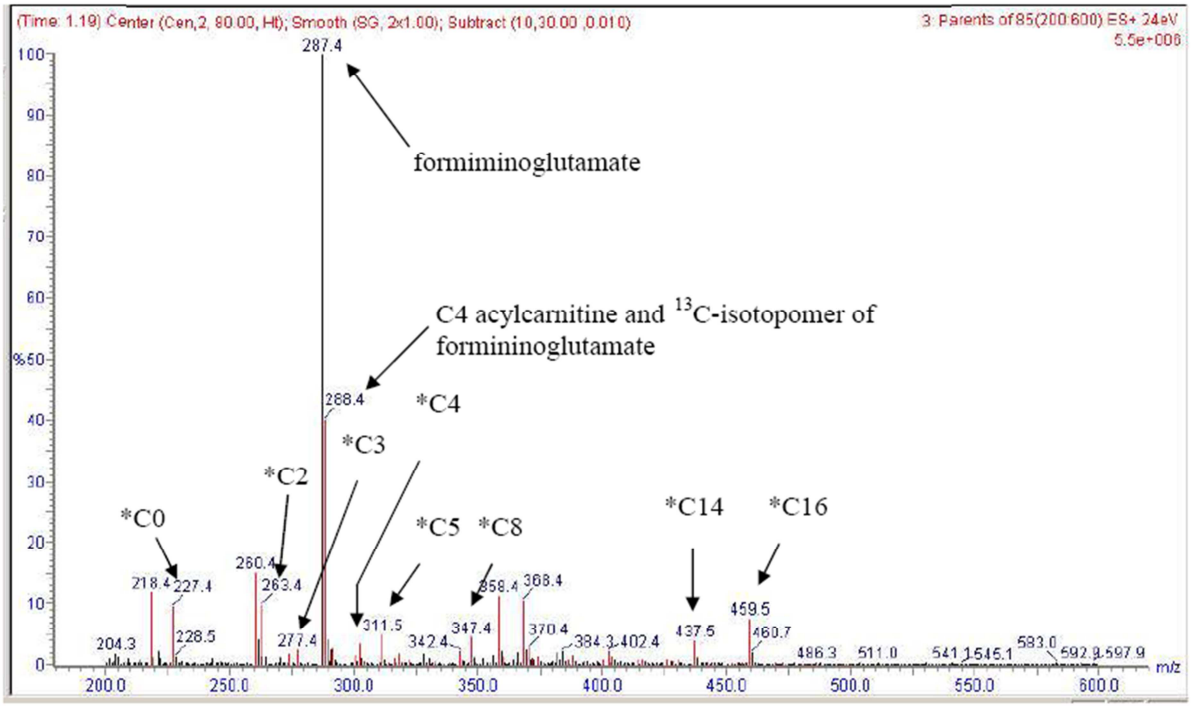
(adenosine kinase deficiency, 3-methylglutaconic aciduria, hypermethioninemia due to vegetarian diet or other causes)

All but 1 participant (who had a problem with his amino acid analyser) performed **amino acid** analysis, and reported:

– **Increase of methionine** (median = 62 mmol/mol creat, range : 30 – 85; CV = 19 %)	**23**
– Increase of methionine sulfoxide	5
– No increase of homocystine	3
– Increase of homocystine (0.8 ; 1.0 mmol/mol creat)	2

Seventeen participants performed organic acids: 17 reported that methylmalonic acid excretion is normal, 8 reported an increase of hydantoin-5-propionic acid, 5 an increase of 3-methylglutaconic acid, and 4 an increase of 3-methylglutaric acid.

The 2 labs who performed acylcarnitines, identified an increase of formiminoglutamic acid (FIGLU). FIGLU gives a signal at *m/z* 287 on acylcarnitine profile using butyl esters, as illustrated on the figure taken below from DPT Switzerland 2010 on DBS:



By amino acid analysis using tandem MS, the scheme organizer obtained a FIGLU concentration of 75 mmol/mol creat, possibly due to low folate levels (as well as for the increase of hydantoin-5-propionic acid) because of the vegetarian diet of this patient.

Scoring

- Analytical performance: increase of methionine (score 2)
- Interpretation of results and recommendations: hypermethioninemia due to methionine adenosyl transferase deficiency (score 2), hypermethioninemia without any precision and/or perform plasma amino acids analysis (score 1).

- **Patient C – Glycerol kinase deficiency (*GK* gene, OMIM #*300474)**

This forty-six year-old patient was treated for many years because of hypertriglyceridemia**.** Otherwise, he had no other symptom. The lab in charge of his follow-up measured urinary triglycerides which were found elevated: 73 g/L. Organic acids were performed at that time.
His brother has the same problem.

