

Clinical and Molecular Characteristics of Russian Patients with Homocystinuria due to Cystathionine Beta-Synthase Deficiency

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Abstract

The aim of this article is to analyze clinical features and molecular and genetic data of the Russian cohort of homocystinuria patients. We present the results of the 45 year clinical observation of 27 Russian homocystinuria patients. The clinical phenotype is a combination of Marfanoid habitus with skeletal deformities, disturbances of the central nervous and cardiovascular systems and ocular pathology.

We made a mutation analysis of the cystathionine beta-synthase deficiency (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.

Keywords: Homocystinuria due to cystathionine beta-synthase deficiency; Clinical presentation; CBS gene; Mutation analysis

Introduction

Classical homocystinuria (OMIM 236200) is an autosomal recessive disorder of sulfur-amino acid metabolism that results from the cystathionine beta-synthase (CBS; EC 4.2.1.22) deficiency. This defect leads to high accumulation of homocysteine and methionine in blood and urine [1,2]. 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 to 1:311,000 [3].

The clinical picture of homocystinuria was described by many researchers [4,5]. 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). 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.

The human CBS gene has been mapped to 21q22.3. 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 B6) as co-factor. As a result, two types of homocystinuria due to CBS deficiency based on its treatment have been distinguished: one is vitamin B6-responsive while the other is not. Usually, patients with the B6-responsive form of the disease have a milder phenotype than patients with the non-responsive form [6,7]. To date, more than 150 different mutations in the CBS gene have been described [8].

The aim of the article - comparative analysis of molecular data and results of the long-lived clinical observation of 27 Russian patients (from 22 unrelated genealogies) with homocystinuria due to CBS deficiency.

Materials and Methods

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.

DNA preparation

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

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.

Mutation analysis

All PCR fragments were subsequently sequenced on an ABI 3130xl 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.

Compliance with Ethics Guidelines

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.

Results

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 of 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. Fifteen patients were B6-responsive.

The height in half of the patients was above average. 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. All patients except two had ocular pathology mostly lens subluxation diagnosed at the age of 5 to 7 years. In 17 patients this condition was complicated by the development of secondary glaucoma that required urgent surgical operation. In two children, the lens removal operation was complicated by transverse venous sinus thrombosis which was successfully cured without clinical consequence.

None of the patients had seizures. The ischemic stroke and central hemiparesis have developed in four patients with the B6-resistant form of the disease at the age of 14 to 17 years. 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. Intellectual development of B6-responsive patients was normal or slightly lower. The pyridoxine non-responders 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 data retrospective analysis of the health condition of 27 patients showed that 3 probands died at the age of 16, 22 and 30 years. The cause of death of these patients was croupous pneumonia, myocardial infarction and stroke, respectively. The proband with the B6-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 B6-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 B6-responsive form of homocystinuria is married and has a healthy son.

Molecular genetic studies of the CBS gene

For 13 patients from 11 unrelated families a DNA analysis of the CBS gene was performed. 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.

Seven out of twenty three mutant alleles were a site splicing mutation IVS11-2a->c resulting in deletion of exon 12 and are prevalent in the population of Eastern Europe [9]. It was revealed that the homozygotes for IVS11-2a->c did not respond to vitamin B6, while in compound heterozygotes the response to vitamin B6 depended on the mutation on the second allele.

The most common panethnic mutation p.Ile278Thr was found only in heterozygous state in one patient with pyridoxine-responsive disease. The second allele was previously described mutation p.Cys109Arg. The mutant with Cys109Arg protein completely lacked catalytic activity [10]. Nevertheless, the presence of the mild mutation Ile278Thr resulted in a B6-responsive form homocystinuria.

One pyridoxine-nonresponsive patient was a compound heterozygote for the novel mutation p.Gln368Term and previously described mutation p.Thr353Met. One new mutation – p.Asp444Tyr – revealed in the patient in the homozygous state, caused the development of the B6-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.

Three novel small deletions were detected in three unrelated patients from three families. The c.1560-1569del CACCGGAAG was determined in two sibs from the family of Armenian origin. 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. The second new deletion was c.216-217delAT which introduces a frameshift and a stop codon at position 104 of protein chain, was found in heterozygous state in pyridoxine-nonresponsive patient. The third deletion was c.1498_1499delT, which introduces a frameshift and, similarly to the c.1560-1569del CACCGGAAG mutation, a stop codon at position 540 of protein chain, was found in heterozygous state in pyridoxine-nonresponsive patient, too.

It is known that the homozygotes for Lys384Asn respond to vitamin B6. Two our patients were heterozygote for Lys384Asn. The second allele was c.1560-1569del CACCGGGAAG. Both patients had B6-nonresponsive form of disease. One patient was compound for Glu302Lys and c.1498_1499delT and also had B6-nonresponsive phenotype. Thus, it can be assumed that the truncated protein in the position 540 completely loses its functional activity and resulted in B6-nonresponsive form of disease.

The previously described Gly305Arg mutation was detected in a homozygous state in a patient with a severe B6-nonresponsive form of homocystinuria.

Discussion and Conclusion

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 B6-responsive and B6-resistant forms of homocystinuria was performed after a 3 day patient intake of vitamin B6 per os at the initial dosage of 100 mg/day.

We determined the activity of the enzyme cystathionine-beta-synthase in liver biopsy samples in nine patients. 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 B6-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 (B6-responsive form of homocystinuria confirmed).

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 [2]. Since then, defining mutations in the CBS gene becomes the final stage in the diagnosis of homocystinuria and identification of its forms (B6-responsive or B6-resistant form). The DNA diagnosis was performed in 13 patients, while in three of them the second mutation could not be determined. 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, leading to a shift in the reading frame and forming the B6-resistant form of the disease. Mutation Gln368Term leads to the synthesis of truncated protein (stop codon), causing also the B6-resistant form of homocystinuria. One new mutation – p.Asp444Tyr – revealed in the patient in the homozygous state, caused the development of the B6-resistant form of the disease.

Splicing site mutation IVS11-2A->C turned out to be the most common mutation in Russian cohort (six patients). Based on the literature data analysis, it is most likely responsible for the formation of the B6-resistant form of homocystinuria. This mutation is most common in patients and heterozygous carriers of the CBS gene defects in Poland, the Czech Republic and Slovakia. So, the high frequency of IVS11-2a->c in Russian patients was not surprising. In one patient, whom we have observed since the age of 5 years, 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 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 the second mutation Trp390Term results in the synthesis of a truncated protein, which promotes the formation of the severe B6-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).

In other patient the second mutation 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 B6-resistant form of homocystinuria.

The patient with homozygosity for mutation Gly305Arg had a very severe B6-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.

In a 6 year old boy with mutation p.Ile278Thr, considered the most frequent among various populations of the world and responsible for the formation of a lighter B6-responsive phenotype, the second mutation Cys109Arg promotes the development of B6-resistant homocystinuria. This second mutation slightly increases the severity of the B6-responsive form of the disease in the child.

The previously unknown mutation (c.1560-569delCACCGGGAAG) in two siblings caused severity of clinical symptoms. The older brother 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).

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 dietician 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, B6, B12, folic acid, antiplatelet agents (acetylsalicylic acid) and drugs aimed at normalizing the mineral metabolism and fighting osteoporosis (metabolites of vitamin D and calcium drugs).

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. It becomes obvious that further development and improvement of treatment methods, especially enzyme-substituting 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.

Conflicts of Interest

All authors contributed with manuscript writing.

Author's Contribution

The authors declare that they have no conflicts of interest.

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