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ERNDIM Diagnostic Proficiency Testing France 2015

ANNUAL REPORT 2015

In 2015, 23 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
Switzerland	1
Total	**23**

Logistic of the scheme

- **2 surveys** 2015-1: patient A, B and C
  2015-2: patient D, E and F

Origin of patients:

- Patient A: **Combined malonic & methylmalonic aciduria** (*ACSF3* gene) – Centre de Biologie Est, Lyon
- Patient B: **Homocystinuria** (CBS deficiency) – Marie-Hélène Read, Caen, France. This sample has been sent to all labs participating to the DPT scheme in Europe
- Patient C: **Mucopolysaccharidosis type VI** (arylsulfatase B deficiency) – Centre de Biologie Est, Lyon
- Patient D: **N-acetylaspartic aciduria** (Canavan disease) – Centre de Biologie Est, Lyon
- Patient E: **D-2-hydroxyglutaric aciduria type II** (de novo heterozygous mutation in *IDH2* gene) – Begoña Merinero, Madrid, Spain
- Patient F: **GM1 gangliosidosis** (beta-galactosidase deficiency) – Thierry Levade, Toulouse, 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

– 31 March 2015	Shipment of samples of Survey 1 and Survey 2 by CSCQ
– 7 April 2015	Clinical data available on CSCQ website and start analysis of samples (Survey 1)
– 23 April 2015	Reminder for result submission by e-mail (Survey 1)
– 30 April 2015	Deadline for result submission (Survey 1)
– 30 April 2015	Interim report of Survey 1 by e-mail
– 1 June 2015	Clinical data available on CSCQ website and start analysis of samples (Survey 2)
– 17 June 2015	Reminder for result submission by e-mail (Survey 2)
– 23 June 2015	Deadline for result submission (Survey 2)
– 6 July 2015	Interim report of Survey 2 by e-mail
– 31 August 2015	Meeting in Lyon
– 17 March 2016	Scientific Advisory Board meeting: final scoring
– 5 April 2016	Annual report with definitive scoring sent by e-mail

Date of receipt of samples

	Survey 1 + 2
+ 24 hours	23

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.**

- 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	23 labs	23 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 Lyon on Tuesday 1 September 2015 from 9.00 to 10.30, before the SSIEM Symposium.

❖ Participants

Representatives from 19 labs were present: A Ribes (Hospital Clinic, Barcelona), JA Arrantz (Vall d'Hebron, Barcelona), I Redonnet-Vernhet (Bordeaux), MH Read (Caen), S Funghini, E Pasquini (Florence), U Caruso (Genova), L Boulet, C Corne, L van Noolen (Grenoble), A PA Binz, O Boulat, O Braissant, C Roux (Lausanne), G Briand (Lille), B Merinero (Madrid), M Gastaldi (Marseille), E Jeannesson-Thivisol (Nancy), T Kolanunnage, G Polo (Padova), JF Benoist, A Imbard, O Rigal (Hôpital Robert Debré, Paris), F Habarou, C Ottolenghi (Hôpital Necker, Paris), D Quelles (Porto) C Rizzo (Rome), S Bekri (Rouen), MD Boveda (Santiago de Compostella).

❖ Information from the Executive Board and the Scientific Advisory Board

- **Scoring and certificate of participation**: scoring is done by 2 scheme organizers, who change every year. The results of DPT France 2015 have been also scored by Dr George Ruijter, from DPT The Netherlands. At the SAB meeting on March 17, 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. Normal samples are usually not eligible for Critical Error. The main argument is that one cannot be absolutely certain that a sample is normal. The patient could, for example, have an IEM that we did not know at the time of analysis, but did result in subtle metabolite abnormalities that the majority of the participants was not aware of. However, when it is clear that the sample was obtained from a patient not suspected of having an IEM and the findings reported were not identified by the rest of the participants then this diagnosis could potentially result in treatment that is harmful for the patient and the findings could constitute a critical error. With effect from 2017, the SAB will agree this on a case by case basis.

  For 2015, the SAB decided that non-identification of an increase of methylmalonic acid for sample A is a critical error, as well as homocystine for sample B, N-acetylaspartic for sample D, and 2-hydroxyglutaric for sample E.

  Certificate of participation for 2015 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). One performance support letter will be sent by the Scheme Advisor for 2015 because of a critical error (sample D).

- **Urine samples**: we remind you that every year, each participant must provide to the scheme organizer at least 300 ml of urine from a patient affected with an established inborn error of metabolism or "normal" urine, together with a short clinical report. If possible, please collect 1500 ml of urine: this sample can be sent to all labs participating to one of the DPT schemes. Each urine sample must be collected from a single patient (don't send urine spiked with pathological compounds). Please don't send a pool of urines, except if urine has been collected on a short period of time from the same patient. For "normal" urine, the sample must be collected from a symptomatic patient (don't send urine from your kids!). Annex 1 gives the list of the urine samples we already sent.

  As soon as possible after collection, the urine sample must be heated at 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.