Diagnosis

Most likely diagnosis

- **Glycerol kinase deficiency** **23**
- No diagnosis 1

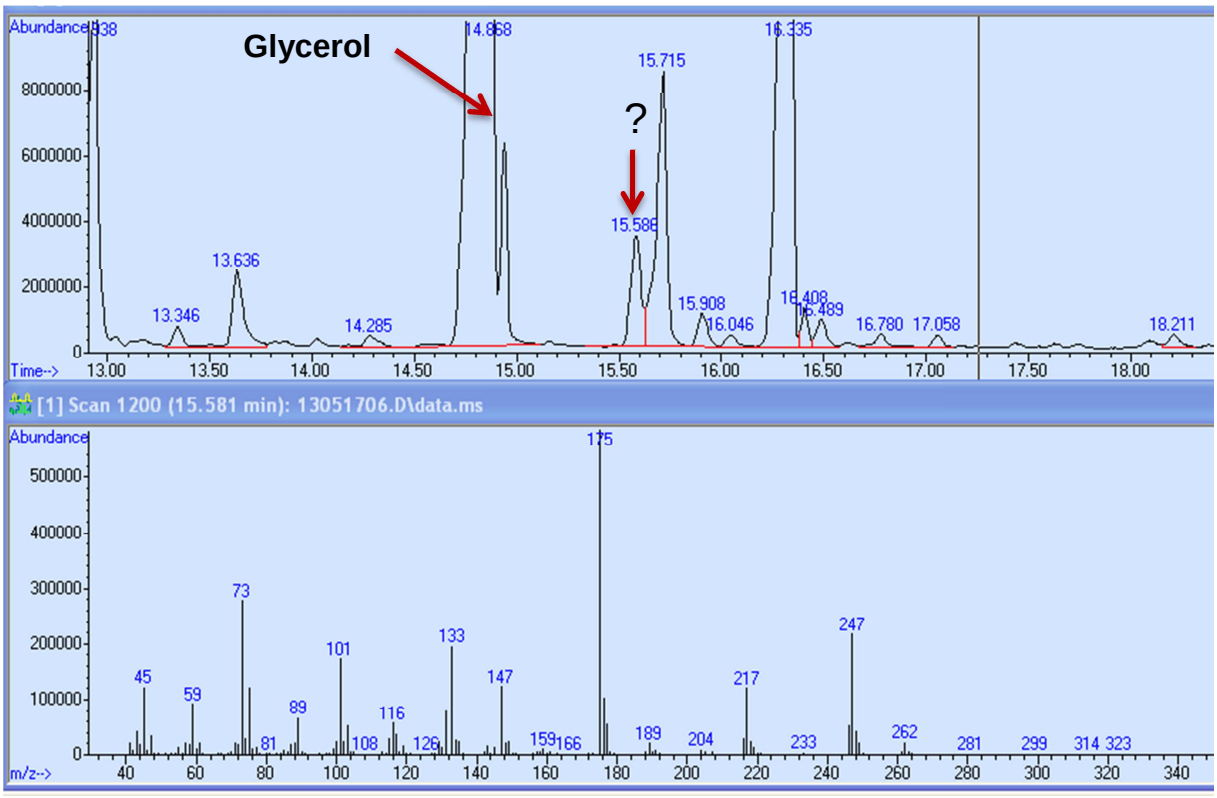
Other possible diagnosis

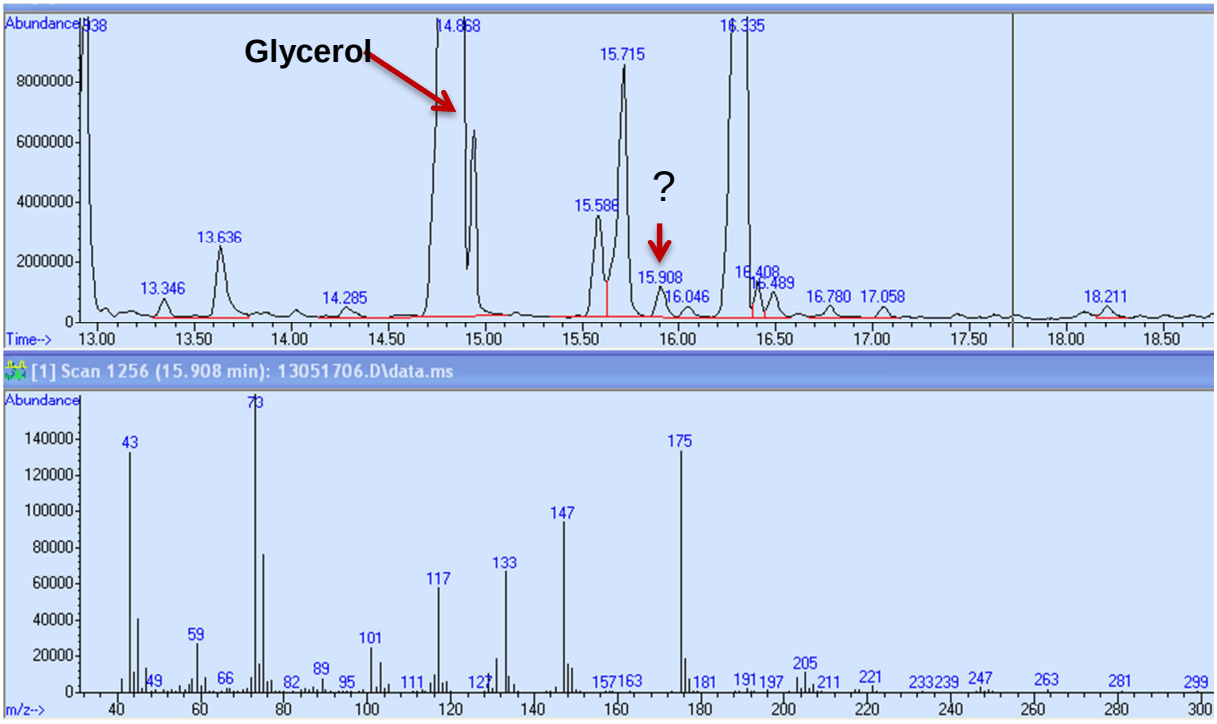
- No other suggestions

All participants performed **organic acid analysis**: all but one reported an **increase of glycerol** (median = 874 mmol/mol creat – mean = 26 731 – range: 138,7 – 191 161; n=8)

All the 21 participants who performed amino acids analysis reported a normal profile or a decrease of some amino acids.

