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Effectiveness of qualifying diagnostics of hereditary metabolic diseases with the use of gas chromatography / mass spectrometry by the example of the HHH syndrome

Abstract. In the process of specifying diagnosis of hereditary metabolic diseases, among others, we use gas chromatography / mass spectrometry. Diagnostic significance of this method was high. On the example of HHH syndrome with hyperornithinemia – hyperammonemia – homocitrullinuria shows the need to use this method in all cases with of episodes of hyperammonemia, there are indications of the disease in the early childhood on the background of triggers (infection).

Keywords: HHH syndrome, hyperornithinemia, hyperammonemia, homocitrullinuria, gas chromatography / mass - spectrometry, rare diseases, thrombophilia.

Problem statement and its significance. Rare hereditary diseases are becoming a global medical problem by their being manifested at all ontogenesis stages. The affirmation of the new 4P medicine paradigm with its predictive, preventive, personalized, and participatory character is largely due to emergence of a great number of hard-to-identify pathologies, which are often a 'conglomerate of diseases' (the phenomenon of genotype and phenotype syntropy). This presents a challenge for early diagnostics, treatment and rehabilitation in case of rare diseases (RD).

In 80 % of cases, RD have been found to be caused by genetic anomalies, and in all other cases, they are the result of infections, allergies and the effect of environmental factors. Among RD, hereditary metabolic disorders are the most widespread ones.

In the early 21st century, about 8 000 RD were described [1], and today, we have information about 50 000 congenital metabolic disorders [2]. Using classical clinical examinations and advanced analytical technologies in medicine is a requisite condition of accurate and timely diagnostics of hereditary diseases. The gas chromatography / mass spectrometry (GC-MS) method allows analysing organic acids (OA) in the urine. OA analysis with the GC-MS method is a necessary stage for qualifying diagnostics in patients with a suspected case of organic aciduria. OA are a component of basic metabolites in actually all paths of small molecule metabolism [3]. The GC-MS method allows detecting and characterising quantitively over 100 substances in micro quantities of a biological material [4].

Introducing effective methods of early diagnostics of RD and improving the level of availability of high-qualified aid for patients with RD is a priority line of activity at the Kharkiv Special Medical-Genetics Centre (KhSMGC). It maintains a record of families with this pathology and conducts their follow-up monitoring. Selective screening of children with hereditary metabolism diseases has been introduced. Amino acids (AA) and OA are analysed for each child with indications of intoxication, cerebral disorders of vague origin, nonspecific mental retardation, convulsive disorder, delayed psychomotor development accompanied by persistent vomiting, food refusal, hypertrophy, respiratory and neuro distress, the Banti syndrome, autistic and aggressive behaviour, muscle tone disorders, and so forth.

The study was conducted using a team approach when a diagnosis is established with participation of a geneticist, neurogeneticist, pediatrician, biochemist, and a molecular geneticist who all are part of the Expert Diagnostic Council.

Objective of the study: investigating the information value of selective screening with the method of gas chromatography / mass spectrometry of organic acids in the urine of patients suspected for a hereditary metabolic disease to develop approaches to pathogenetic rehabilitation.

Materials and methods: Children were selected for risk groups based on suspected manifestation of hereditary metabolic diseases (HMD) during medical consultations at the Kharkiv Special Medical-Genetic Centre (KhSMGC), as well as when children were examined at regional children's clinics, perinatal centres, and intensive care units at children's clinical hospitals. Each year, the Centre helps 35 000 patients suspected for different hereditary pathology (of this number, 6 000 are those who were subject to initial examination), and over 300 different nosological RD units were identified.

Chromatographic analysis is done with GC-MS **(**Agilent, GC 6890, MS 5975C).

During 2010 to June 2014, the Centre conducted 6 000 tests for organic acids in the urine of patients suspected for HMD. The tests identified 139 clinically significant organic compounds.

In so doing, the probable origin of metabolites was differentiated.

Thus, the following variations of metabolites not related to HMD were distinguished: bacterial contamination of a sample (including excessive growth of yeast); intake of food abundant in tartaric (nutrition additives), apple and citric acid; heavy metal intoxication (aluminium, lithium, arsenic, mercury, and lead); parathyroidectomy; hyperparathyroidism; anoxia; AC metabolic disorder and deficiency of cofactors.