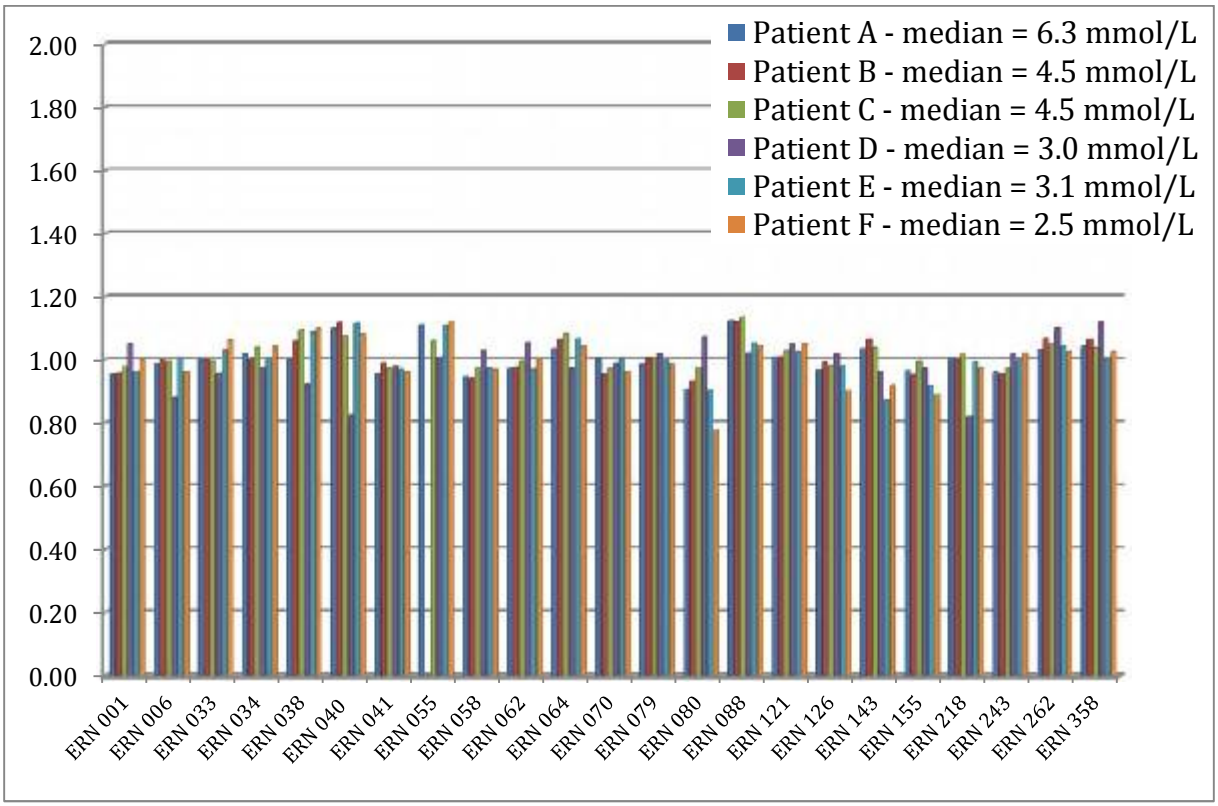
- **Lab identification**: since 2007, it has been accepted that the ERNDIM number is used for "in centre" communication but anonymous identification is used for the Annual Report on the website or other purposes.

❖ Discussion of results

- **Creatinine measurement**

Creatinine determination was satisfying for all labs this year. There were no wrong values, nor systematic error. Creatinine values are expressed in the figure as the ratio of each measurement over the median for all labs.
CV is < 8 % for all samples (4.7 – 7.8 %), and this is rather similar to the interlab CV 2014 for Special Assay in urine (3.4 %, n = 122), and the interlab CV 2013 for Quantitative organic acids (4.9 %, n = 67).

Creatinine: ratio to median



- **Patient A – Combined malonic and methylmalonic aciduria (CMAMMA - *ACSF3* gene – OMIM #*614245)**

The patient is a 47 year old man, born from consanguineous parents. He was investigated because he presented from the age of 45 years with asthenia, weight loss, recurrent episodes of headache, polyarthralgia, and pancreatitis. Diagnosis of combined malonic and methylmalonic aciduria (CMAMMA) was suspected by urinary organic acid analysis: **methylmalonic acid = 178 mmol/mol creat** (controls < 5) and **malonic acid = 13 mmol/mol creat** (controls < 2). **Plasma methylmalonic acid** level was **11.7 µmol/L** (controls <0.5). Plasma amino acids were normal (Cys= 28 µmol/L and Met=22 µmol/L), as well as plasma total homocysteine = 11 µmol/L (controls <15). Urinary methylmalonic and malonic levels remained unchanged with vitamin B12 supplementation.

The diagnosis has been confirmed by mutation analysis of *ACSF3* gene (Dr JF Benoist, Robert Debré, Paris): the patient is compound heterozygote for 2 variants of this gene: c.1672C>T (p.Arg558Trp) / c.1689G>C (p.Lys563Asn). Parents have not been investigated.

Exome sequencing identified *ACSF3* as the cause of Combined Malonic and Methylmalonic Aciduria (Sloan et al. Nat Genet. 2011; 43: 883–88). Nine patients were described. After uneventful early decades, four patients were diagnosed in adulthood with neurological manifestations (seizures, memory problems, psychiatric disease, and/or cognitive decline) without vitamin B12 deficiency. Methylmalonic acid levels ranged from 29 to 206 mmol/mol creat, malonic acid from 2.9 to 26 mmol/mol creat, and the ratio MMA/MA from 8 to10.

The *ACSF3* gene is an orphan member of the acyl-coenzyme A synthetase gene family, enzymes that thioesterify substrates into CoA derivatives. ACSF3 is a mitochondrial malonyl-CoA and methylmalonyl-CoA synthetase (MCS), an enzyme postulated to catalyze the first step of intramitochondrial fatty acid synthesis. It is the first disease association with a member of the acyl-CoA synthetase (ACS) family. A short communication has been presented during the SSIEM symposium in Lyon (Levtova et al 2015 J Inher Metab Dis 38(suppl.1):S46): several cases have been diagnosed through the neonatal screening in Canada. The authors speculated that CMAMMA is probably a benign condition or may predispose to late onset disease.