Interestingly, Dr Soumeya Bekri from Rouen reported in 2013 the occurrence of two unknown peaks that we observed in all our patients with glycerol kinase deficiency.





Scoring

- Analytical performance: increase of glycerol (score 2).
- Interpretation of results and recommendations: glycerol kinase deficiency (score 2).

A similar urine sample has been distributed in 2013. The overall performance has improved.

	2013	2015
Analytical performance	87 %	96 %
Interpretative performance	87 %	96 %
Overall performance	87 %	96 %

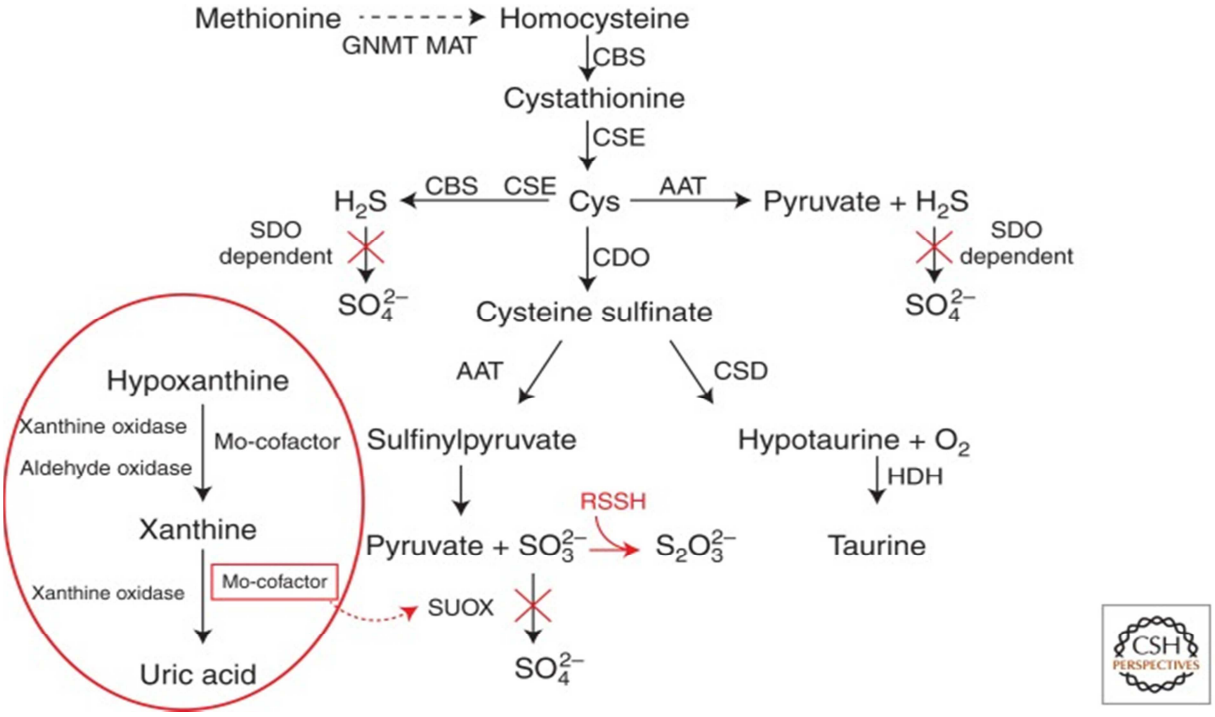
- **Patient D – Ethylmalonic encephalopathy (*ETHE1* gene - OMIM #*608451)**

The patient is a girl, 3rd child from consanguineous parents. She was born after an uneventful pregnancy and delivery. From birth, she had generalized hypotonia and failure to thrive. At 5 months of age, her weight was -1.5 SD, contrasting with a normal growth. She had bilateral epileptoid tremor, increased deep tendon reflexes, acrocyanosis, petechiae, and ecchymoses of all limbs.
Biochemical investigation at diagnosis (5 months) was:

- Acylcarnitines: butyrylcarnitine (C4) = 2.4 µmol/L (controls <0.6), isovaleryl/2-methylbutyrylglycine (C5) = 0.3 µmol/L (controls <0.3)
- Organic acids
  - Ethylmalonic acid = 357 mmol/mol creat (controls <15)
  - Methylsuccinic acid = 31 mmol/mol creat (controls <5)
  - Isovalerylglycine = 34 mmol/mol creat (controls <5)
  - 2-methylbutyrylglycine = 6 mmol/mol creat (controls <2)
  - Isobutyrylglycine : ++
  - Butyrylglycine : +
- Redox status: lactate = 3 150 µmol/L (controls < 1700), pyruvate = 173 µmol/L (controls < 140), L/P = 18 (controls < 20)

EEG was well organized, with no paroxystic elements. ETF showed hyperechogenicity, and IRM abnormal signal intensity of basal ganglia.
She died at 6.5 months of age.