The metabolites not related to HMD are also depletion or deficiency of glutathione: (↓ 5-oxyprolin, ↓ citric acid, ↑ or N(?) – aconitic acid,↑ or N - isocitric, 2-oxoglutaric, succinic, and fumaric acids).

The detection of citric, aconitic, and isocitric acids was evaluated depending on many factors (formation from acetyl-CoA, a metabolite of oxidation of fatty acids, glycolysis, glycogenesis, alanine, aspartate, glutamine, leucine, isoleucine, valine; increase in substances caused by deficiency of cofactors; aconitic – iron deficiency; isocitric – B3, magnesium, and manganese deficiency).

The detected 2-oxoglutaric acid demanded accounting for the fact that it is a metabolite of the cycle of ammonia detoxification via glutamine and glutamate; the alanine-aspartate cycle; the metabolism of ascorbic acid and alderates; or it can be a product of breakage of glutamine, glutamic acid, arginine, histidine, proline; and a lipoic acid increase with deficiency of cofactors B1, B2, B3, B5, and Mg.

It was taken into account that succinic acid is a metabolite of degradation of leucine, isoleucone, and valine. It changes with deficiency of cofactors B2 (riboflavin), iron, and coenzyme Q10. Fumaric acid is a metabolite of oxidation of phenylalanine, tyrosine, arginine and proline, and it is detected with deficiency of cofactor B3. It was found that apple acid is involved in a complex metabolic process: in ionised form as malate – an intermediate component of the tricarboxylic acids cycle, following fumarate, a precursor of oxalacetic acid. It supports NADH delivery to the mitochondria. Besides, it can be formed from pyruvate as a anaplerotic reaction. It has been identified with deficiency of cofactors B3, niacin, and coenzyme Q10.

Metabolites related to HMD:

The majority of disorders in the Krebs metabolic cycle and respiratory chain enzymes are accompanied by a high level of blood and urine lactic acid, and metabolite change. According to www.geonme.jp data, these are deficiency of fumarase, the 2-ketoglutarate-dehydrogenase complex, succinate dehydrogenase, pyruvate dehydrogenase, pyruvate carboxylase, cytochrome C oxydase, the Coven-similar syndrome, and 2-hydroxyglutaric aciduria.

- **2-hydroxyglutaric aciduria:** three forms are known. They involve a significant 2-hydroxyglutaric acid increase. It does not involve a lactate level increase.

- **Fumaric aciduria:** ↑↑↑ Fumaric acid 3,000-4,000 mmol/mol creatinine; hyperammonemia; lactate-acidosis.

- **Complex 2-ketoglutarate dehydrodenase deficiency:** ↑↑↑ 2-oxoglutaric acid; lactate-acidosis; glucose – normal or ↑.

- **Cytochrome C oxydase deficiency:** simultaneous increase of apple, citric, fumaric and 2-ketoglutaric acids.

- **Pyruvate carboxylase deficiency:** hyperammoniemia; lactate-acodosis; glucose – normal or ↑; alanine, citrulline, and lysine increase in plasma.

- **Pyruvate dehydrogenase (E3) deficiency:** hyperammoniemia; lactate-acidosis; glucose – normal or ↑; isoleucine, leucine and valine increase in plasma; in urine, metabolites of AA isoleucine, leucine, and valine.

Since 2011, the gas chromatography unit of the KhSMGC biochemistry laboratory has been participating in the program ERNDIM Qualitative Organic Acid QA Scheme (Germany)

conducted at the Centre for Metabolic Diseases Heidelberg, supervised by Dr. C. D. Langhans, Dr. V. Peters, and Prof. Dr. G.F. Hoffmann.

Results and discussion: The organic compounds, which were identified in the urine of patients with GC-MS, were metabolites formed due to the activity of intestinal microflora, pathogenic microflora (in presence of bacilluria), exogenic origin substances (metabolites of medicinal preparations, metabolites of pesticides, the results of a specific diet and of metabolism of specific toxins, etc.).

Most often, test personnel identified metabolites of the Krebs cycle and the respiratory chain, and fatty acids oxidation with a test frequency of 1:75. Moderate increases in the level of fatty acids oxidation metabolites in the mitochondria occurred with a test frequency of 1:120. We interpreted these indicators as the result of a secondary mitochondrial dysfunction. A moderate increase of methyl-malonic acid (1:10 of tests) was found in patients with vitamin B12 deficiency, gastro-intestinal disorders and cobalamin deficiency. The increased level of branch chain AA oxidation metabolites (1:50 of tests) was a result of deficiency of cofactors of metabolic processes: vitamins B1, B2, B3, B5, biotine, lipoic acid, and magnesium.