Diagnosis

Most likely diagnosis

- Combined malonic and methylmalonic aciduria 8

- Isolated methylmalonic aciduria 7
- Methylmalonyl-CoA mutase deficiency 5
- Methylmalonyl-CoA epimerase deficiency 3
- CblD deficiency 2
- Other: 1

(B12 malabsorption or deficiency, CblA, CblB, transcobalamine I deficiency)

Alternative diagnosis

- Disorder of cobalamin metabolism 5
- B12 malabsorption or deficiency 4
- SUCLA2/SUCLG1 3
- Other: 1

(Transcobalamine I or II deficiency, methylmalonyl-CoA mutase or epimerase deficiency)

All 23 participants performed **organic acid** analysis. They reported:

- Increase of **methylmalonic acid** : 23 labs
  (median 144 mml/mol creat – range: 57.9 - 220)
- Increase of **malonic acid** : 8 labs
  (median 13 mml/mol creat – range: 3 – 28,8)
- No increase of methylcitric acid : 9 labs
- No increase of 3-hydroxypropionic acid : 5 labs
- No increase of propionylglycine : 3 labs

Seventeen participants performed amino acids and all of them reported a normal profile. Six measured total homocysteine which was reported to be normal.

Scoring

- Analytical performance: Increase methylmalonic acid (score 1), Increase of malonic acid (score 1).
- Interpretation of results and recommendations: Combined malonic and methylmalonic aciduria (score 2), isolated mild methylmalonic aciduria (score 1)

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

- **Patient B – Cystathionine beta-synthase (CBS) deficiency (*CBS* gene, OMIM #236200)**

This urine sample has been distributed to all labs in Europe participating to DPT scheme. Details of this patient are available on the ERNDIM website: Meetings & Reports, ERNDIM Workshop Lyon 2015, Common sample 2015.

The patient is a 34 year-old woman, with a normal psychomotor development. She was investigated because of phlebitis at the age of 30 (under oestrogens). The diagnosis of cystathionine beta-synthase deficiency was suspected on the results of plasma and urine amino acids. Under pyridoxine (vitamin B6) treatment, together with vitamin B9 and B12, plasma homocystine and methionine concentrations normalized. Spontaneously, the patient had a high protein diet (134 g/day). She felt much better (less anxiety and irritability) under vitamin supplementation and a normoproteic diet (80 g/day).

Mutation analysis of *CBS* gene identified 2 variants: c.146C>T (p.Pro49Leu) and c.374G>A (p.Arg125Gln). No measurement of CBS activity has been performed.

Diagnosis

Most likely diagnosis

– CBS deficiency	20
– Homocystinuria	2
– Remethylation defect	1

Other possible diagnosis

– CBS deficiency	1
– Methylene tetrahydrofolate reductase def.	5
– CblG	3
– CblD	2
– CblE	2
– Other	1

(Vitamin deficiency, Cbl defects, remethylation defect)

All but 2 participants performed **amino acid** analysis, and reported:

– **Increase of homocystine** (median = 37 mmol/mol creat, range : 24.5 – 79 - CV = 31%)	19
– **Increase of methionine** (median = 17 mmol/mol creat, range : 11.9 – 25 - CV = 29%)	13
– **Increase of cysteine-homocysteine mixt disulfide**	7
– Decreased or normal level of cystine (range : 0 – 8 mmol/mol creat)	7
– Normal level of methionine	4

Ten participants measured **total homocysteine** and identified an **increase of total homocysteine** (median = 69.5 mmol/mol creat, range : 30 – 130).

The ten participants who performed organic acids reported a normal profile or no increase of methylmalonic acid.

Scoring

- Analytical performance: Increase of homocystine or of total homocysteine (score 2).
- Interpretation of results and recommendations: Cystathionine beta-synthase deficiency as first or alternative diagnosis (score 2), Homocystinuria without any precision (score 1).

To miss the increase of homocyst(e)ine has been considered by the SAB as a critical error.

A similar urine sample has been distributed in 2007: the overall performance has improved.

	2007	2014
Analytical performance	95 %	96 %
Interpretative performance	93 %	96 %
Overall performance	92 %	96 %

- **Patient C – Mucopolysaccharidosis type VI (arylsulphatase B deficiency, OMIM #253200)**

This boy was investigated at 4.5 years of age. He presented with dysmorphic features, thoracic and spinal dysgenesia but normal intelligence.

Urinary mucopolysaccharide profile was in agreement with MPS VI (Maroteaux-Lamy syndrome).

Arylsulfatase B activity was severely decreased in leukocytes:
- Flurorimetric method = 0.1 µkat/kg, simultaneous control = 4.0 ; total hexosaminidase = normal
- Colorimetric method = 8.0 µkat/kg, simultaneous control = 32.4 (controls: 16.0-77.0) ; total hexosaminidase = normal

The urine sample has been collected at 15 years of age.

Diagnosis

Most likely diagnosis

– Mucopolysaccharidosis type VI	18
– Mucopolysaccharidosis type I, II or VI	1
– Mucopolysaccharidosis	3
– No diagnosis	1