Ethylmalonic encephalopathy is due to mutations in *ETHE1* gene (Tiranti et al 2004). The Ethe1p, a 30 kDa polypeptide located in the mitochondrial matrix, functions in vivo as a homodimeric, Fe-containing sulfur dioxygenase (SDO) activity (Tiranti et al, 2009), which is one of the reactions involved in the catabolic oxidation of sulfide to sulfate. Impaired activity of ETHE1-SDO leads to the accumulation of H_2S in critical tissues, including colonic mucosa, liver, muscle, and brain, up to concentrations that inhibit short-chain acyl-CoA dehydrogenase (SCAD), branched-chain acyl-CoA dehydrogenases and COX activities, therefore accounting for EMA, butyryl-, isobutyryl-, 2-methybutyryl- and isovalerylglycine in urine, C4 and C5 acylcarnitines in plasma, EMA and lactate in plasma.
Besides these enzymes deficiency, other symptoms of EE are explained by accumulation of H_2S, including damage of endothelial cells and vasodilation, which account for the petechiae and the acrocyanosis.



From Tiranti & Zeviani, Cold Spring Harb Perspect Biol. 2013 Jan 1;5(1):a011437

Diagnosis

Most likely diagnosis

- **Ethylmalonic encephalopathy** **22**
  (ETHE1 gene, EPEMA)
- Multiple acyl-CoA dehydrogenase deficiency 2

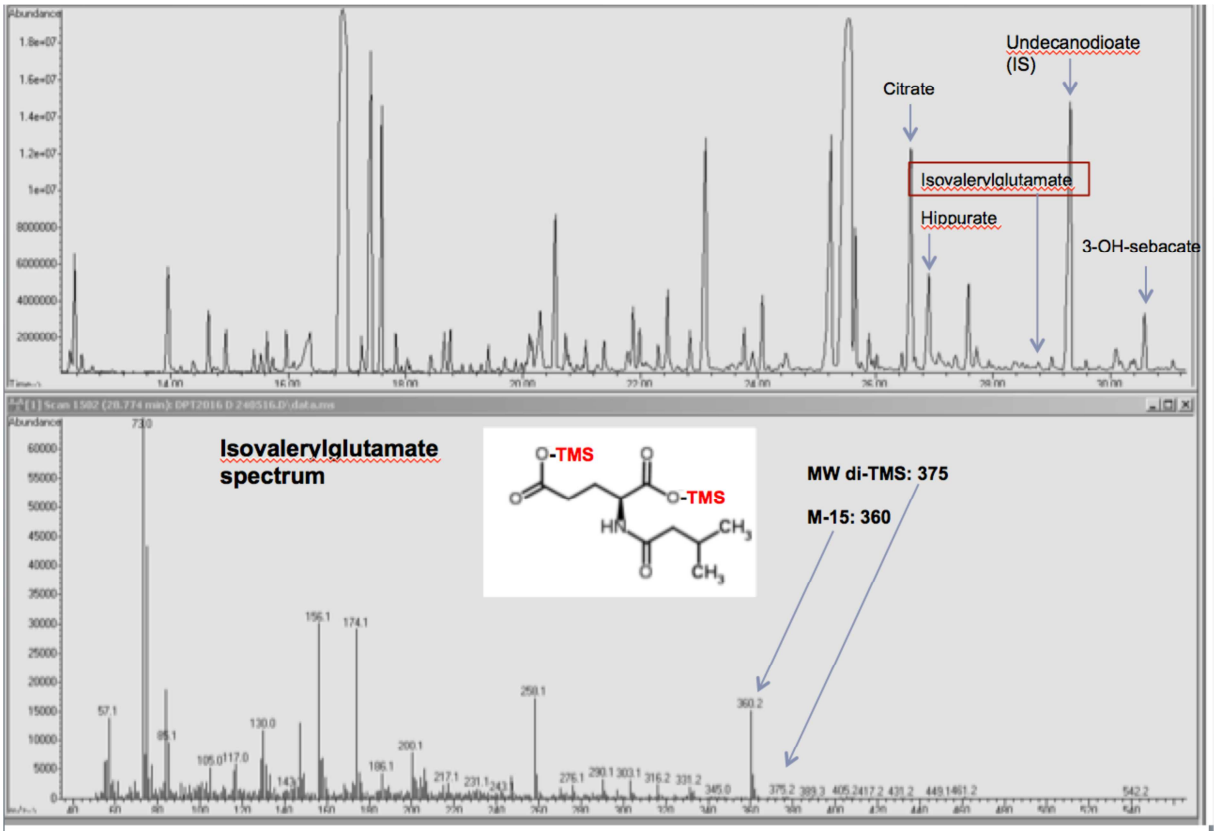
Alternative diagnosis

- SCAD deficiency 9
- Multiple acyl-CoA dehydrogenase deficiency 7
- Riboflavin transporter defects 2
- Respiratory chain deficiency 2

All participants performed **organic acid analysis:**

- Increase of ethylmalonic acid 24
  (median = 464 mmol/mol creat, range : 169 – 1 400 ; n = 21)
- Increase of methylsuccinic acid 24
  (median = 57.5 mmol/mol creat, range : 31 – 462.7 ; n = 14)
- Increase of isovalerylglycine 14
  (median = 54 mmol/mol creat, range : 13 – 718 ; n = 7)
- Increase of isobutyrylglycine 14
  (median = 26 mmol/mol creat, range : 6.9 – 44.7 ; n = 5)
- Increase of 2-methylbutyrylglycine 7
  (median = 26 mmol/mol creat, range : 6.9 – 44.7 ; n = 5)
- Increase of 2-hydroxyglutaric acid 6
  (77 ; 92 ; 151 mmol/mol creat)
- Increase of butyrylglycine 4
- Increase of isovalerylglutamate 1

Thanks to Antonia Ribes and Judit Garcia Villoria who provided the following spectrum of isovalerylglutamate.



