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Homocystinuria due to cystathionine beta-synthase (CBS) deficiency in Russia: Molecular and clinical characterization

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ABSTRACT

We present the results of the 45-year clinical observation of 27 Russian homocystinuria patients. We made a mutation analysis of the *CBS* gene for thirteen patients from eleven unrelated genealogies. All patients except for the two were compound heterozygotes for the mutations detected. The most frequent mutation in the cohort investigated was splice mutation IVS11-2a- > c. We detected one new nonsense mutation, one new missense-mutation and three novel small deletions. We also report the clinical case of the B_6-responsive patient genotyped as Ile278Thr/Cys109Arg.

1. Introduction

Health problems of children are often caused by hereditary factors and significantly reduce the life quality not only for the sick child, but also for all members of his/her family. Scientific advances in human genetics have allowed clinical genetics to become one of the most rapidly developing areas of modern medicine. The successful search for novel therapeutic options for inborn errors of metabolism is impossible without studies elucidating the underlying pathogenic mechanism of the diseases. In large number of adult, seriously handicapped patients, the disease has onset in childhood, but can be diagnosed early and thus many serious clinical complications could be averted. Homocystinuria due to cystathionine beta-synthase deficiency (CBS) deficiency is an example of the inherited disease which manifests itself in adults with life-threatening thromboembolic events also in asymptomatic patients during childhood period [1].

Homocystinuria due to CBS deficiency (OMIM 236200) is an autosomal recessive disorder of sulfur- amino acid metabolism that results from the CBS (EC 4.2.1.22) deficiency. This defect leads to high accumulation of homocysteine and methionine in blood and urine [2,3]. Mutations in the *CBS* gene lead to a substantial reduction of cystathionine beta-synthase activity. The incidence in the general population by various authors ranges from 1:50,000–1:250,000, 1:311,000 [4].

The clinical picture of homocystinuria was described by many researchers [5–7]. All the authors draw attention to the clinical heterogeneity and progression of the disease, but the majority of them consider that homocystinuria is characterized by a peculiar syndrome of "marfanoid" features, mental retardation, with the formation of focal neurological symptoms, optic lens dislocation, osteoporosis and skeletal deformations, thromboembolism, and cardiovascular disease (myocardial infarction). Children with homocystinuria usually have blond or light brown, soft and slightly curly hair, delicate blush on the cheeks and blue iris. Usually they are tall, with asthenic constitution. Patients recorded long thin limbs, arachnodactyly of hands and feet, valgus knee setting, kyphoscoliosis, funnel or pigeon chest deformation, and moderate osteoporosis. Due to osteoporosis patients with homocystinuria often have a history of fractures. Along with this, there are descriptions of these forms of the disease with minimum or completely absent skeletal abnormalities.

The human *CBS* gene has been mapped to 21q22.3 [8]. The *CBS* encodes a protein of 551 amino acids. In its biochemical activity the enzyme requires pyridoxal 5′-phosphate (PLP, which is an active form of vitamin B_6) as co-factor. As a result, two types of homocystinuria due to CBS deficiency based on its treatment have been distinguished: one is vitamin B_6-responsive while the other is not. Usually, patients with the B_6-responsive form of the disease have a milder phenotype than patients with the non-responsive form. But, severity of the disease varies from mild to severe even in patients with the B_6-responsive form [9–12].

To date, more than 150 different mutations in the *CBS* gene have been described [13]. The Ile278Thr is the most frequent mutation among the various populations of the world and causes a mild form of the disease [14]. The Gly307Ser mutation is responsible for the formation of severe clinical symptoms of the disease and is detected mainly in patients of Celtic origin [15,16]. It was determined that the

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Czech Republic, Slovakia and Poland are countries with the most common mutations Ile278Thr and IVS11-2a- > c [17].

In the present study, we describe 27 Russian patients from 22 unrelated genealogies. We investigated the molecular basis of homocystinuria due to CBS deficiency in 13 of them from 11 families. We also report in detail the clinical case of the homocystinuria patient with genotype Ile278Thr/Cys109Arg.

2. Material and methods

2.1. Patients

All patients were observed at different times at the Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University of Moscow. The diagnosis was confirmed by positive routine nitroprusside test, high levels of methionine and homocysteine in plasma and urine and excretion of homocysteine in the urine. For 13 patients from 11 families a DNA analysis of the *CBS* gene was performed.

2.2. DNA preparation

DNA was isolated from whole peripheral blood leukocytes using the DNAPrep100 Kit (IsoGene, Moscow, Russian) according to manufacturers' recommendations and stored at −20C prior to the analysis.

2.3. Oligonucleotide primers and PCR

All primers were synthesized by a commercial company (“Syntol”, Moscow, Russia). Nucleotide sequences of primers were complementary to the sequences of the introns flanking of each coding exons of the *CBS* gene (according to Electronic-Database Information (NCBI)). PCR was performed as described characterized mutation.

2.4. Mutation analysis

All PCR fragments were subsequently sequenced on an ABI 3130*xls* automated DNA sequencer (Applied Biosystems) with the Taq Dye Deoxy Terminator Cycle Sequencing Kit. All PCR fragments were sequenced on both strands, and mutations were confirmed by restriction enzyme analysis or second DNA sequencing. Restriction enzymes were purchased from Sibenzyme (Russia) and used according to the manufacturers' recommendations.