Selective screening detected the following hereditary metabolic diseases (Table 1):

Table 1. Identified hereditary metabolic diseases

Ref. No.	Name of disease	Identified metabolites	Patient rate
1	Sulphite oxidase deficiency	Sulphite	1
2	Propion aciduria	↑↑↑ 3-hydroxypropionic , 2-hydroxyisovaleric, propionylglycine, methylcitric acid	1
3	Methylmolon aciduria	Methylmalonic acid (› 500 mmol/mol KREA)	3
4	Isovaleric aciduria	Isovalerylglycine, 3-hydroxyisovaleric acid	1
5	Glutaric aciduria type 1	glutaconic, 3-hydroxyglutaric, 2-methylglutaconic, 3-hydroxyisovaleric acid	5
6	Maple syrup urine disease	2-hydroxyisovaleric ↑↑↑, 3-hydroxyisovaleric, 2-hydroxy-4-methylvaleric acid, N-acetyl-L-isoleucine	2
7	Carnitine metabolic disorder	↑ metabolites of fatty acids oxidation (3-hydroxSebacic, Adipic), ↓ Krebs cycle metabolites (citric, fumaric) lactic (N, ↓)	1
8	Ornithine carbomoiltransferase deficiency	↑ pyrimidines (↑uracil, ↑orotic), ↓ citrulin, ↓ornithine	2
9	Pyrimidine metabolic disorder (uracil dehydropyrimidine dehydrogenase)	↑uracil, ↑thymine	2
10	5-oxyprolinemia	↑↑↑ 5-oxoproline (› 1000 mmol/mol KREA), γ-Glutamil cycle disorder	2
11	Biotinidase deficiency	↑↑↑ 3-hydroxypropionic, methylmalonic acid, 3-hydroxyisovaleric	5

12	Deficiency of holocarboxylase synthetase	↑↑↑ 3-hydroxypropionic, methylmalonic, 3-hydroxyisovaleric acid ↑↑; change: leucine, isoleucine, valine, Lactic; Krebs cycle disorder	3
13	Canavan disease	N-acetyl-L-aspartic (› 200 mmol/mol KREA)	2
14	2-hydroxylglutaric aciduria	2-hydroxyglutaric acid	1
15	HMD neurotransmitters	↓ homovanilic, ↓ vanylmandelic acid	2
16	Alkaptonuria	↑↑↑ homogentisic acid	1
17	Tyrosinemia type II	↑ N-acetyl-L-tyrosine, 4-hydroxyphenylpyruvic, 4-hydroxyphenyllactic; ↑ Phenylalanine, 2-hydroxyphenylacetic acid	1
18	Disorder of metabolism of fatty acids with a long carbon chain	3-hydroxysebacic, 2-hydroxysebacic, 3-hydroxydodecanedioc; suberic, sebaric acid	4
19	Tyrosinemia type 1	↑ N-acetyl-L-tyrosine, 4-hydroxyphenylpyruvic 4-hydroxyphenyllactic acid, succinylacetone	1
20	Deficiency of succinic acid semialdehyde (4-hydroxybutyro aciduria)	4-hydroxybutyric, glycolic, lactic, 2,4-dihydroxybutyric, 3,4- dihydroxybutyric acid	2
21	Glycerolemia	Glycerol (›10000 mmol/mol KREA)	2
22	HHH syndrome	↑pyrimidines (↑uracil, orotic), ↑ornithine, ↑homocytruelin	1