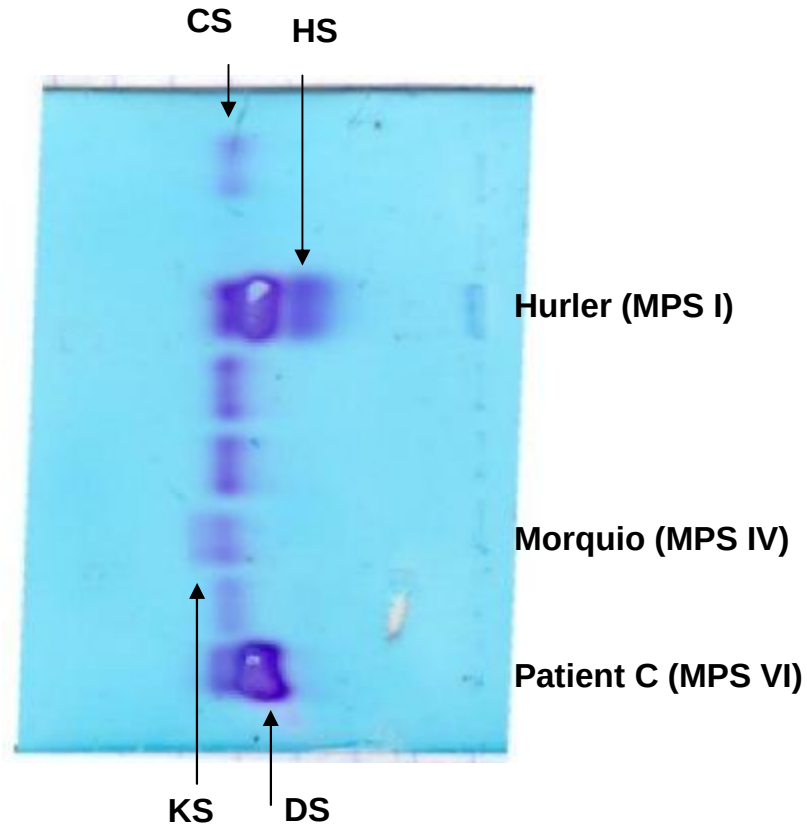
Other possible diagnosis

– Mucopolysaccharidosis type I	2
– According to clinical signs, MPS type IV or VI	1
– Mucopolysaccharidosis type IV	1
– Multiple sulfatase deficiency	1

Nineteen participants over 23, performed **GAG quantification**: 14 of them reported an **increase of GAGs**. All the 17 participants who performed **GAG fractionation** reported an abnormal profile but 14 of them specified that an **increase of dermatan sulphate** was observed. The only lab who performed a screening test for GAGS reported a positive test.

All the 14 participants who performed oligosaccharide analysis reported a normal profile, as well as the 4 labs who performed salic acid quantification.

The figure below illustrates the urinary mucopolysaccharide electrophoretic profile of the patient.



KS: keratan sulphate
CS: chondroitin sulphate
DS: dermatan sulphate
HS: heparan sulphate

Scoring

- Analytical performance: increase of dermatan sulphate (score 1), increase of glycosaminoglycans without GAGs fractionation (score 1).
- Interpretation of results and recommendations: mucopolysaccharidosis type VI (score 2), unspecified or wrong mucopolysaccharidosis, or diagnosis according to the clinical presentation (score 1).

A similar urine sample has been distributed in 2006. The overall performance has slightly improved.

	2006	2015
Analytical performance	84 %	87 %
Interpretative performance	84 %	89 %
Overall performance	84 %	88 %

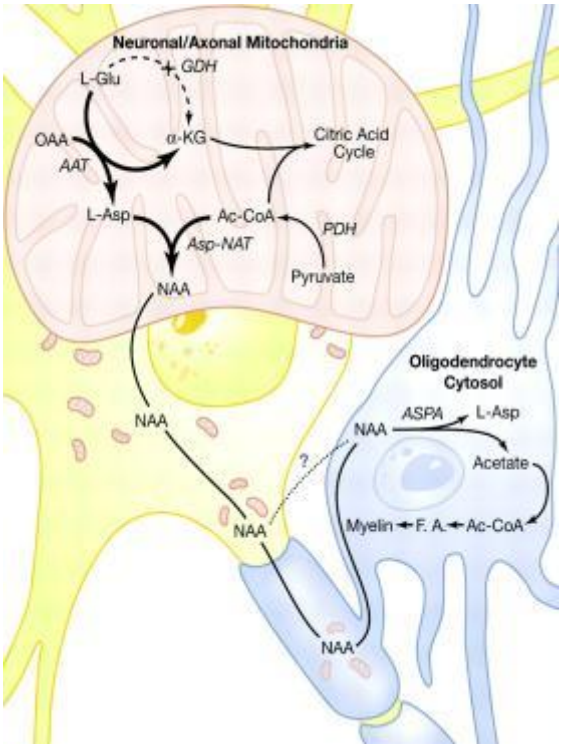
- **Patient D – N-acetylaspartic aciduria (aspartoacylase deficiency OMIM #271900)**

The patient is a girl, born from consanguineous parents. She was hospitalized at 5 months of age because of bronchiolitis. At clinical examination, she presented with axial hypotonia, abnormal eye movements, delayed psychomotor development and poor contact. MRI revealed hydrocephaly, with dilated lateral ventricles. Urinary organic acids at diagnosis were strikingly abnormal with a high increase of N-acetylaspartic acid (= 1481 mmol/mol creatinine - controls <40). Mutation analysis revealed that the patient is homozygous for the c.79G>A variation (p.Gly27Arg) in exon 1 of *ASPA* gene, mutation already described (Kaul et al, Am J Hum Genet, 1996). Parents are heterozygous for this mutation

The urine sample has been collected at 11 months of age.

N-acetylaspartic aciduria is caused by mutations in the *ASPA* gene that encodes the enzyme aspartoacylase. Its clinical spectrum varies between severe forms with leukodystrophy, macrocephaly and severe developmental delay, and a very rare mild/juvenile form characterized by mild developmental delay. The resulting deficiency of aspartoacylase leads to accumulation of N-acetylaspartic acid (NAA) in the brain and to oligodendrocyte dysfunction, spongiform changes, and absence of myelin. However, the precise mechanisms causing spongiform degeneration are uncertain. The *ASPA* gene is located on chromosome 17pter-p13. Several mutations have been defined in *ASPA*, but just 4 of them account for >99 % of aspartoacylase deficiency cases in Ashkenazi Jews.