Among the 19 labs who performed amino acid analysis, 10 reported an increase of glycine (median = 844 mmol/mol creat, range: 620 – 1006), and 6 an increase of β-alanine (median = 257 mmol/mol creat, range: 160 – 266).

Scoring

- Analytical performance: increase of ethylmalonic acid (score 1), increase of acylglycine derivatives (score 1).
- Interpretation of results and recommendations: ethylmalonic encephalopathy (score 2), multiple acyl-CoA dehydrogenase deficiency or inborn error of riboflavin metabolism (score 1).

- **Patient E – Mucopolysaccharidosis type IVA (Morquio syndrome A, OMIM #253000)**

The patient, a boy, is born from non-consanguineous parents. From the age of 1 month, he presented with a slight hypotonia, He could walk at 1 year but with an abnormal tiptoes walk. He had several otitis, and pain with ambulation from the age of 5 years. At 8 years, he had short trunk, dorsal kyphosis, and a limited walking range. X ray revealed epiphyseal dysplasia, and platyspondyly. He had hip surgery at 11 years. At 13 years, he is measuring 1.66 m, his weight is 41kg, and he presents with carinate breast, school difficulties because of frequent absences.
He was investigated at 13 years of age. Mucopolysaccharides analysis showed an increase of keratane sulphate. Galactose-6-sulfate sulfatase activity was decreased in leukocytes as well as the ratio Gal6sulf / total hexosaminidase.
The urine sample has been collected at 13 years of age.

Diagnosis

Most likely diagnosis

- **Mucopolysaccharidosis type IV** **23**
- No diagnosis 1

Alternative diagnosis

– MPS IV on clinical presentation	1
– MPS I	2
– MPS VI	2
– MPS II	1
– MPS III	1
– MPS VII	1
– Hypophosphatasia	1

GAGs quantification was performed by 21 out of 24 participants:

– Increase of GAGs	16
– Normal results	5

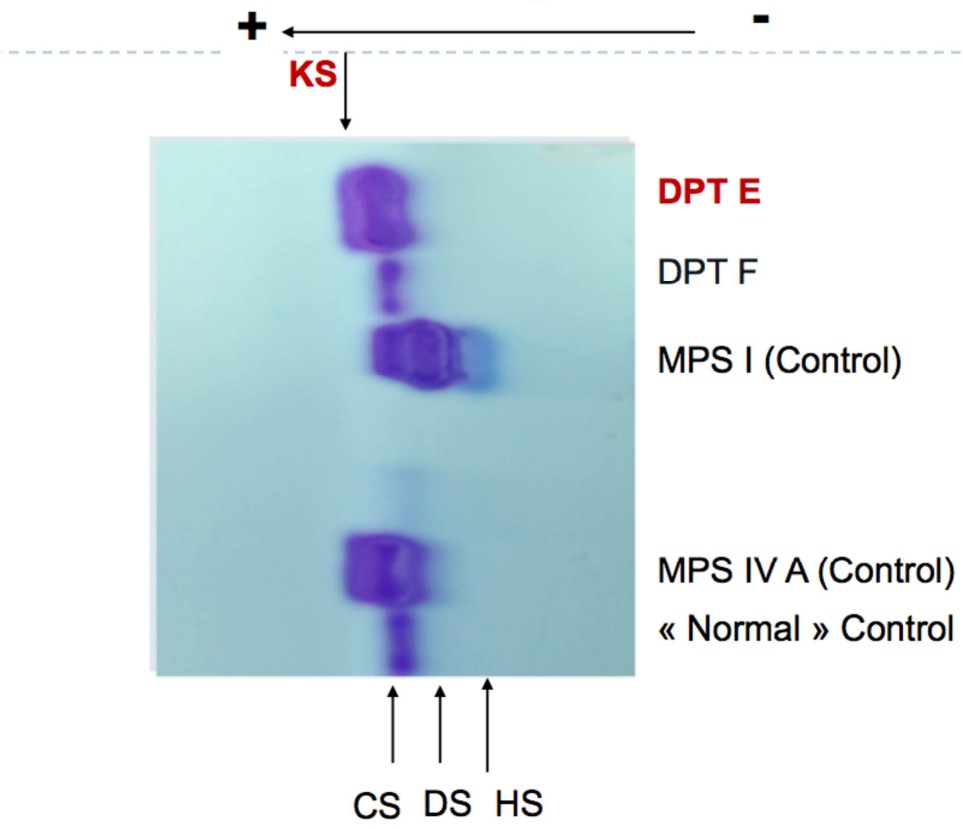
GAGs fractionation by 18 participants:

– **Increase of keratan sulphate**	**15**
– **MPS IV profile**	**2**
– Increase of chondroitin sulphate	1

Screening test MPS: 3

– Positive	2
– Negative	1

MPS electrophoresis of patient E is presented below.



