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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