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Vascular presentation of cystathionine beta-synthase deficiency in adulthood

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Abstract Several recent studies describing a solely vascular presentation of cystathionine beta-synthase (CBS) deficiency in adulthood prompted us to analyze the frequency of patients manifesting with vascular complications in the Czech Republic. Between 1980 and 2009, a total of 20 Czech patients with CBS deficiency have been diagnosed yielding an incidence of 1:311,000. These patients were divided into three groups based on symptoms leading to diagnosis: those with vascular complications, with connective tissue manifestation and with neurological presentation. A vascular event such as a clinical feature leading to diagnosis of homocystinuria was present in five patients, while two of them had no other symptoms typical for CBS deficiency at the time of diagnosis. All patients with the vascular manifestation were diagnosed only during the past decade. The median age of diagnosis was 29 years in the vascular, 11.5 years in the connective tissue and 4.5 years in the neurological group. The ratio of pyridoxine responsive to nonresponsive patients was higher in the vascular (4 of 5 patients) and connective tissue groups (6 of 7 patients) than in the neurological group (2 of 8 patients). Mutation c.833T>C (p.I278T) was frequent in patients with vascular (6/10 alleles) and connective tissue presentation (8/14 alleles), while it was not present in patients with neurological involvement (0/16 alleles). During the last decade, we have observed patients with homocystinuria diagnosed solely due to vascular events; this milder form of homocystinuria usually manifests at greater ages, has a high ratio of pyridoxine responsiveness/nonresponsiveness, and the mutation c.833T>C (p.I278T) is often present.

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Abbreviations
CBS Cystathionine beta-synthase

Introduction

Homocystinuria due to cystathionine β-synthase (CBS; EC 4.2.1.22) deficiency (OMIM# 236200) is the most common inborn error of methionine metabolism (Mudd et al. 2001). The disease was discovered in 1962, when mentally retarded individuals were screened for abnormal excretion of urinary amino acids. An accumulation of homocysteine in tissues leading to the formation and urinary excretion of homocysteine disulfide, homocystine, was observed (Gerritsen et al. 1962), and subsequently CBS deficiency has been shown as the cause of the disease (Mudd et al. 1964).

The clinical phenotype of homocystinuria was largely documented in the group of 629 patients by Mudd et al.

(1985). The most common symptoms include neurological complications such as mental retardation and epilepsy, marfanoid features with lens ectopia, and vascular changes represented mostly by thromboembolic events. These symptoms were present separately or in various combinations. Despite relatively frequent occurrence of vascular events in untreated homocystinuric patients, only 1.1% of patients in this large cohort manifested solely with a vascular symptom. The authors postulated that the observed incidence of thromboembolism in this disease might be falsely low because some patients presenting with solely thromboembolic events and manifesting no other feature of the CBS deficiency could have remained undiagnosed (Mudd et al. 1985).

The above hypothesis has been supported by recent publications describing young adult patients with homocystinuria diagnosed solely on the basis of thromboembolic events with no other symptoms (Gaustadnes et al. 2000; Linnebank et al. 2003). In this work, we focused on examining the frequency of vascular manifestation in CBS-deficient patients diagnosed in the Czech Republic over the last three decades.

Methods and patients

All patients have been followed in the Department of Pediatrics and in the Institute of Inherited Metabolic Disorders of First Faculty of Medicine of Charles University in Prague. To our knowledge, the reported 20 patients represent the absolute majority of Czech patients diagnosed with homocystinuria in the past 30 years.

At least two out of the following three diagnostic criteria had to be met: (1) metabolic abnormalities (hypermethioninemia with a simultaneous presence of elevated total plasma homocysteine above 100 μmol/l, or elevated homocystine in urine and/or free homocystine in blood, and/or decreased plasma cystathionine), (2) CBS deficiency in cultured fibroblasts, or (3) the presence of two pathogenic CBS mutations in *cis*. Family screening was carried out in all patients and two affected sib-pairs were identified (patients 2 and 4, and 8 and 9 in Table 1).

Until 1985, free homocystine was detected in plasma deproteinated by sulfosalicylic acid using the aminoacid analyser. Between 1989 and 1993, plasma level of total homocysteine was determined using the ion-exchange chromatography after dithiotreitol reduction (Brattström et al. 1988). Since 1994, high performance liquid chromatography (HPLC) with SBDF derivatization has been used to determine plasma total homocysteine (Araki and Sako 1987; Krijt et al. 2001). The urinary homocystine concentration and plasma methionine level were determined by ion-exchange chromatography with postcolumn ninhydrine derivatization. The enzyme activity was measured in cultured skin fibroblasts radiometrically according to Kraus (1987). The mutation analysis was carried out using either genomic DNA and/or RNA isolated from fibroblasts (Janošík et al. 2001) in all patients except for patient no. 1.