Proposed model for NAA synthesis and degradation



Chikkathur N. Madhavarao et al. PNAS 2005;102:5221-5226

Diagnosis

Most likely diagnosis

- N-acetylaspartic aciduria 22
  (aspartoacylase deficiency, Canavan disease)
- Succinate dehydrogenase deficiency 1

Alternative diagnosis

- Secondary respiratory chain complex II deficiency 1

Among the 23 participants who performed **organic acid analysis** (22) **or screening** (1), all but one reported an **increase of N-acetylaspartic acid** (median = 928 mmol/mol creat, range : 131 – 7 621 ; n = 12), and 2 reported metabolites of paracetamol.

The 4 labs who performed amino acid analysis reported a normal profile.

Scoring

- Analytical performance: increase of N-acetylaspartic acid (score 2)
- Interpretation of results and recommendations: N-acetylaspartic aciduria (score 1), perform measurement of aspartoacylase activity or mutation analysis of *ASPA* gene to confirm the diagnosis (score 1).

To miss the increase of N-acetylaspartic acid has been considered by the SAB of ERNDIM as a critical error.

- **Patient E – D-2-hydroxyglutaric aciduria type II due to isocitrate dehydrogenase 2 deficiency (*IDH2* gene, OMIM *147650)**

The patient, a girl, was investigated at 5 months of age because of developmental delay, hypotonia, and mild facial dysmorphism. Cerebral MRI showed white matter abnormalities. At 17 months of age, urinary 2-hydroxyglutaric acid and 2-hydroxyglutaric lactone were highly elevated: 4003 and 431 mmol/mol creat, respectively. D-2-hydroxyglutaric acid was confirmed by HPLC-MS/MS. Mutation analysis revealed that the girl is heterozygous for the previously described *de novo* gain-of-function mutation p.Arg140Gln in *IDH2* gene.

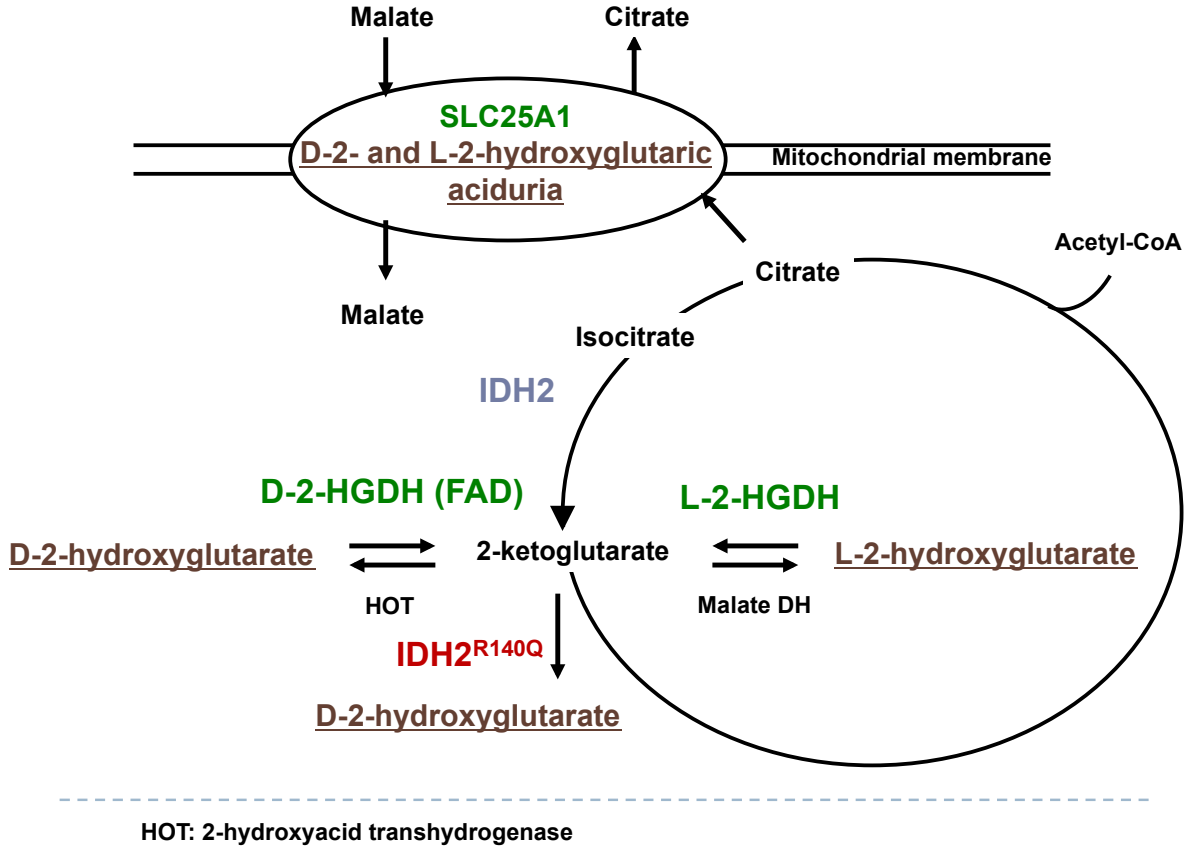
Heterozygous mutations that alter residues p.Arg140 and p.Arg172 of mitochondrial isocitrate dehydrogenase 2 (IDH2) have been detected in acute myeloid leukemia and gliomas. These mutations also lead to abnormal production of D-2-HG. These mutations disable the enzyme's normal ability to convert isocitrate to 2-ketoglutarate (2-KG) and confers on it a new function: the ability to convert 2-KG to D-2-hydroxyglutarate (D-2-HG).