3. Compliance with ethics guidelines

3.1. Informed consent

All procedures were performed in accordance with the institutional and national ethical standards and the 1975 Helsinki Declaration revised in 2000. The informed consent was obtained from all patients included in the study. The additional informed consent was obtained from all patients whose personal information may be identified in this article.

4. Results

4.1. Patients

For the past forty years under the supervision of the Department of Clinical Genetics of the Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University there were 27 patients aged from 3 to 21 years with homocystinuria due to CBS deficiency. The male–female ratio was 11:16. Five families had two affected siblings. Sixteen patients were B_6-responsive.

The clinical data of the homocystinuria patients are summarized in Table 1. The height in half of the patients was above average. All patients except two had ocular pathology mostly lens subluxation.

In 17 patients this condition was complicated by the development of secondary glaucoma that required urgent surgical operation (Fig. 1). The lens subluxation was diagnosed at the age of 5 to 7 years.

Twenty one patients exhibited a skeletal pathology, such as valgus deformity of shins increase in knees and their installation, kyphoscoliosis, chest deformity, clubfoot, several previous fractures, and moderate osteoporosis. None of the patients had seizures. Four patients after ischemic stroke developed central hemiparesis. One patient had a stroke of the pancreas. Psychic abnormalities were observed in 7 patients and included stubbornness, inadequacy, attacks of aggression and sexual promiscuity. Eighteen probands demonstrated mental retardation. Two children underwent the operation that was complicated by thrombosis of sinus venosus transversus and successfully treated without clinical consequences.

The central hemiparesis after ischemic stroke has been observed in 4 patients. Intellectual development of B_6-responsive patients was normal or slightly lower. The pyridoxine-nonresponders had moderate mental retardation.

The mitral valve prolapse was observed in twenty three patients, the transient cardiac arrhythmia in eight, and the arterial hypertension in ten patients. The ischemic stroke has developed in seven patients with the B_6-resistant form of the disease at the age of 14 to 17 years. In two children, the lens removal operation was complicated by transverse venous sinus thrombosis which was successfully cured without clinical consequence.

The data retrospective analysis of the health condition of 27 patients showed that 3 probands died at the age of 16 (patient 8), 22 (patient 10) and 30 years (patient 11^c). The cause of death of these patients were croupous pneumonia, myocardial infarction and stroke, respectively. The proband with the B_6-resistant form of homocystinuria, who died from myocardial infarction at age 22, had expressed neurological symptoms, which manifested itself mainly as a violation of the gait (the patient could only move with a wheelchair). The sister of the proband, also suffering from homocystinuria, is currently 51 years old. Like her brother, she experiences difficulties with independent movement, has pronounced personality traits and periodic attacks of aggression. The parents of the siblings are obligate and heterozygous mutation carriers of the *CBS* gene. The father suddenly died at the age of 40 years from a stroke. The mother of the patients has a group II disability in connection with the pathology of the cardiovascular system (ischemic heart disease), and her own sister being an aunt of the sibs died of the 5th stroke, at the age of 80. The death of another patient's father also occurred suddenly because of stroke. The father of one child with the B_6-resistant form of homocystinuria ended his life with suicide at the age of 42 and also suffered from alcoholism.

The 28 year old woman with the B_6-responsive form of homocystinuria (Patient 12) is married and has a healthy son (Fig. 2, Panels A and B).

4.2. Molecular genetic studies of the CBS gene

For 13 patients from 11 unrelated families a DNA analysis of the *CBS* gene was performed. The results of the DNA analysis presented in Table 2. Twenty three mutant alleles were identified. The second allele in three patients from two unrelated genealogies was not identified. The non-coding exons and deep introns areas have escaped DNA analysis. The large deletion and rearrangements also have not been investigated. The full sequencing of coding exons of the *CBS* gene has revealed only one mutation in heterozygous state. The family analysis had confirmed inheritance: one of the parents was a carrier for the mutation found. Seven out of twenty three mutant alleles were a site splicing mutation IVS11-2a- > c resulting in deletion of exon 12 and is prevalent in the population of Eastern Europe [18,19]. So, the high frequency of IVS11-2a- > c in Russian patients was not surprising. For the detection of

Table 1
Clinical presentation of CBS deficiency patients.