Oligosaccharide analysis allows differentiating MPS IVA from MPS IVB. An abnormal oligosaccharide profile (similar to GM1 gangliosidosis) is observed in MPS IVB, while it is normal in MPS IVA. Ten labs performed this test: all but one reported a normal profile.

Scoring

- Analytical performance: increase of keratan sulphate (score 2), increase of glycosaminoglycans quantification or positive screening test, without fractionation of GAGs (score 1).
- Interpretation of results and recommendations: mucopolysaccharidosis type IV on analytical analysis (score 2), mucopolysaccharidosis type IV on increase of glycosaminoglycans and clinical presentation or other mucopolysaccharidosis (score 1).

A similar urine sample has been distributed in 2013: the overall performance is identical.

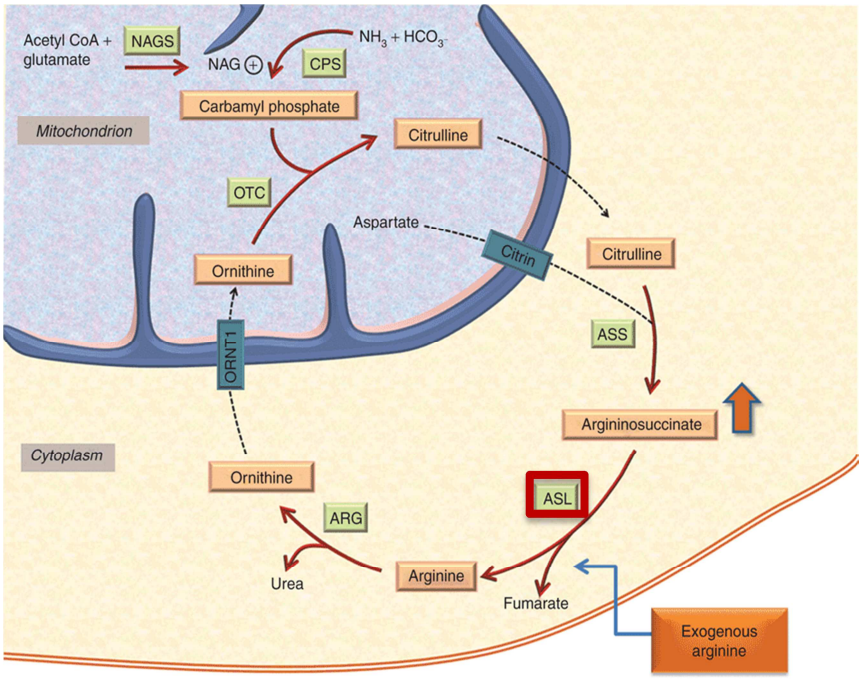
	2013	2016
Analytical performance	80 %	79 %
Interpretative performance	83 %	88 %
Overall performance	82 %	83 %

- **Patient F – Argininosuccinic aciduria (argininiosuccinate lyase deficiency, *ASL gene*, OMIM *608310)**

This 27-year patient was investigated at 6 years of age because of slight hepatomegaly, a moderate elevation of transaminases, and a partial alopecia with trichorrhexis nodosa. He did not have hyperammonemia.
Plasma amino acids results were:
- Cit = 181 µmol/L
- ASA + anhydrides = 173 µmol/L
- Glu + Gln = 733 µmol/L

Urinary amino acids:
- Cit = 15 mmol/mol creat (reference values <5)
- ASA + anhydrides = 2 358 mmol/mol creat

The urine sample has been collected at 27 years of age. He has a slight mental retardation with some behavioural abnormalities. His is treated with low doses of arginine.



From Erez, Genet Med 2013;15:251-257

Patients with argininosuccinic aciduria present with fewer hyperammonemic episodes than other urea cycle disorders such as OCT or CPSI deficiency, because excreted ASA is a nitrogen-rich compound. But there is a greater risk for poor neurocognitive outcome, seizures, hypertension and liver disease, despite early treatment.

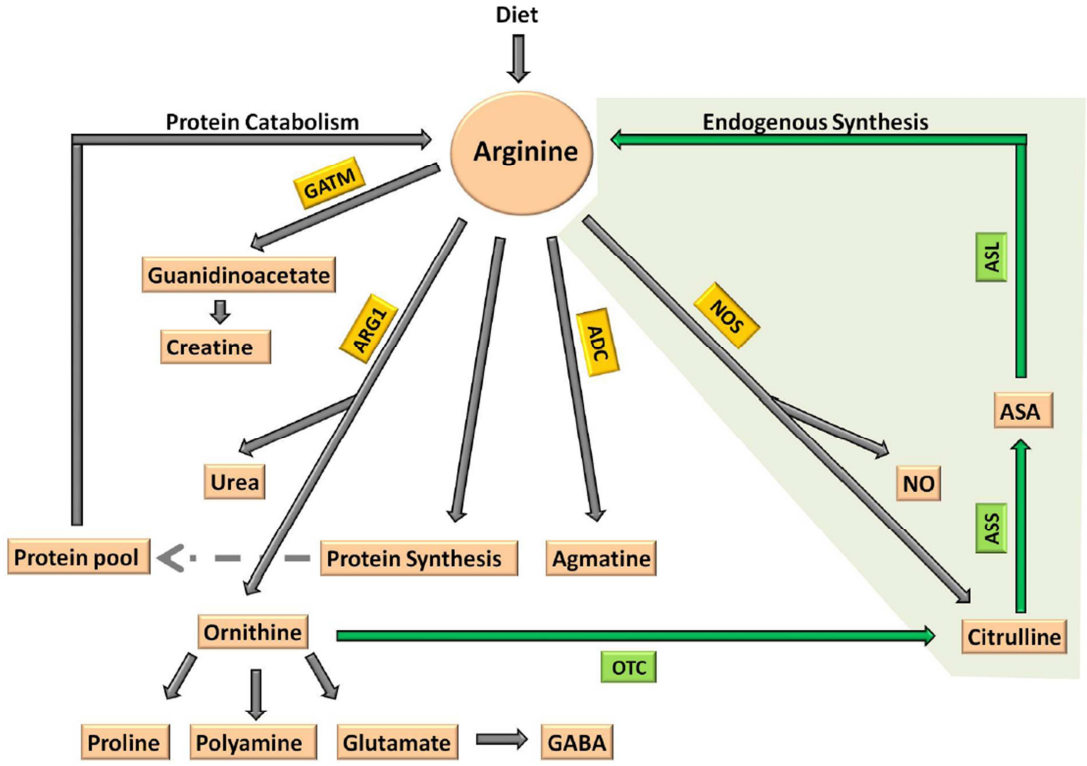
The pathophysiology is mainly due to the deficiency of the endogenous synthesis of arginine, a substrate for the generation of multiple metabolites:

- Nitric oxide (NO), catalysed by nitric oxide synthase (NOS): NO is involved in cell signalling and survival. Decrease of NO production increases production of free radicals and this can explain hypertension,
- Polyamines, proline, creatine, glutamate, agmatine (cell signalling)

The decrease of endogenous synthesis of fumarate possibly affects the Krebs cycle.

Moreover argininosuccinate lyase is structurally required to maintain a complex that facilitates the channelling of exogenous arginine to NOS for NO synthesis.

Supplementation with high doses of Arg is harmful because it induces an increase of guanidinoacetate, known as a cellular and neuronal toxin. Conversely treatment with NO can be helpful.



From Erez, Genet Med 2013;15:251-257

Diagnosis

Most likely diagnosis

- **Argininosuccinic aciduria** 22
- No or wrong diagnosis 2

Alternative diagnosis

- None

All participants performed **amino acids**, and 22 of them reported a high increase of **argininosuccinic acid** (median = 1468 mmol/mol creat – range: 267- 3843; n = 16).

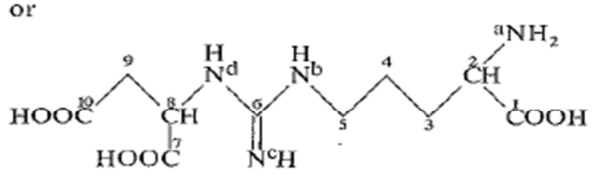
The 20 participants who performed organic acids reported a normal profile. When quantified (15 labs), orotic acid excretion was normal.

Quantification of argininosuccinic acid

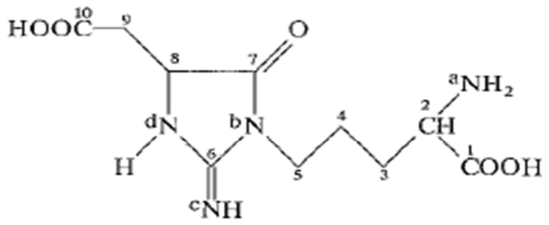
ASA is strongly hygroscopic and is only stable in its anhydrous form. In aqueous solutions, depending on pH and temperature, 2 anhydrides are formed:

- Anhydride I: neutral amino acid (pI=5.7)
- Anhydride II: more acidic (pI=4.2)

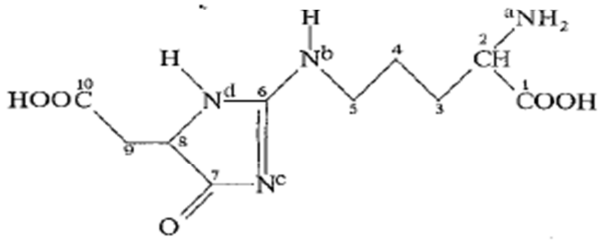
or



argininosuccinic acid



anhydride I



anhydride II

For quantification, ASA and anhydride II have to be converted into anhydride I in acidic conditions:

- Buffer LiS: lithium citrate pH 2.2
- Plasma: 50 µL + 150 µL buffer (dilution 1/4)
- Urine: 20 µL + 180 µL buffer (dilution 1/10)
- Calibration: 200 µL ASA solution in LiS buffer (0, 250, 500 µmol/L)

Incubate the samples and the calibration solutions 1 hour at 100°C in Eppendorf tubes with clamps.

Scoring

- Analytical performance: identification of argininosuccinic acid or its anhydrides (score 2).
- Interpretation of results and recommendations: argininosuccinic aciduria (score 2), other urea cycle disorder (score 1)

Missing the identification of argininosuccinic acid has been considered by the SAB as a **critical error**.

A similar urine sample has been distributed in 2003! The overall performance has improved.