In 2010, Kranendijk et al (Science 2010;330:336) identified heterozygous *de novo* mutation(s) in *IDH2* gene in 15 unrelated idiopathic D-2-HGA patients (i.e., normal D-2-HGDH enzyme activity / no mutations in *D2HGDH* gene and consistently increased D-2-HG levels in body fluids): the known heterozygous c.419G>A (p.Arg140Gln), and a novel heterozygous c.418C>G (p.Arg140Gly) in one patient. These patients were identified as D-2-hydroxyglutaric acid type II.

Urinary excretion of D-2-hydroxyglutaric acid is higher in type II than in type I (due to D-2-hydroxyglutarate dehydrogenase deficiency):

- Type I: mean = 969 mmol/mol creat (n=20)
- Type II: mean = 2153 mmol/mol creat (n=14)
- Controls <17.0 mmol/mol creat

The figure below illustrates the metabolism of D- and L-2hydroxyglutaric acids:



HOT: 2-hydroxyacid transhydrogenase

Diagnosis

Most likely diagnosis

– D-2-hydroxyglutaric aciduria		10
– 2-hydroglutaric aciduria		9
– D-2-hydroxyglutaric aciduria type I	3	
– Combined D, L-2-hydroxyglutaric aciduria	1	

Alternative diagnosis

– L-2-hydroglutaric aciduria	8	
– Combined D, L-2-hydroxyglutaric aciduria	4	
– D-2-hydroxyglutaric aciduria type II	3	
– D-2-hydroxyglutaric aciduria		1

Recommendations

– Perform enantiomers identification	18

All 23 participants performed **organic acid** analysis: all of them reported an increase of **2-hydroxyglutaric acid** (median = 600 mmol/mol creat, range : 215 – 3 550; n = 15), 11 an increase of 2-hydroxyglutaric lactone, and 5 an increase of succinic acid. The 2 labs who performed separation of D- and L- enantiomers, identified an increase of D-2-hydroxyglutaric acid.

No significant abnormality was reported by the 7 participants who performed amino acids analysis.

Scoring

- Analytical performance: increase of 2-hydroxyglutaric acid (score 2).
- Interpretation of results and recommendations: D-2-hydroxyglutaric aciduria type I or type II (score 2), 2-hydroxyglutaric aciduria and perform enantiomers identification (score 2), D,L-2-hydroxyglutaric aciduria due to mutations in *SLC25A1* gene (score 1). .

To miss the increase of 2-hydroxyglutaric acid acid would have been considered by the SAB of ERNDIM as a critical error.

A similar urine sample has been distributed in 2014 (L-2-hydroxyglutaric aciduria): the overall performance is the same.

	2014	2015
Analytical performance	96 %	100 %
Interpretative performance	100 %	98 %
Overall performance	98 %	99 %

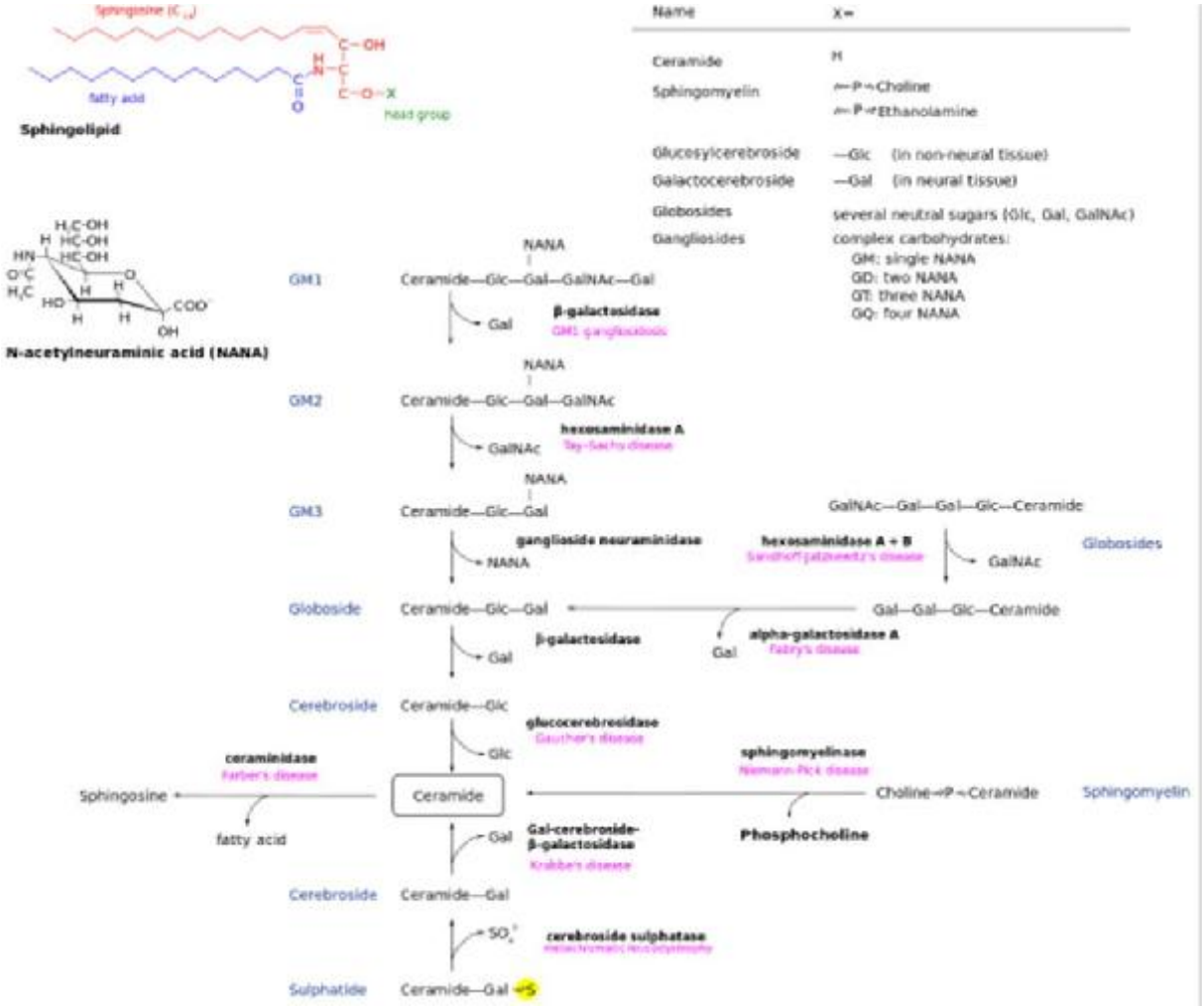
- **Patient F – GM1 gangliosidosis (-galactosidase deficiency, *GLBI gene*, OMIM #230500)**