Fragment of the organic acid examination report

11.1. Metabolites of AA Phenylalanine (Phe) and Tyrosine (Tyr)

11.1 Метаболиты АК Фенилаланина (Phe), Тирозина (Tyr)					
2-hydroxyphenylacetic (**Phe**, Tyr)	8, 13	n.d.		0 - 11	Umol/mmol KREA
p-hydroxyphenylacetic (**Phe**, Tyr)	8	82.17		0 - 837.9	Umol/mmol KREA
Phenylactic (**Phe**, Tyr)	14			-	Umol/mmol KREA
Mandelic (**Phe**, Tyr)	14			-	Umol/mmol KREA
Phenylpiruvic (**Phe**, Tyr)				-	Umol/mmol KREA
Phenyllactic (**Phe**, Tyr)				-	Umol/mmol KREA
↓ Sumiki's (5-hydroxymethyl-2-furoic) (Phe)		1.7		0 - 55.12	Umol/mmol KREA
N-acetyltyrosine (Tyr)	15	1.77	↑	n.d.	mmol/mol KREA
4-hydroxyphenylpyruvic (Phe, **Tyr**)	12, 13, 15	10.40		0 - 28.57	Umol/mmol KREA
Hydroxyphenyllactic (Phe, **Tyr**)	12, 13, 15	37.62		0 - 167.01	Umol/mmol KREA
Homogentisic (Phe, **Tyr**)	12, 13	present	↑↑↑	-	Umol/mmol KREA
4-hydroxybenzoic (Phe, Tyr)	8	2.52		15.92 - 273.2	Umol/mmol KREA
p-hydroxyhippuric (Phe, Tyr)	8, 16	5.25		0 - 405.21	Umol/mmol KREA
3-hydroxyhippuric (Phe, Tyr)	8	n.d.		n.d.	Umol/mmol KREA
Hippuric (Phe, Tyr)	8, 9, 16, 16	606.71		0 - 2181.85	Umol/mmol KREA
4 hydroxycyclohexylcarboxylic (Phe, Tyr)	8	n.d.		0 - 2.02	Umol/mmol KREA
4-hydroxycyclohexylacetic (Phe, Tyr)	8			n.d.	Umol/mmol KREA
Fumaric (Phe, Tyr)	1, 7, 12	4.62		1.2 - 25.25	mmol/mol KREA

11.2. Metabolites of AA Tryptophan (Trp), Lysine (Lis), Histidine (His) and Arginine (Arg)
11.3. Ketosis; metabolites of AA with a branch chain: Leucine (Leu), Isoleucile (Ile) and Valine (Val)
Test for keto acids in case of leucinosis neg. negative

11.2 Метаболиты АК Триптофана (Trp), Лизина (Lis), Гистидина (His), Аргинина (Arg)					
Pimelic (Lys)	4	6.55	↑	0 - 2.77	mmol/mol KREA
Glutaric (Lys, Trp, B2)	12, 14	2325.59	↑↑	0 - 3.62	mmol/mol KREA
5-Hydroxyindoleacetic (Trp)	8, 10	225.64		43.4 - 463.14	Umol/mmol KREA
Indoleacetic (Trp)	8	187.2		0.98 - 225.13	Umol/mmol KREA
Indolelactic (Trp)	8			-	Umol/mmol KREA
Oxoglutaric (His, Arg, Pro)	1, 7, 9, 11, 12, 13, 14	615.92	↑	4.9 - 110.94	Umol/mmol KREA
11.3 Кетоз; метаболиты АК с разветвленной цепью: Лейцина (Leu), Изолейцина (Ile), Валина (Val)					
Тест на кетокислоты при лейцинозе		отр.		отрицательный	-
3-methylglutaric (Leu)		9.56	↑	0 - 0.54	Umol/mmol KREA
3-Methylglutaconic (Leu)		99.21	↑	5.78 - 38.65	Umol/mmol KREA
Isovalerilglicine (Leu)				0 - 4.13	Umol/mmol KREA
3-methylcrotonylglycine (Leu)				-	Umol/mmol KREA
2-Hydroxyisovaleric (Leu)				0 - 11.8	Umol/mmol KREA
3-hydroxyisovaleric (Leu)	12	251.66	↑	5.44 - 59.09	Umol/mmol KREA
3-hydroxymethylglutaric (Leu)	1, 7, 12	26.29	↑	0 - 3.67	Umol/mmol KREA
Hydroxyisobutyric (Ile)		24.22	↑	1.75 - 21.61	Umol/mmol KREA
Erythronilic (Ile)		270.88	↑	0 - 76.19	Umol/mmol KREA
2-Ethylhydracrylic (Ile)		42.13	↑	0 - 12.76	Umol/mmol KREA
Tyglylglycine (Ile)	1, 2			0 - 11.14	Umol/mmol KREA
2-Methylbutyrylglycine (Ile)				-	Umol/mmol KREA
3-Hydroxyisobutyric (Val, тимин)		42	↑	1.26 - 13.73	Umol/mmol KREA
Isobutyrylglycine (Val)				0 - 6.04	Umol/mmol KREA
Succinic (Leu, Ile, Val)	1, 2, 12, 13	20.59		1.28 - 49.7	mmol/mol KREA

Hence, using the GC-MS method, RD was found in 45 patients suspected for a hereditary metabolic disease. Besides, this method allowed identifying secondary metabolic disorders in the patients. Their correction improved the effectiveness of treatment of the main condition.