Patient	Age at diagnosis	Gender	Height	Lens subluxation	Secondary glaucoma	Increase in knee and their valgus installation	Kyphoscoliosis	Chest deformation	Moderate osteoporosis	Fractures
1	7 years	F	Normal	+	−	−	−	−	+	−
2[a]	8 years	F	Normal	+	+	+	−	−	+	−
3[a]	3 years	M	Normal	−	−	+	−	+	+	−
4	14 years	F	Normal	+	+	+	−	−	+	+
5	15 years	F	High	+	−	+	−	−	+	−
6[b]	10 years	M	High	+	+	+	+	−	+	−
7[b]	7 years	F	High	+	−	−	−	−	−	−
8	11 years	F	High	+	−	−	−	−	−	−
9	6 years	M	Normal	+	–	−	−	−	−	−
10[c]	12 years	M	Normal	+	+	−	−	−	−	−
11[c]	10 years	M	Normal	+	+	−	−	−	−	−
12	5 years	F	Normal	+	+	−	−	−	−	−
13[d]	7 years	F	Normal	+	+	+	+	−	+	−
14[d]	5 years	M	Normal	−	−	+	−	−	−	−
15	21 years	F	Normal	+	+	+	+	+	+	+
16	10 years	F	Normal	+	+	+	+	+	+	+
17	10 years	M	High	+	+	+	+	+	+	+
18[e]	15 years	M	High	+	+	+	+	−	+	−
19[e]	13 years	F	High	+	+	+	−	−	+	−
20	6 years	M	High	+	+	+	−	−	+	+
21	5 years	F	High	+	+	+	−	−	+	+
22	10 years	M	High	+	+	+	+	−	+	+
23	12 years	M	High	+	+	+	−	−	+	+
24	12 years	F	High	+	+	+	−	−	+	+
25	12 years	F	High	+	−	+	+	−	+	−
26	13 years	F	Normal	+	−	+	+	+	+	−
27	10 years	F	Normal	+	+	+	+	−	+	−

Patient	Mitral valve prolapse	Transient arrhythmias	Arterial hypertension	Ischemic stroke	Hemiparesis	Thrombosis of venous sinuses	Seizure	Psychological abnormalities	Mental retardation	Pyridoxine-responsiveness
1	+	−	−	+	+	−	−	−	+	B_6-resistant
2[a]	+	−	−	−	−	−	−	+	+	B_6-resistant
3[a]	−	−	−	−	−	−	−	−	+	B_6-resistant
4	+	+	+	+	+	−	−	+	+	B_6-resistant
5	+	+	+	+	−	−	−	+	+	B_6-resistant
6[b]	+	−	−	−	−	−	−	−	−	B_6-resistant
7[b]	+	−	−	−	−	−	−	−	−	B_6-resistant
8	+	−	−	−	−	−	−	−	−	B_6-responsive
9	+	−	−	−	−	−	−	−	−	B_6-responsive
10[c]	−	−	−	−	−	−	−	−	−	B_6-responsive
11[c]	−	−	−	−	−	−	−	−	−	B_6-responsive
12	+	−	+	−	−	−	−	−	+	B_6-resistant
13[d]	+	−	−	−	−	−	−	−	−	B_6-responsive
14[d]	+	−	−	−	−	−	−	−	−	B_6-responsive
15	+	+	+	+	+	−	−	+	+	B_6-resistant
16	+	+	+	+	+	−	−	+	+	B_6-resistant
17	+	+	+	−	−	−	−	−	+	B_6-resistant
18[e]	+	−	−	+	−	−	−	+	−	B_6-responsive
19[e]	+	−	−	−	−	−	−	−	−	B_6-responsive
20	+	−	−	−	−	−	−	−	−	B_6-responsive
21	+	−	−	−	−	+	−	−	−	B_6-responsive
22	+	−	−	−	−	+	−	−	−	B_6-responsive

(continued on next page)

Table 1 (*continued*)

Patient	Mitral valve prolapse	Transient arrhythmias	Arterial hypertension	Ischemic stroke	Hemiparesis	Thrombosis of venous sinuses	Seizure	Psychological abnormalities	Mental retardation	Pyridoxine-responsiveness
23	+	−	+	−	−	−	−	−	+	B_6-resistant
24	+	−	−	−	−	−	−	+	+	B_6-resistant
25	+	+	+	+	−	−	−	+	+	B_6-resistant
26	+	+	+	−	−	−	−	−	+	B_6-resistant
27	+	+	−	−	−	−	−	−	+	B_6-resistant

[a] Siblings.
[b] Siblings.
[c] Siblings.
[d] Siblings.
[e] Siblings.