The patient is a girl born from consanguineous parents (gipsy family). At 4 months of age, she was referred to the hospital for bronchiolitis. Clinical examination revealed hypotonia, nystagmus, moderate hepatomegaly, no facial dysmorphism, but mongoloid spot. The first urine sample was collected at 6 months of age, and the urinary oligosaccharides profile was consistent with GM1 gangliosidosis. Measurement of enzyme activities confirmed the diagnosis:

- β-galactosidase activity in leucocytes = 4 nmol/h.mg prot – simultaneous control = 281
- Neuraminidase activity in leucocytes: normal

Mutation analysis revealed that the patient is homozygous for the mutation c.202C>T in exon 2 *GLBI* gene. She died few months later with respiratory failure.

GM1 gangliosidosis is due to β -galactosidase deficiency, the first step in ceramide synthesis (see figure below).



Diagnosis

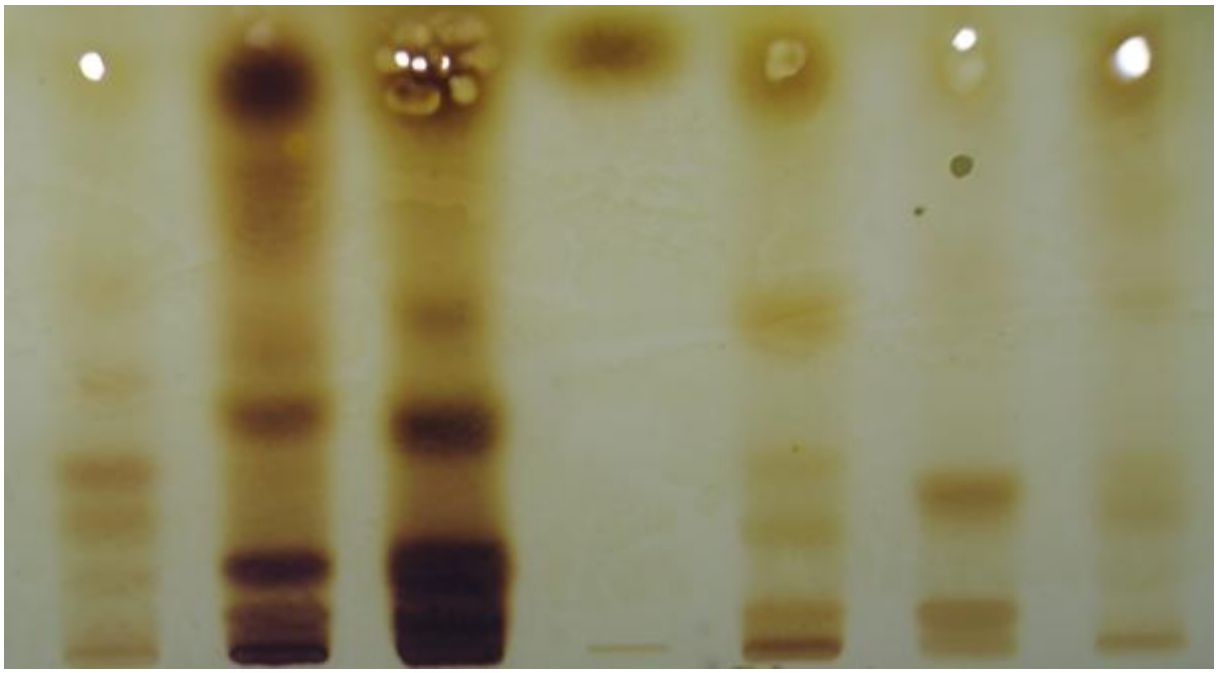
Most likely diagnosis

– GM1 gangliosidosis	18
– Investigate for a LSD	1
– No diagnosis, no IEM, normal profile	3
– SCAD deficiency	1

Alternative diagnosis

– Galactosialidosis	1
– Possible mucopolysaccharidosis	1
– Multiple acyl-CoA dehydrogenase deficiency	1
– Ethylmalonic encephalopathy (*ETHE1*)	1

Heighteen participants performed **oligosaccharides analysis** and all of them reported an **abnormal profile evocative GM1 gangliosidosis**.



Aspartyl-gluco saminuria	GM1 Gangliosidosis	**DPT France E**	Lactose	Fucosidosis	Sialidosis	Control urine

An **Educational Oligosaccharide Kit** containing 6 common oligosaccharidoses samples is now available to purchase on a not-for-profit basis from MCA Laboratories in the Netherlands. MCA Laboratories (a sub-section of SKML) organise some of ERNDIM's EQA schemes on our behalf. The samples included in the educational kit are: GM1 gangliosidosis, GM2 gangliosidosis, M. Pompe (infantile), Aspartylglucosaminuria, alpha-mannosidosis and Schindler disease. A protocol for qualitative TLC oligosaccharide analysis and also a picture of a TLC separation of the Oligosaccharide kit are both available on the ERNDIM website under Training/ Educational Documents. Further details of the Educational Oligosaccharide kit are available from MCA Laboratories:

http://cms.erndimqa.nl/Educational-Panels.aspx

Sixteen out of the 18 labs who performed GAG quantification reported a normal result. And 7 of the 11 participants who performed GAG fractionation reported a normal profile.