The hyperornithinemia-hyperammonemia-homocitrullinuria syndrome is rare, with about 100 patients described worldwide. The disease is linked to a defect of ornithintranslocase, It is characterised by a high level of ammonia ions and ornithine in the blood, and elevated kidney excretion of homocitrullin. The mode of inheritance is autosomal recessive. Gene ORNT1 mut (T32R) is localised in the long chromosome arm 13, in the region 13q14; del F188 mutation in SLC25A15 [5, 6, 7].

Insufficient delivery of ornithine into the mitochondrial matrix has been found to disturb the functioning of the urea synthesis cycle. A consequence of this is disturbed utilisation of nitrogen compounds and occurrence of hyperammoniemia. Absence of ornithine activates transformation of lysine into homocitrullin and increases its blood and urine level [8, 9].

The age of occurrence of first indications is known to vary largely from the neonatal period and to an age of 18. The course is paroxysmal. Often early symptoms are not specific, and therefore they are easy to detect. The triggers can be infection, anaesthesia, super stress, transfer to artificial feeding, and introducing high protein-content food products to the diet.

In early childhood, the symptoms are usually less acute and more variable than in the neonatal period. They include anorexia, lethargy, vomiting, delayed psychomotor and speech development, and short stature. The symptoms occur episodically in the setting of 'metabolic stresses'.

This example of one of our observations of the HHH (hyperornithinemia-hyperammonemia-homocitrullinuria) syndrome demonstrates GC-MS effectiveness in diagnosing aciduria.

Boy S., age of 4, was admitted with complaints of frequent vomiting, atony, asthenia, sleeping disorder, and delayed rate of psycho and speech development.

The child was delivered by a second physiologically passing pregnancy, I term birth with a gestation term of 36–38 weeks, by caesarean section, the indications for which were *spina bifida occulta* of the mother's lumbar spine region L5-S1. The newborn body mass was 4 350 g, the body length was 55 cm, with immediate vagitus. The newborn was breast fed up to 2 months. Head control started at 2-3 months, and the baby started sitting at 6.5 months and walk at 10 months. The psychomotor development at the first year of life corresponded to the age. At 1.5 years, the child started experiencing delayed speech development, motor hyperactivity, and low attentiveness. At 2 years and 7 months, the child had glandular fever, adenoiditis, and was treated at an infectious diseases hospital. During the neonatal period, the child's development was apparently normal, though he sometimes refused to eat. According to N. Blau et al. (2003), during the neonatal period, children with an HHH syndrome appear to be normal, but soon they refuse to eat, and nausea and vomiting, general hypotony and neurological and vegetative disorders appear, including vasomotor instability, and apneic spells and coma occur [3]. Such peculiar manifestations were also evident during our attendance.

The mother considered the child ill since the age of three when, after a contused wound and suturing under general anaesthesia, the following manifestations gradually appeared: delayed psycho-motor development, growth retardation, anorexia, vomiting, dullness, and prolonged sleep. The mother noticed that the provoking factor of vomiting was eating cheese, which the child liked very much.

The child was examined by a gastroenterologist who diagnosed biliary dyskinesia and pancreatopathy. After a temporary improvement, a fluctuating level of consciousness progressed with local neurological symptoms, and difficulties in learning appeared. The neurological condition demonstrated enhanced tendon reflexes, a Babinski reflex, feet clonuses, spastic paraparesis, ataxia, choreoathetosis, and symptomatic epilepsy. During attack-free intervals, the neurological symptoms disappeared almost completely, though hepatosplenomegaly developed.

Neurologist and psychiatrist consultations diagnosed a minimal cerebral dysfunction, the syndrome of delayed rate of speech and psycho-motor development, a mild vestibulo-ataxic syndrome, hyperactivity with lack of attentiveness and liquid-venous distention. The dismetabolic state was of vague origin.