Pyridoxine responsiveness of patients was tested by an oral administration of pyridoxine in doses of at least 5–10 mg/kg per day for at least 2 weeks. Pyridoxine responsive patients were classified as those with a decrease of plasma total homocysteine to levels below 50 μmol/l, patients with no decrease were classified as nonresponders, patients with intermediate changes as partial responders.

Results

Between 1980 and 2009, a total of 20 Czech patients with cystathionine beta-synthase deficiency have been diagnosed in the Czech Republic (Table 1). The number of diagnosed patients did not differ significantly among the decades—seven, six and seven patients were diagnosed between 1980 and 1989, between 1990 and 1999, and between 2000 and 2009, respectively. The incidence of clinically diagnosed patients with CBS deficiency in the Czech Republic was 1:311,000 with the 95% confidence interval of 1:201,000–1:509,000. The median age at diagnosis was higher in women (i.e. 15 years), than in men (i.e. 8.5 years).

For each patient, we analyzed the symptoms leading to the plasma homocysteine level determination, and we divided the patients into three groups. Patients in the vascular group were ascertained due to thromboembolic events and/or thrombophilia screening, or due to intracranial bleeding, while patients in the neurological group were ascertained due to the delayed psychomotor development. The symptoms leading to homocysteine analysis in the connective tissue patient group were myopia, lens ectopia or marfanoid features.

A vascular event as a clinical feature leading to the diagnosis of homocystinuria was present in 5 of the 20 patients. Two patients from the vascular group had no other symptoms typical for CBS deficiency at the time of diagnosis. All patients ascertained for vascular complication as the leading symptom have been diagnosed only in the decade between 2000 and 2009.

The median age at diagnosis was 29 years (range 17–32) in the vascular group, 11.5 years (range 8–47) in the connective tissue group and 4.5 years (range 3–16) in the neurological group. The ratio of pyridoxine responsive to nonresponsive patients was higher in the vascular (4 of 5 patients) and connective tissue (6 of 7 patients) groups than in the neurological group (2 of 8 patients).

The mutation analysis revealed a considerable genetic heterogeneity among Czech patients with CBS deficiency.

Table 1 List of patients

Patient	Gender	Year of diagnosis	Age at diagnosis	Symptom leading to diagnosis	Group	Other symptoms at time of diagnosis	Pyridoxine responsiveness	Mutations observed	References
1	M	1981	5	Psychomotor delay	Neurological	Lens ectopia, marfanoid features	+/-	- -	A patient 14; B patient 13
2[a]	M	1981	4	Psychomotor delay	Neurological	-	-	r.[210_235del26]+[28delG] c.[210-1G>C]+[28delG]	A paTient 10; B patient 10
3	M	1982	3	Psychomotor delay	Neurological	Kyphoscoliosis	+	p.[A114V]+[W409X] c.[341C>T]+[1226A>G]	A patient 18; B patient 17
4[a]	M	1983	3	Psychomotor delay	Neurological	-	-	r.[210_235del26]+[28delG] c.[210-1G>C]+[28delG]	A patient 8; B patient 8
5	M	1983	16	Psychomotor delay	Neurological	Lens ectopia, quadruspasticity kyphoscoliosis, marfanoid features	-	p.[H65R]+[?] [c.194A>G]+[?]	A patient 11
6	M	1987	21	Myopia	Connective tissue	Marfanoid features thrombosis	+	p.[I278T]+[I278T] c.[833T>C]+[833T>C]	A patient 20; B patient 19
7	F	1987	13	Myopia	Connective tissue	-	+	p.[I278T]+r.[1224_1358del135] c.[833T>C]+[1224-2A>C]	-
8[b]	F	1992	10	Lens ectopia	Connective tissue	Kyphoscoliosis	+	p.[I278T]+[E144K;A155T] c.[833T>C]+[430G>A; 463G>A]	A patient 17; B patient 16
9[b]	M	1993	8	Lens ectopia	Connective tissue	Marfanoid features, kyphoscoliosis	+	p.[I278T]+[E144K;A155T] c.[833T>C]+[430G>A; 463G>A]	A patient 16; B patient 15
10	M	1995	10	Psychomotor delay	Neurological	Lens ectopia, marfanoid features	-	r.[1224_1358del135]+[737_828del 92] c.[1224-2A>C]+[828+1G>A]	A patient 4; B patient 4
11	M	1998	9	Lens ectopia	Connective tissue	-	-	r.[1224_1358del135]+p. [E144K; A155T] c.[1224-2A>C]+[430G>A; 463G>A]	A patient 2; B patient 2
12	M	1999	47	Lens ectopia	Connective tissue	Suffered from myocardial infarction	+	p.[I278T]+[I278T] c.[833T>C]+[833T>C]	A patient 21
13	F	1999	5	Psychomotor delay	Neurological	Lens ectopia marfanoid features	-	p.[C165Y]+[C165Y] c.[494G>A]+[494G>A]	A patient 1; B patient 1
14	F	2003	29	Intracranial bleeding	Vascular	Epilepsy as a complication of bleeding	+/-	p.[I278T]+[?] c.[833T>C]+[?]	-
15	F	2003	3	Psychomotor delay	Neurological	-	-	r.[1224_1358del135]+[1224_1358del135] c.[I1224-2A>C]+[1224-2A>C]	-
16	F	2006	32	Sterility	Vascular	Myopia	+	p.[I278T]+[I278T] c.[833T>C]+[833T>C]	-
17	F	2007	17	Thrombosis	Vascular	Lens ectopia myopia, scoliosis	-	p.[?]+[E144K; A155T] c.[?]+[430G>A; 463G>A]	-
18	F	2007	19	Thrombosis	Vascular	-	+/-	p.[I278T]+[E144K; A155T] c.[833T>C]+[430G>A; 463G>A]	-
19	F	2007	10	Marfanoid features	Connective tissue	-	+/-	p.[I278T]+[D376N] c.[833T>C]+[1126G>A]	-
20	F	2008	32	Thrombosis	Vascular	Lens ectopia, scoliosis	+	p.[I278T]+[I278T] c.[833T>C]+[833T>C]	-