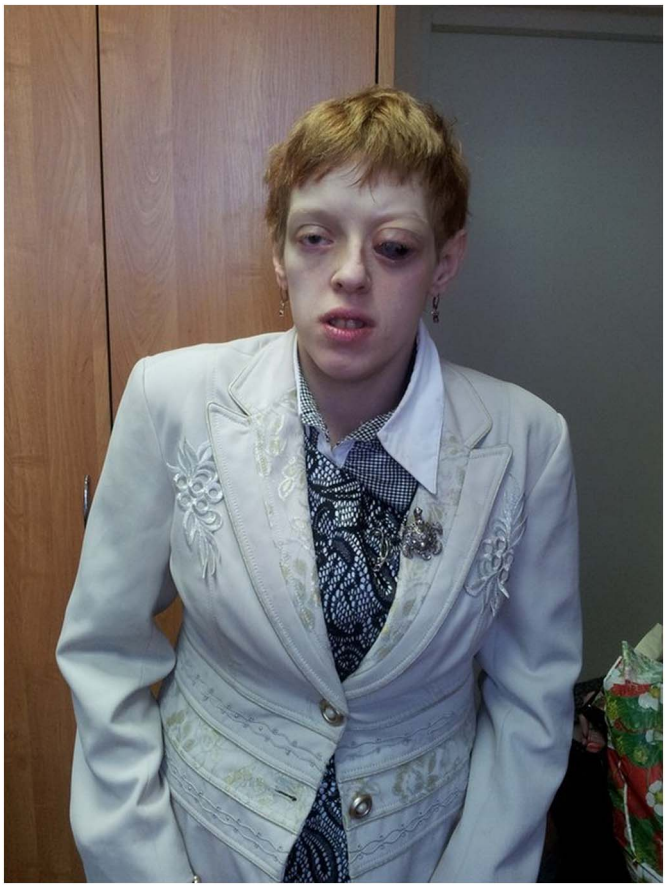

Fig. 1. Patient 15, homozygous for Gly305Arg mutation, with malignant secondary glaucoma as a complication of pyridoxine-nonresponsive homocystinuria.

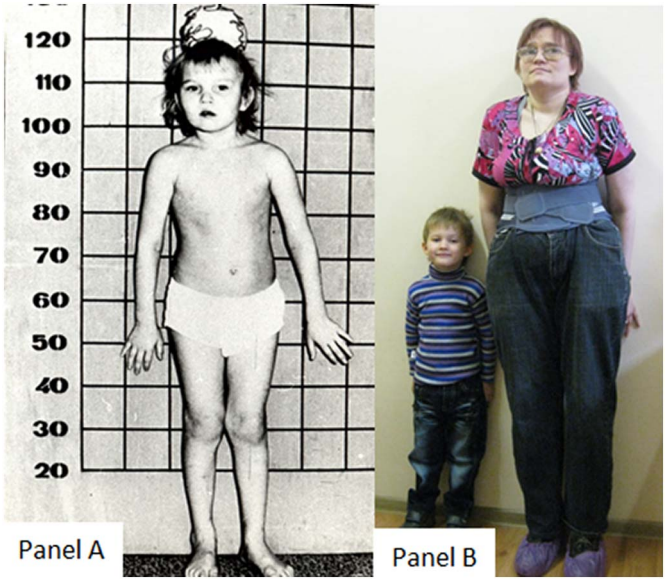

Fig. 2. Patient 12, homozygous for IVS11-2a- > c mutation, suffer from pyridoxine-responsive homocystinuria and shows minimal changes in musculoskeletal system at age of 5 years (panel A) and 23 years later, at age of 28 years (panel B, with her healthy son).

mutation IVS11-2a- > c the fast PCR of the *CBS* gene's exon 12 with subsequent treatment with *Msp*I enzyme was developed. The mutation forms the additional restriction site for MspI (Fig. 3).

It was revealed that the homozygotes for IVS11-2a- > c did not respond to vitamin B_6, while in compound heterozygotes the response

Table 2
The *CBS* mutations spectrum.

Patients[a]	Mutation	
	Allele 1	Allele 2
21	*IVS11-2a- > c*	*?*
13[b]	*IVS11-2a- > c*	*?*
14[b]	*IVS11-2a- > c*	*?*
6[c]	*Lys384Asn*	*c.1560-1569del CACCGGGAAG Novel*
7[c]	*Lys384Asn*	*c.1560-1569del CACCGGGAAG Novel*
20	*Ile278Thr*	*Cys109Arg*
12	*IVS11-2a- > c*	*IVS11-2a- > c*
23	*IVS11-2a- > c*	*c.216-217delAT Novel*
24	*Thr353Met*	*Gln368Term Novel*
15	*Gly305Arg*	*Gly305Arg*
25	*IVS11-2a- > c*	*Trp390Term*
26	*Glu302Lys*	*c.1498_1499delT Novel*
27	*Asp444Tyr Novel*	*Asp444Tyr Novel*

[a] Patients IDs correspond to those in Table 1.
[b] Siblings.
[c] Siblings.

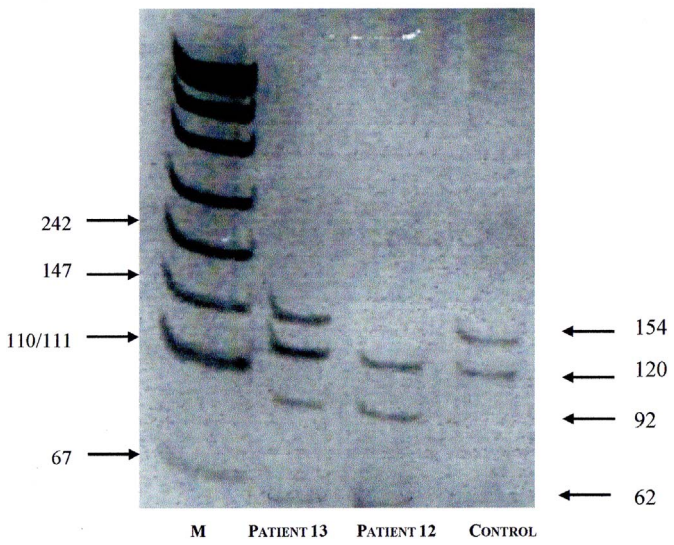

Fig. 3. Detection of IVS11-2a- > c mutation. Exon 12 of the CBS gene was PCR amplified and digested with *Msp*I restriction enzyme. The mutation forms a recognition site for the enzyme. The products were resolved on 8% polyacrylamide gel. M – molecular weight marker (pUC19 cleaved with MspI). Arrows designate the product of WT (154 and 120 bp) and mutant allele (120, 92 and 62 bp), respectively.

to vitamin B_6 depended on the mutation on the second allele [20].