At 3 years and 9 months, a geneticist consulted the child for the first time. Basic examination revealed the following: hyperhomocysteinemia, and polymorphism MTRR 66AG, MTR 2756AG. Hyperhomocysteinemia treatment was prescribed with betaine, and vitamins B_6 and B_{12} for two weeks. The child's condition improved fast. The homocysteine level normalised. The mother was offered follow-up examination aimed at searching for the biochemical "target" of the disease because the child evidently had a hereditary metabolic disease. However, the family failed to take the recommended examination. In two months, after a condition of acute bronchitis, the mother noticed a deterioration in the child's state, viz. onset of hyperactivity and aggressive behaviour, which were replaced with dullness and vomiting, and decided to continue the examination.

Examination in the Medical-Genetics Centre revealed increased transaminase activity, alkali phosphatase, with a normal level of gamma glutamyl transpeptidase, and a high ammonia

level of 260.99 µmol/L (normal range 18-72 µmol/L). The child was admitted to a multiprofile regional in-patient hospital because hyperammoniemia and a behavioural disorder was revealed. The hospital performed differential diagnostics for hepatitis and the Wilson-Konovalov disease, and imbalance of metabolism of sulphur-containing amino acids.

Test results: functional liver tests - cytolysis up to 7 (N); copper in blood serum - 20.5 µmol/L (N 10 - 18), copper in urine - 1.17 µmol/L (N 0.03 - 1.26), and blood ceruleoplasmin - 219.6 mg/L (N 180- 450).

Disturbed forms of behaviour and the syndrome of lack of attentiveness along with hyperactivity were diagnosed. Hepatitis and the Wilson-Konovalov disease were excluded. Protein intake to 1.2 g/kg, and Pantogam, Betargin, and carnitine were recommended. The child was discharged in a better condition.

In three months, in the setting of the therapy prescribed by pediatricians, repeated daily vomiting occurred up to seven times in the evening and night time. Hyperactivity increased and it alternated with dullness, drowsiness, shaky walk, missing objects when attempting to take them, and episodes of mental fog. The mother once again applied to KhSMGC only after these symptoms appeared again. The ammonia level was tested urgently and found to be up to 391.49 µmol/L (N 18-72 µmol/L). The child was presented to an international council of physicians represented by the Director of the Ukrainian Institute for Clinical Genetics, Prof. Grechanina Ye.Ya., and Professor Matalon with the Pediatrics Department at the Galveston University (U.S.A.), Prof. Emeritus of the Kharkiv National Medical University. With account of the above complaints, case history, clinical-genealogical analysis, somatic and neurological condition data, additional examination methods (high blood ammonia level – from 391.49 to 89.44 µmol/L (normal range 18-72 µmol/L), hyperornithinemia 1.373 mg (N 0.345-1.008), hypercitrullinuria 737.18 mmol/mol KREA), and no earlier treatment effect, the following was diagnosed: the HHH syndrome (hyperornithinemia, hyperammoniemia, homocitrullinuria); polymorphism of folate cycle genes (heterozygote compound MTRR 66AG/MTR 2756AG), and hyperhomocysteinemia.

The child's status stabilised in the treatment setting. However, in short time, the disease progressed again.

The status demonstrated increasing drowsiness, ataxia, hypersalivation, and declining appetite. Again, there appeared pronounced aggression, convulsive twitches, and loss of acquired skills. This episode was also linked to a past respiratory disease.

Follow-up examination revealed the following:

- US examination of inner organs: enlarged liver by +4 cm, diffuse parenchyma changes, high exogeneity, venous pattern invariable, gall bladder wall oedema, spleen with increased echogeneity, veins in hilus are convoluted, the splenic vein is 6-7 mm, kidneys – oedema and parenchyma ischemia, and moderate dextral pyelectasia.
- EEG: indications of decreased level of bioelectric activity in all derivations. Paroxysmal activity in the form of low-amplitude diffuse sharp waves in the setting of dysfunction of lower stem structures.
- EEG: Mechod = Mechos= 67 mm. Mecho width 6.0. Displacement not revealed. Indications of liquor hypertension.
- Ultrasound Doppler sonography of cerebral vessels: regional angiodystonia in the medial cerebral artery, anterior cerebral artery with increased blood flow and vasospastic responses.