Scoring

- Analytical performance: abnormal oligosaccharide profile evocative of GM1 gangliosidosis (score 2).
- Interpretation of results and recommendations: GM1 gangliosidosis (score 2), perform oligosaccharides (score 1).

Scores of participants

- **Survey 2015-1**

Lab n°	Patient A CMAMMA			Patient B CBS deficiency			Patient C MPS VI		
	A	**I**	**Total**	**A**	**I**	**Total**	**A**	**I**	**Total**
1	2	2	**4**	2	2	**4**	2	2	**4**
2	2	2	**4**	2	2	**4**	2	2	**4**
3	2	2	**4**	2	2	**4**	2	2	**4**
4	1	1	**2**	1	2	**3**	2	2	**4**
5	1	1	**2**	2	2	**4**	1	1	**2**
6	1	1	**2**	2	2	**4**	1	1	**2**
7	1	1	**2**	2	2	**4**	2	2	**4**
8	1	1	**2**	2	2	**4**	2	2	**4**
9	1	1	**2**	2	2	**4**	2	2	**4**
10	1	1	**2**	2	2	**4**	2	2	**4**
11	1	1	**2**	2	2	**4**	2	2	**4**
12	2	2	**4**	2	2	**4**	2	2	**4**
13	2	2	**4**	2	2	**4**	2	2	**4**
14	1	1	**2**	2	1	**3**	0	1	**1**
15	1	1	**2**	2	2	**4**	1	1	**2**
16	1	1	**2**	2	2	**4**	1	1	**2**
17	1	1	**2**	2	2	**4**	2	2	**4**
18	2	2	**4**	2	2	**4**	2	2	**4**
19	2	2	**4**	2	2	**4**	2	2	**4**
20	1	1	**2**	1	1	**2**	2	2	**4**
21	1	1	**2**	2	2	**4**	2	2	**4**
22	1	1	**2**	2	2	**4**	2	2	**4**
23	2	2	**4**	2	2	**4**	2	2	**4**

❖ **Survey 2015-2**

Lab n°	Patient D N-acetylaspartic aciduria			Patient E D-2-hydroxyglutaric aciduria type II			Patient F GM1 gangliosidosis		
	A	**I**	**Total**	**A**	**I**	**Total**	**A**	**I**	**Total**
1	2	2	**4**	2	2	**4**	2	2	**4**
2	2	2	**4**	2	2	**4**	2	2	**4**
3	2	2	**4**	2	2	**4**	2	2	**4**
4	2	2	**4**	2	2	**4**	2	2	**4**
5	2	2	**4**	2	2	**4**	0	0	**0**
6	2	2	**4**	2	2	**4**	0	0	**0**
7	0	0	**0**	2	2	**4**	0	1	**1**
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**	2	2	**4**	2	2	**4**
14	2	2	**4**	2	1	**3**	2	2	**4**
15	2	1	**3**	2	2	**4**	0	0	**0**
16	2	2	**4**	2	2	**4**	0	0	**0**
17	2	2	**4**	2	2	**4**	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**	2	2	**4**	2	2	**4**

❖ **Total scores**

Lab n°	Survey 2015-1	Survey 2015-2	Cumulative score (max = 24)	Cumulative score (%)
1	12	12	**24**	100%
2	12	12	**24**	100%
3	12	12	**24**	100%
4	9	12	**21**	88%
5	8	8	**16**	67%
6	8	8	**16**	67%
7	10	5 **Critical error**	**15**	63% **Critical error**
8	10	12	**22**	92%
9	10	12	**22**	92%
10	10	12	**22**	92%
11	10	12	**22**	92%
12	12	12	**24**	100%
13	12	12	**24**	100%
14	6	11	**17**	71%
15	8	7	**15**	63%
16	8	8	**16**	67%
17	10	12	**22**	92%
18	12	12	**24**	100%
19	12	12	**24**	100%
20	8	12	**20**	83%
21	10	12	**22**	92%
22	10	12	**22**	92%
23	12	12	**24**	100%

Performance

	Number of labs	% total labs
Excellent performers (100 % of good responses)	**8**	**35 %**
Poor performers (< 62 % good responses) or critical error	**1**	**4 %**
Partial non submitters	**0**	**0**

❖ **Summary of scores**

Sample	Diagnosis	Analytical (%)	Interpretation (%)	Total (%)
Patient A	CMAMMA	**67 %**	**67 %**	**67 %**
Patient B	CBS deficiency	**96 %**	**96 %**	**96 %**
Patient C	MPS VI	**87 %**	**89 %**	**88 %**
Patient D	N-acetylaspartic ac.	**96 %**	**94 %**	**95 %**
Patient E	L-2-hydroxyglutaric ac.	**100 %**	**98 %**	**99 %**
Patient F	GM1 gangliosidosis	**78 %**	**80 %**	**79 %**

DPT-scheme in 2016

- 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 2016

It will take place during the SSIEM meeting in Rome **Tuesday 5 September 2016** from 9.00 to 10.30 am. Information concerning the venue is available on http://www.ssiem2016.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

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**
• **2008:1**	**P1**	**Cbl C/D**
	P2	**Mucopolysaccharidosis type III (common sample)**
	P3	**2-hydroxyglutaric aciduria**
• **2008:2**	**P4**	**Glycerol kinase deficiency**
	P5	**~-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**