The most common panethnic mutation p.Ile278Thr was found only in one patient in heterozygous state. Two previously described mutations p.Cys109Arg and p.Thr353Met, associated with pyridoxine-responsive and nonresponsive form of the disease, respectively, were found each once in heterozygous state. The patient 24 is a compound heterozygote for the mutations p.Gln368Term and p.Thr353Met and is pyridoxine-nonresponsive. The second allele was Ile278Thr. Thus, even the presence of the mild mutation Ile278Thr does not change the response to vitamin B_6. The mutant with Cys109Arg protein completely lacked catalytic activity [13,20,21,22]. The patient 24 is a compound heterozygote for the mutations Gln368Term and Thr353Met and is pyridoxine-nonresponsive. It is highly probable that the mutation Gln368Term is also associated with B_6-nonresponsive form of the disease.

Three novel small deletions were detected in three unrelated patients from three families. The *c.1560-1569del CACCGGGAAG* was determined in two sibs from the family of Armenian origin (Patients 18 and 19). This deletion introduces a frameshift and a stop codon at position 540 of protein chain. The second mutant allele in the patients was the previously described and characterized mutation Lys384Asn [18]. The second new deletion is *c.216-217delAT* which introduces a frameshift and a stop codon at position 104 of protein chain, was found in heterozygous state in patient 23. The third deletion is *c.1498_1499delT*, which introduces a frameshift and, similarly to the *c.1560-1569del CACCGGGAAG* mutation, a stop codon at position 540 of protein chain, was found in heterozygous state in patient 26. The homozygotes for Lys384Asn in homozygote state respond to vitamin B_6[13]. The patients 6 and 7 were heterozygote for Lys384Asn. The second allele was *c.1560-1569del CACCGGGAAG. Both patients had* B_6-nonresponsive form of disease. Patient 26 was compound for *Glu302Lys and c.1498_1499delT and also had* B_6-nonresponsive phenotype. Thus, it can be assumed that the truncated protein in the position 540 completely loses its functional activity and resulted in B_6-nonresponsive form of disease.

The previously described Gly305Arg mutation [23] was detected in patient 15 in a homozygous state. The patient is the offspring of a consanguineous marriage that has a severe B_6-nonresponsive form of the disease.

4.3. Case report

Here is a case report of the boy (patient 20) with the B_6-responsive form of homocystinuria (Fig. 4). The patient is the offspring of a non-consanguineous marriage from the second physiological pregnancy. At birth, his weight was 3500 g and his length was 50 cm. The early neonatal period was uneventful. At the age of 5.5 years a decrease in visual acuity was observed and bilateral lens subluxation was found. The preliminary diagnosis was Marfan syndrome. At the age of 8 years, the patient was admitted to our clinic. He presented high anthropometric parameters corresponding to the 90th percentile, an increase in the knees and their valgus installation, and incorrect posture.

ECG revealed ectopic right-atrial rhythm, bradycardia, incomplete right bundle branch block; echocardiography revealed dysfunction of the mitral valve, diagonal trabecula in the left ventricle. Ultrasound study revealed a slight hepatomegaly and increased mobility of kidneys. Ophthalmological findings were lens subluxation of II-III degree, and myopic astigmatism in both eyes. His mental development was normal. However, there were manifestations of emotional and volitional immaturity, weakness of self-regulation of behavior and arbitrary action. The tics and moderate fatigue were also found.

MRI revealed areas of increased MR signal in the white matter in the posterior horns of the lateral ventricles, retrocerebellar cyst and instability in the cranio-cervical junction. MRI of the brain vessels stated knee-bend of the right vertebral artery and angle-like bend of the left vertebral artery before its communicative department. Malformations were not found.

The X-ray study revealed osteoporosis and valgus shins. Biochemical findings included parameters of the key types of metabolism (protein, fat, carbohydrate, and mineral) consistent with normal values.

Studies of amino acid spectrum of urine by high-performance liquid chromatography-tandem mass spectrometry revealed high levels of methionine (432 umol/L); (N = 4–130 μmol/L) and homocysteine (541 umol/L, normally absent).

The DNA analysis of the *CBS* gene revealed two mutations in heterozygous state: Ile278Thr and Cys109Arg. (Fig. 5, Panels A and B). The Ile278Thr mutation always resulted in a B_6-responsive phenotype whereas Cys109Arg leads to a complete loss of protein function.