- Cerebral NMRI: symmetrical lesion in the form of oedema of the cortical-subcortical sections of the frontal, temporal and parietal lobes (of hypoxic or dismetabolic origin), and with a mass-effect. Ventricular system not changed, lateral ventricles D>S, liquor outflow from ventricles maintained. Midline structures not displaced. Cortex of hemispheres beyond zones of described lesions is without change. Subarachnoid spaces without features. Retrocerebral cistern dilated to 1.11 cm (arachnoid cyst) with cerebellar vermian hypoplasia.

The child was consulted by a neurosurgeon. Cerebral MRI revealed zones of cortical ischemia in both hemispheres in the basin of the medial cerebral artery.

- Platelet aggregation: at an ADP concentration of 0.625 µmol/L, single-wave aggregation with disaggregation was observed. At an ADP concentration of 1.25 µmol/L and 2.5 µmol/L, a second wave of aggregation with partial disaggregation was observed. At an ADP concentration of 5.0 µmol/L, single-wave aggregation with partial disaggregation was observed.

Clotting system indicators

Ref. No.	Metabolites	Result	Reference values
1	APTT (activated partial thromboplasin time)	49.4 s	30.5-38.5
2	International normalised ratio (INR)	1.77	0.8-1.3
3	Prothrombin time	24.7 s	10.5-15.8

- Blood lactate 0.97 mmol/L (N 0.2-2.2).

Analysis of blood serum amino acids

Ref. No.	Metabolites	Result	Reference values
1	Lysine	1.608 mg ↓	1.825-3.106
2	Threonine	0.397 mg↓	0.89-1.483
3	Glycine	0.824 mg	1.106-2.12
4	Alanine	1.034 mg↓	2.163-3.922
5	Valine	0.965 mg	2.065-2.95
6	Isoleucine	0.149 mg↓	0.484-0.936
7	Leucine	0.616 mg↓	1.275-2.009
8	Phenylalanine	0.513 mg↓	0.750-1.442
9	Ornithine	1.373 mg↑	0.345-1.008
10	Methionine	0.511 mg↑	0.167-0.400
11	Ammonia	1.634 mg↑	0.382-1.147

Biochemical profile

Ref. No.	Metabolites	Result	Reference values
1	AspAT	46.48 U/L↑	0-36
2	ALAT	69.17 U/L↑	0-29
3	Creatine kinase	155.91 U/L↑	0-149

- Uric acid 1.22 U/L (N 1.68-3.84).
- Blood homocysteine – 5.1 (normal 4.3).

Follow-up gas chromatography of the urine and organic amino acids revealed the following: a significant uracil and citrulline increase, and changes in pyrimidine metabolites and the Krebs cycle.

The child received the following treatment: a protein-limited diet (a special Renilon nutrition mixture by Nutricia); an antibacterial therapy (ceftriaxone, amicile, meronem, and vancomec); an immunomodulatory therapy (bioven mono); a pathogenetic therapy with the application of hepa-merz, betargin, glutargyn, riboflavin, cytoflavin, pyridoxine, cyanocobalamine, tivortin; a cerebral oedema-brain swelling therapy (L-lysine escinate and magnesium sulfate); drugs for improving cerebral metabolic processes (Mexidol, Ceraxon), detoxication therapy, supporting the plasma colloid-oncotic pressure, stabilising cell membranes, correcting diselectrolytic disorders, improving blood rheological properties, for preventive treatment of stress ulcers (gastrocepin), controlling the DIC syndrome, and providing myocardium inotropic support.

On the third day after admission to the in-patient department, augmentation of pathological neurological symptoms was noticed: dextral hemiparesis, the pseudobulbar syndrome and somnolence episodes appeared. Cerebral oedema-brain swelling therapy was conducted; actions were taken to improve cerebral hemodynamics and blood rheological properties, and correct electrolytic and dismetabolic disorders. Data of follow-up EEG and NMRI indicated pathological process progression: EEG showed the emergence of a locus of slow high-amplitude waves in the derivations of the right hemisphere and a site of pronounced decrease of bioelectric activity and functional activity in the derivations of the left hemisphere. A gross mediobasal dysfunction was detected. Cerebral NMRI determined an extensive lesion in the form of an oedema of cortical-subcortical sections of the frontal, temporal and parietal lobes on the left (with involvement of the basal section of the frontal and temporal lobes), as well as a lesion of the cortical-subcortical sections of the frontal and temporal lobes on the right in the Sulvian fissure projection. The lesion described has no clear contours and has a diffuse oedema form. The NMRI determined an increasing zone of lesion in the left hemisphere and an augmentation of the mass-effect, viz. the left lateral ventricle is compressed, the medial structures are displaced to the right up to 5 mm, and the right lateral ventricle is moderately dilated. The inner liquor flow is maintained. Geneticists suspect a total cerebral occlusion.