Pyridoxine responsiveness of patients was tested by oral administration of pyridoxine in doses of at least 5–10 mg/kg per day for at least 2 weeks. Pyridoxine responsive patients were classified as those with decrease of plasma homocysteine levels below 50 μmol/l, patients with no decrease were classified as nonresponders, patients with some decrease as partial responders.

References previous publications where patients were originally reported: *A* Janošík et al. 2001, *B* Orendáč et al. 2000

[a,b] Sibling pairs identified through family screening

The most common CBS mutation c.833T>C (p.I278T) was frequent in patients with the vascular (6 of 10 alleles) and connective tissue presentation (8 of 14 alleles), while it was not present in patients with neurological involvement (none of 16 alleles; Table 1). As in other populations, the c.833T>C allele was predominantly detected in Czech patients with the milder phenotype of CBS deficiency.

Case reports of patients with vascular manifestation of homocystinuria

Patient no. 14 This woman suffered from abdominal pain and frequent vomiting in childhood. An episode of left-sided hemiparesis with impaired consciousness and generalized seizures appeared at the age of 29 years. Two intraparenchymal bleeding lesions in the central cortical area on the right side were observed on the CT. Subsequent laboratory analysis revealed plasma total homocysteine level of 329 μmol/l. No signs of intracranial thrombosis were detected on digital subtraction angiography.

Patient no. 16 This 32-year-old woman has been treated for hypothyroidism since 10 years of age. The CBS deficiency was ascertained due to thrombophilia screening performed because her first gravidity resulted in spontaneous abortion 3 weeks after in vitro fertilization and embryo transfer. At the time of diagnosis, the patient exhibited moderate myopia.

Patient no. 17 The reason for plasma total homocysteine analysis in this female patient was the thrombosis of the sagittal sinus. This was manifested by a sudden headache at the age of 17 years with focal seizures of the right arm developing after the event. Since school age, the patient has been followed up for visual problems—lens subluxation and myopia–-and for scoliosis.

Patient no. 18 The plasma total homocysteine level in this 19-year-old woman was analysed during the diagnostic work-up after an ischemic lesion of medial cerebral artery with a subsequent mild central paresis of the facial nerve and an integral aphasia. She was on a hormonal contraception at the time of event. No other symptoms of CBS deficiency were present at the time of diagnosis.

Patient no. 20 This woman underwent a surgery for dislocated lenses at the age of 30 years, which was complicated by thrombosis of the right sigmoideal and transversal sinuses. Plasma total homocysteine was analyzed only 2 years later following a delivery of a dead fetus in the 32nd week of gestation. Scoliosis was present at the time of diagnosis.