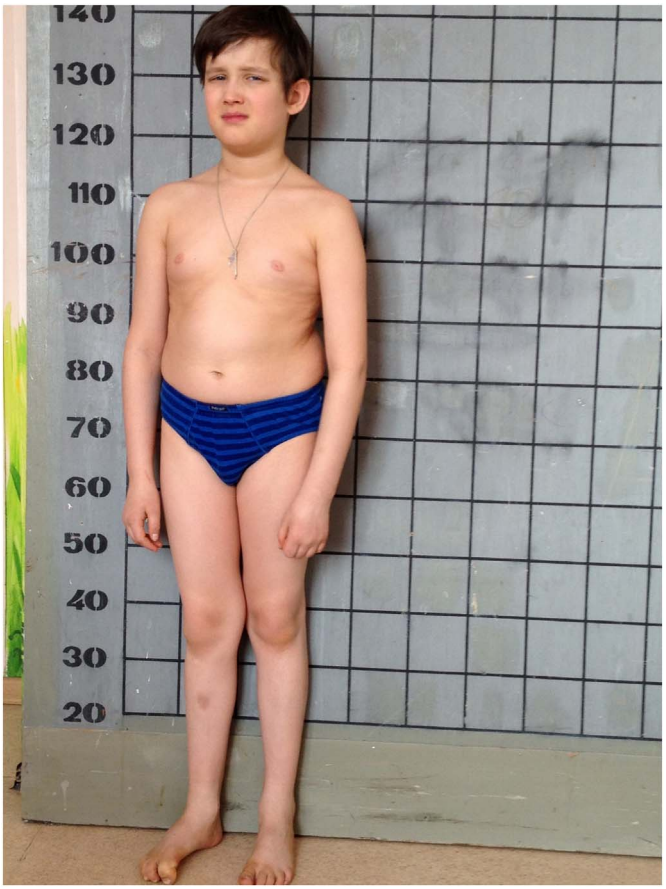

Fig. 4. Patient 20, a compound heterozygote for Ile278Thr and Cys109Arg, with pyridoxine-responsive homocystinuria at age of 6 years.

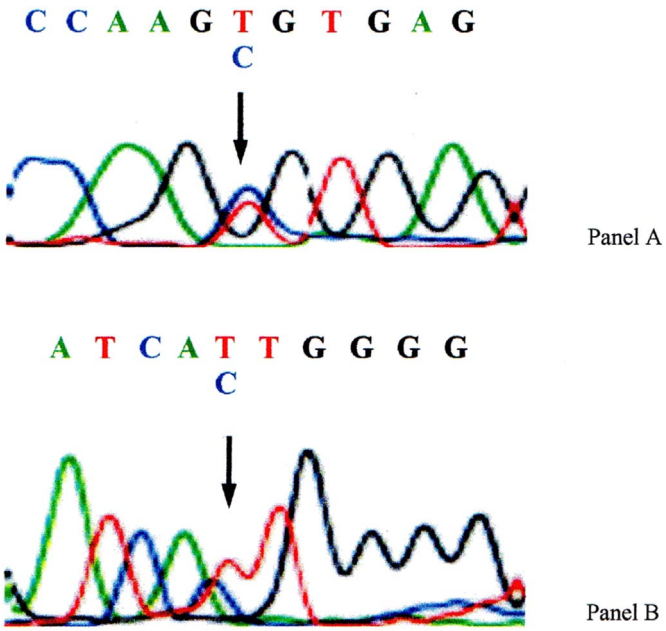

Fig. 5. Identification of pathogenic mutations in *CBS* gene in patient 20: c.325T- > C (Cys109Arg) in exon 3 (panel A) and c.833T- > C (Ile278Thr) in exon 8 (panel B).

Studies have led to the definitive diagnosis of the proband which was homocystinuria due to CBS deficiency in the vitamin B_6-responsive form.

5. Conclusion and discussion

Over the past 45 years, we observed 27 patients with homocystinuria due to CBS deficiency. In the 1970s, the diagnosis of homocystinuria in Russia was based on a combination of clinical symptoms confirmed by appropriate biochemical analysis of blood serum and urine. Analysis of the amino acid spectrum of biological fluids was made by the automatic amino acid analyzer and later by tandem chromatography-mass spectrometry. The criteria for diagnosis were high levels of methionine and homocysteine, the presence of homocystine and low level of cystine in blood serum and urine. The diagnosis of B_6-responsive and B_6-resistant forms of homocystinuria was performed after a 3-day patient intake of vitamin B_6 per os at the initial dosage of 100 mg/day. The normalization of these biochemical parameters with the disappearance of homocystine in biological fluids indicated a B_6-responsive form of the disease. Preservation of biochemical changes in the patient required an increasing dosage of vitamin B_6 up to 500 mg/day, which the patient used to take for three more days. Static biochemical parameters used to prove the B_6-resistant form of the disease in a patient.

We determined the activity of the enzyme cystathionine-beta-synthase in liver biopsy samples in nine patients (patients 1, 2^a, 3^a, 10^c, 11^c, 12, 16, 17 and 21 in Table 1). In all these patients, the activity of the enzyme was sharply reduced and approached zero values. The direct effect of pyridoxal phosphate on the enzyme under study revealed a complete lack of stimulating effect of the cofactor on the activity of cystathionine synthase in eight patients, which objectively proved the presence of the B_6-resistant form of homocystinuria. In one child, the presence of the cofactor stimulated the activity of cystathionine synthase in the liver biopsy to 55–65% of the norm (B_6-responsive form of homocystinuria confirmed).