Child observation over time, fever periods, progressing changes, CNS changes, and bulbar disorders required conducting a differential diagnosis for viral encephalitis. Child consultancy with a neuro-infectionist showed that encephalitis was unlikely.

Follow-up tests showed an increasing ammonia level - 460.9 µmol/L, 820.57 µmol/L, and 1 000.14 µmol/L (normal range 18-72 µmol/L).

Despite the therapy being conducted, the child failed to thrive: the patient was inaccessible for oral contact; the response to noxious stimuli was chaotic movement of the limbs; the vision is not fixed, anisocoria was detected, the guttural and throat reflexes were depressed, and breathing was shallow. With account of the rapidly progressing cerebral insufficiency and depressed stem reflexes, the child was intubated and placed under artificial pulmonary ventilation. Consultations were conducted in real time with participation of the republican child neurologist, the republican anesthesiologist, a toxicologist, and a university centre geneticist

(Amsterdam). Continuous monitoring by Prof. Ye.Ya. Grechanina and Prof. R. Matalon (the latter, in the on-line mode) was provided. The extreme severity of the patient's condition was assessed as a result of progressing metabolic encephalopathy with an evolving cerebral coma III in the setting of the HHH (hyperornithinemia, hyperammoniemia, homocitrullinuria) syndrome. At the same time, indications of cobalamin deficiency, pronounced anemia, hyperhomocysteinemia, indications of thrombophilia in the setting of a genetic compound of polymorphism MTRR 66 AG/MTR 2756 AG were revealed. This fact suggested the possibility of changed functions of neutral alleles MTRR and MTR to a 'risk allele', and subsequently to a clinically significant allele under the repeated effect of a trigger – a relapsing infection. This idea was also supported by the patient's positive response to a short-time correction of hyperhomocysteinemia with a folate therapy, and the presence of a myelocele in the mother. Since the activity of the above-mention enzymes was not investigated, this suggestion was not confirmed. However, we are continuing to search for the indicated mechanism, which changes its clinical manifestations in other rare diseases associated with different variants of cobalamine deficiency in the presence of respective polymorphisms.

The clinical diagnosis was formulated as follows: ornithine metabolism disorder – the HHH (hyperornithinemia, hyperammoniemia, homocitrullinuria) syndrome. Polymorphism MTRR 66 AG / MTR 2756 AG. Hyperhomocysteinemia. Metabolic encephalopathy. Stage III cerebral coma.

In spite of providing a protein-limited diet (the Renilon nutrient mixture by Nutricia), etiotropic, pathogenetic and symptomatic therapy, the patient's condition degraded progressively, the syndrome of polyorgan insufficiency (cerebral, respiratory, cardiovascular, and acute renal failure evolved), and cardiac standstill occurred. The child was subject to resuscitation procedures in full scope; however, to no avail, and biological death was registered on the 28th day after admission to hospital and the 38th day of diagnosing the HHH syndrome.

Post-mortem diagnosis: inborn error of metabolism: HHH syndrome (hyperornithinemia, hyperammoniemia, homocitrullinuria).

Complications: thrombohemorrhagic syndrome with massive progressive cerebral venous sinus thrombosis, thrombosis of meninx vasculosa, and intracerebral vessels. Total softening of the brain. This finding once again focused our attention on the possible role of thrombophilia associated with discovered polymorphism in the development of a severe clinical picture.

Conclusions: The study performed has demonstrated the high effectiveness of GC/MS in qualifying diagnostics of HMD provided classical methods of clinical genetics (detailed information about the case history and life of the family, analysis of ancestry, and quality somato-genetical investigation with a syndromological analysis) are combined with advanced hi-tech methods.

For unambiguous prognosis of progeny in this family, preconception preventive treatment and molecular-genetic investigations of mutations associated with the HHH syndrome are conducted.

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