Discussion

Patients ascertained due to vascular events represent a substantial proportion of Czech patients with homocystinuria. In this subgroup of CBS-deficient patients, the disease manifested relatively late in life and the course of the disease was milder than in patients diagnosed due to neurological or connective tissue symptoms. The observed proportion of patients diagnosed solely due to vascular events (2 of 20) was one order of magnitude higher than the 1.1% observed in the large group of Mudd et al. (1985).

The recently observed high number of CBS-deficient patients with vascular manifestation might be explained by an increased availability of plasma total homocysteine measurement in routine thrombophilia screening in the last decade. This trend is demonstrated by a marked increase in number of total homocysteine assays in the Czech Republic between the years 2003–2007 compared to the period of 1998–2002 demonstrated by the data from the largest national health insurance company (Fig. 1).

Ascertainment of adult patients with CBS deficiency due to vascular events has been reported in the past decade also by other authors. Gaustadnes et al. (2000) described three CBS deficient sisters aged 58, 56 and 55, respectively, who manifested by repeated vascular events since the third decade of life. Ophtalmologic examination revealed no lens ectopia or other visual problems, the mental state of the patients was normal and none of the three patients had

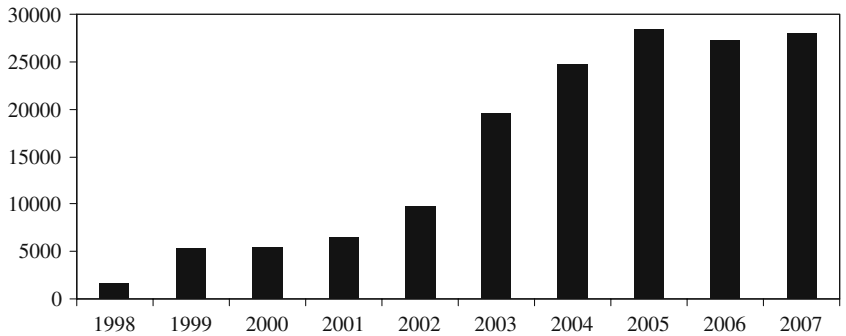

Fig. 1 Number of total homocysteine assays between 1998 and 2007. Data provided by the Všeobecná zdravotní pojišťovna (VZP) which provides health insurance for 65–75% of the population of the Czech Republic

reduced bone mineral content as determined by dual-energy X-ray absorptiometric scanning at the time of diagnosis. Linnebank et al. (2003) screened for the c.833T>C allele in 225 DNA samples of young adults with ischemic stroke and in 46 samples of patients with sinus thrombosis. They found a 34-year-old male who suffered from bilateral occlusion of the carotid arteries and of one vertebral artery, and with three previously documented stroke episodes. The second patient was a 24-year-old woman who developed thromboembolic symptoms after delivery of a healthy daughter via Ceasarian section. These two patients did not present any other nonvascular symptoms of homocystinuria.

All the patients with only vascular manifestation presented by Gaustadnes and Linnebank (Gaustadnes et al. 2000; Linnebank et al. 2003) were homozygotes for the c.833T>C allele. The high frequency of c.833T>C was also seen in our patients manifesting with vascular events. Recently, Skovby et al. (2010) observed a large difference between the number of diagnosed Danish homocystinuric patients and the incidence of predicted homozygosity for the c.833T>C allele (1:20,400; 95% CI 1:4,800–1:128,000). Only 10 patients were diagnosed in contrast to about 270 such individuals that were expected based on the molecular screening of the Danish population (95% CI 43–1,148). Two of those 10 patients were clinically unaffected and diagnosed by family screening because of an affected sibling (Skovby et al. 2010). The large discrepancy between the number of diagnosed homocystinuric patients compared to a predicted frequency of homozygosity was also seen in the Czech population (Sokolová et al. 2001; Janošík et al. 2009). These studies and our observation of patients with CBS deficiency diagnosed in adulthood support the hypothesis proposed by Skovby et al. (2010) that the predominant portion of homozygotes for the c.833T>C allele may be clinically unaffected, or may be ascertained for thromboembolic events occurring no sooner than in the third decade of life.

Conclusion

Vascular manifestation was a leading symptom initiating diagnostic work-up in one-quarter of Czech patients with CBS deficiency. These patients have been diagnosed due to vascular complications only in the last decade, and it can be hypothesized that this was most likely due to the increased availability of plasma homocysteine determination. This milder form of the disease usually manifested at a greater age, had a high ratio of pyridoxine responsiveness, and the mutation c.833T>C (p.I278T) was often present. Observation of a high proportion of patients with a vascular presentation of the disease emphasizes the importance of the total homocysteine plasma level determination in patients with thromboembolic events in adulthood.

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