There is no screening for homocystinuria in Russia. To identify patients with homocystinuria among 280 pupils of the boarding school for visually impaired children, a qualitative urine sample with cyanide nitroprusside was performed. If the exchange of sulfur-containing amino acids is disrupted, the addition of the reagent will turn the urine into an intense beetroot color. At the same time, a positive qualitative test was identified in the eight-year old girl (patient 2 in Table 1). The child had an eye pathology (lens subluxation and secondary glaucoma) and was taught on a special education program at the school. In the quantitative study of the urine homocystine (the automatic amino analyzer), the diagnosis of homocystinuria was confirmed. Vitamin B_6 in doses of 100 and 500 mg/day did not reduce the levels of methionine and homocystine in biological fluids, which allowed diagnosing the B_6-resistant form of the disease in the girl. The activity of the cystathionine-beta-synthase enzyme in the liver biopsy specimen in the child was almost completely absent (0.008 nmol/1 mg of liver tissue per minute, whereas the control number is 1.380 nmol/1 mg of liver tissue per minute). The effect of pyridoxal phosphate on the enzyme in the biopsy material led to the increase in the enzyme's activity by only 0.002 points reaching 0.010 nmol/1 mg of liver tissue per minute. The results obtained confirmed once again the B_6-resistant form of the disease in the girl. The sibling of this child (patient 3 in Table 1) was considered healthy by the parents, however, during the examination, the behavioral specifics, delay in speech development, funnel-shaped 1st degree deformity of the thorax, irregular bite, a slight increase in the size of knees and valgus installation of knee joints were revealed. The study of the amino acid spectrum of blood serum and urine also allowed us to diagnose a B_6-resistant form of homocystinuria.

It should be noted, that until 2005 no single patient entered the clinic with correct diagnosis. The most frequent diagnosis of the patients entered the clinic is Marfan syndrome (60%); oftentimes children were hospitalized for ocular pathology (lens subluxation and glaucoma), delayed speech development, and child cerebral palsy. In recent years, the situation has changed for the better, and three children have been hospitalized to a genetic clinic for the first time with the correct

guiding diagnosis, i.e. homocystinuria.

In 2012, for the first time in Russia, the DNA diagnosis of homocystinuria caused by the deficiency of cystathionine beta-synthase was developed (Voskoboeva E.Yu.). Since then, defining mutations in the *CBS* gene becomes the final stage in the diagnosis of homocystinuria and identification of its forms (B_6-responsive or B_6-resistant form). As was already noted in Table 2, the DNA diagnosis was performed in 13 patients, while in three of them the second mutation could not be determined (patient 21 and siblings 13^d and 14^d). Among the mutations identified, five have not been described in the literature; three of them were deletions (c.1560-1569delCACCGGGAAG; c.216-217delAT; c.1498_1499delT, patients 6^b, 7^b, 23, 26, table No. 1), leading to a shift in the reading frame and forming the B_6-resistant form of the disease. Mutation Gln368Term leads to the synthesis of truncated protein (stop codon), causing also the development of the B_6-resistant form of homocystinuria (patient 24, Table 1). One new mutation – p.Asp444Tyr (patient 27, Table 1) – revealed in the patient in the homozygous state, caused the development of the B_6-resistant form of the disease. The child has skeletal deformities, typical eye pathology (lens subluxation lens and secondary glaucoma), mitral valve prolapse, borderline intelligence, which allows him to study according to the regular school curriculum, however with the parents' help.

As was already noted, splicing site mutation IVS11-2A- > C turned out to be the most common mutation in Russian patients. Based on the literature data analysis, it is most likely responsible for the formation of the B_6-resistant form of homocystinuria [20]. This mutation is most common in patients and heterozygous carriers of the *CBS* gene in Poland, the Czech Republic and Slovakia. To detect this mutation, a PCR method was also developed with subsequent restriction analysis (Fig. 3). Such a mutation was detected in six patients (patients 21, 13^d, 14^d, 12, 23, 25, Table No. 1). In one patient, whom we have observed since the age of 5 years (patient 12, Table 1, Fig.2, Panel A), this mutation was detected in the homozygous state. A relatively favorable course of homocystinuria should be noted in this patient. At present, she is 29 years old; the patient never experienced marked skeletal changes; at 7 years, the displaced lenses complicated by secondary glaucoma were removed. Changes in the cardiovascular system remain minimal (small prolapse of the mitral valve); the IQ level is at the lower border of the norm. She is married and has a healthy son of 5 years old. However, it is somewhat difficult for her to lead a household and take care of the family members, so she constantly seeks help from her mother.

The course of the underlying disease in the other three patients with mutation IVS11-2a- > c also proceeds relatively favorably. This may be due to the positive effect of the second, milder, unidentified mutations in these patients. Three children with mutation IVS11-2a- > c are distinguished by height, scoliosis of the 1st or 2nd degree of the thoracic spine, typical ocular pathology complicated in one patient (patient 21, Table No. 1) with transverse sinus thrombosis during the removal of the displaced lens (the thrombosis was successfully liquidated by conservative means). All children are very sociable, friendly and peaceful demonstrating a relatively good academic standing in their school programs (rarely B and mostly C grades).