	2013	2015
Analytical performance	89 %	92 %
Interpretative performance	87 %	92 %
Overall performance	88 %	92 %

Scores of participants

- **Survey 2016-1**

Lab n°	Patient A Hyperoxaluria type II			Patient B MAT deficiency			Patient C Glycerol kinase def.		
	A	I	Total	A	I	Total	A	I	Total
1	2	2	**4**	2	2	**4**	2	2	**4**
2	1	2	**3**	2	1	**3**	2	2	**4**
3	2	2	**4**	2	2	**4**	2	2	**4**
4	2	2	**4**	2	2	**4**	2	2	**4**
5	1	2	**3**	2	0	**2**	2	2	**4**
6	1	2	**3**	2	0	**2**	0	0	**0**
7	2	2	**4**	2	0	**2**	2	2	**4**
8	1	1	**2**	2	1	**3**	2	2	**4**
9	2	2	**4**	2	2	**4**	2	2	**4**
10	2	2	**4**	2	0	**2**	2	2	**4**
11	1	2	**3**	2	2	**4**	2	2	**4**
12	1	2	**3**	2	0	**2**	2	2	**4**
13	2	2	**4**	2	2	**4**	2	2	**4**
14	2	2	**4**	2	1	**3**	2	2	**4**
15	2	2	**4**	2	2	**4**	2	2	**4**
16	1	2	**3**	2	0	**2**	2	2	**4**
17	1	2	**3**	2	0	**2**	2	2	**4**
18	1	2	**3**	2	1	**3**	2	2	**4**
19	1	2	**3**	2	2	**4**	2	2	**4**
20	1	2	**3**	2	2	**4**	2	2	**4**
21	1	2	**3**	2	0	**2**	2	2	**4**
22	2	2	**4**	2	2	**4**	2	2	**4**
23	1	2	**3**	2	2	**4**	2	2	**4**
24	2	2	**4**	0	1	**1**	2	2	**4**

❖ **Survey 2016-2**

Lab n°	Patient D EMA encephalopathy			Patient E MPS IVA			Patient F Argininosuccinic ac.		
	A	**I**	**Total**	**A**	**I**	**Total**	**A**	**I**	**Total**
1	2	2	**4**	1	2	**3**	2	2	**4**
2	2	2	**4**	0	1	**1**	2	2	**4**
3	2	1	**3**	2	2	**4**	2	2	**4**
4	2	2	**4**	2	2	**4**	2	2	**4**
5	2	2	**4**	1	1	**2**	2	2	**4**
6	2	2	**4**	2	2	**4**	2	2	**4**
7	2	1	**3**	2	2	**4**	**Critical error**		
8	2	2	**4**	2	2	**4**	2	2	**4**
9	2	2	**4**	2	2	**4**	2	2	**4**
10	2	2	**4**	2	2	**4**	2	2	**4**
11	2	2	**4**	2	2	**4**	2	2	**4**
12	2	2	**4**	2	2	**4**	2	2	**4**
13	2	2	**4**	1	2	**3**	2	2	**4**
14	2	2	**4**	2	2	**4**	2	2	**4**
15	2	2	**4**	1	1	**2**	2	2	**4**
16	2	2	**4**	1	1	**2**	**Critical error**		
17	2	2	**4**	1	1	**2**	2	2	**4**
18	2	2	**4**	2	2	**4**	2	2	**4**
19	2	2	**4**	2	2	**4**	2	2	**4**
20	2	2	**4**	2	2	**4**	2	2	**4**
21	2	2	**4**	2	2	**4**	2	2	**4**
22	2	2	**4**	2	2	**4**	2	2	**4**
23	2	2	**4**	0	1	**1**	2	2	**4**
24	2	2	**4**	2	2	**4**	2	2	**4**

❖ **Total scores**

Lab n°	Survey 2016-1	Survey 2016-2	Cumulative score (max = 24)	Cumulative score (%)
1	12	11	**23**	96%
2	10	9	**19**	79%
3	12	11	**23**	96%
4	12	12	**24**	100%
5	9	10	**19**	79%
6	5	12	**17**	71%
7	10	7 **Critical error**	**17**	71% **Critical error**
8	9	12	**21**	88%
9	12	12	**24**	100%
10	10	12	**22**	92%
11	11	12	**23**	96%
12	9	12	**21**	88%
13	12	11	**23**	96%
14	11	12	**23**	96%
15	12	10	**22**	92%
16	9	6 **Critical error**	**15**	62% **Critical error**
17	9	10	**19**	67%
18	10	12	**22**	79%
19	11	12	**23**	96%
20	11	12	**23**	96%
21	9	12	**21**	88%
22	12	12	**24**	100%
23	11	9	**20**	83%
24	9	12	**21**	88%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	**3**	**13 %**
Poor performers (< 62 % good responses) or critical error	**2**	**8 %**
Partial or non submitters	**0**	**0**

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
Patient A	Hyperoxaluria type II	**73 %**	**98 %**	**85 %**
Patient B	MAT deficiency	**96 %**	**56 %**	**76 %**
Patient C	Glycerol kinase def.	**96 %**	**96 %**	**96%**
Patient D	EMA encephalopathy	**100 %**	**96 %**	**98 %**
Patient E	MPS IVA	**79 %**	**88 %**	**83 %**
Patient F	Argininosuccinic ac.	**92 %**	**92 %**	**92 %**

DPT-scheme in 2017

- 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

If you are not performing one of these assays, or if purine and pyrimidine analysis is required, 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 2017

Since there will not be a SSIEM meeting in 2017 (ICIEM in Rio de Janeiro), a separate ERNDIM meeting will be organized probably on 21-22 November 2017 in Manchester. Further information will be available on http://www.erndim.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.

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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**