In one patient (patient 25, Table 1) the second mutation Trp390Term results in the synthesis of a truncated protein, which promotes the formation of the severe B_6-resistant form of homocystinuria. The girl has great height, typical changes in the skeleton, mitral valve prolapse, transient heart rhythm disturbances, lens subluxation, secondary glaucoma and decreased intelligence (special education programs offered).

The second mutation in patient 23 (Table 1) is represented by deletion c.216-217delAT, which also disrupts the synthesis of the full-length protein. Clinical symptomatology in the child completely corresponds to the B_6-resistant form of homocystinuria.

Patient 15 (Table 1) is homozygous for mutation Gly305Arg which has a very severe B_6-resistant form of the disease reflected by kyphoscoliosis of 3rd degree of thoracic spine, decompensated secondary glaucoma not susceptible to conservative and operative methods of treatment, a marked decrease in intelligence accompanied by a number of psychological patterns such as stubbornness, conflict, and lack of communication skills (Fig. 1).

In a 6-year-old boy (patient 20, Table 1, Figs. 4, 5) with mutation p.Ile278Thr, considered the most frequent among various populations of the world and responsible for the formation of a lighter B_6-responsive phenotype, the second mutation Cys109Arg promotes the development of B_6-resistant homocystinuria. The second mutation slightly increases the severity of the B_6-responsive form of the disease in the child. Thus, the patient's parents pay special attention to the medical and dietary correction of the boy, fully complying with all the recommendations and prescriptions of doctors. Nonetheless, achieving normalization of biochemical parameters, increasing attention and perseverance, improving memory and academic performance of the child is extremely difficult.

The previously unknown mutation in two siblings (c.1560-569delCACCGGGAAG, patients 6^b и 7^b, Table 1) caused severity of clinical symptoms. The child suffered from an early and severe manifestation of ocular pathology (lens subluxation with malignant secondary glaucoma) and cardiovascular disorders with the development of vascular crises (confusion and delusions, lasting for 3 or 4 days and poorly docking by medicine). All this testified the severe B_6-resistant form of homocystinuria in children.

The therapy of patients with homocystinuria was accompanied by dietary treatment with a significant restriction of products of animal origin rich in methionine. The diet was compiled by a dietitian individually for each patient in accordance with the child's actual nutrition and body weight. The daily content of methionine in the diet of the sick child should be extremely reduced and should not exceed 10–15 mg/1 kg of body weight per day. The protein supply necessary for the normal development of the patient was achieved by introducing a mixture of amino acids deprived of methionine, which is XMET HOMIDON.

Medication included vitamins C, B_6, B_{12}, folic acid, antiplatelet agents (acetylsalicylic acid) and drugs aimed at normalizing the mineral metabolism and fighting osteoporosis (metabolites of vitamin D and calcium drugs).

It is established that vitamin C reduces endothelial dysfunction in patients with homocystinuria and significantly extends the time of formation and manifestation of arterial thrombosis.

In addition, we widely used psychostimulants and nootropic drugs (gopanthenic acid, pyracetam, etc. in the absence of convulsive syndrome), cerebral circulation correctors (cinnarizine, vinpocetine), antioxidants and antihypoxants (levocarnitine, deproteinized blood calves (Actovegin)), and hepatoprotectors (phospholipids - Essentiale forte). We also prescribed massage and therapeutic gymnastics.

Betaine, successfully used to treat patients with homocystinuria in other countries, was practically not used in Russia, as this medicine is not registered in Russia. However, now there is an option of importing betaine based on the resolution of medical counsels. Thus, we hope to receive our own objective information about the effect of this drug.

More recently (2016), there were reports of the development for patients with homocystinuria of an enzyme-substituting drug capable of fulfilling the functions of the enzyme cystathionine-beta-synthase. In mice experiments under the influence of this enzyme-substituting drug, the level of homocysteine in the blood decreased below 120 and 100 nmol/l [11]. It becomes obvious that further development and improvement of this treatment method, its introduction into practical health care will make a significant contribution to the treatment of this serious hereditary disease.

Thus, the detection of mutations of the *CBS* gene in homocystinuria allows us to verify the diagnosis, predict the course of the disease, select individual optimal therapy, and conduct effective medical and genetic counseling for families.

Authors' contributions

Elena Voskoboeva conducted laboratory research, interpreted data and wrote the manuscript;

Alla Semyachkina conceived the research, examined the patients, provided treatment, analyzed the results and wrote the manuscript; Maria Yablonskaya examined the patients, provided treatment, analyzed the results and wrote the manuscript; Elena Nikolaeva examined the patients, provided treatment, analyzed the results, and wrote the manuscript.

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Competing interests

Elena Voskoboeva, Alla Semyachkina, Maria Yablonskya, and Ekaterina Nikolaeva declare that they have no competing interests.

Authors have obtained an informed consent for publishing photos and clinical and laboratory data of the patients.

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