Vascular and connective tissue features in 5 Italian patients with homocystinuria

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Abstract

Homocystinuria is a metabolic disorder associated with defects in genes encoding for methionine metabolism enzymes. Vascular and connective tissue manifestations such as deep venous thrombosis, ectopia lentis and skeletal alterations are the major clinical features.

We investigated the clinical manifestations of 5 Italian homocystinuric patients, performed mutation screening analysis on cystationine beta-synthase (CBS) gene and searched for genotype/phenotype correlations.

We detected mild cardiovascular and skin connective tissue stigmas in these patients, never reported in homocystinuric patients before. We found 1 novel and 7 known mutations. Our patients carried no other mutation associated with venous thrombosis. Our data stress the importance of extending the clinical investigation for connective tissue manifestations in homocystinuric patients to all the organs/systems involved in Marfan syndrome, also suggesting long term follow-ups for cardiovascular manifestations.

Keywords: Connective tissue manifestations; CBS gene; Homocystinuria; Vascular clinical manifestations

Homocystinuria (MIM# 236200) is a rare metabolic disorder (1:344.000) mainly due to cystathione beta-synthase (CBS) deficiency which causes increased urinary homocysteine (Hcy) and methionine excretion [1]. Major clinical manifestations involve eyes, skeleton, central nervous and vascular systems. Ocular and skeletal manifestations are shared by both homocystinuria and Marfan syndrome (MFS) patients, while aortic dilatation and valve prolapse and regurgitation, have been reported only in Marfan patients.

Patients are diagnosed during infancy, mostly after the onset of ectopia lentis (EL) [1,2], or because of venous thrombosis in later age. The homotetramer CBS (EC 4.2.1.22; chr.21q22.3) (Fig. 1), enzyme in the transulfuration pathway, converts the potentially toxic Hcy into cysteine [3]. A continuously updated CBS website (http://www.uchsc.edu/sm/cbs/cbs data/cbsmain.htm) lists 140 mutations in 624 patient alleles [4]. Homocystinuria can also be due to mutations in 5–10, Methylenetetrahydrofolate reductase (MTHFR; MIM#236250)[5], methionine synthase reductase (MTRR) [6] and methionine synthase (MTR)[7] genes.

We investigated 5 homocystinuric Italian patients and an echocardiographic study/examination was performed according to the guidelines of the American Society of Echocardiography [8]. Controls were selected among apparently healthy Italian subjects with no history of sudden death, without venous thrombosis, cardiovascular, skeletal or eyes Marfan or homocystinuric manifestations. Biochemical and genetic factors predisposing to thrombotic events were evaluated as previously reported [9–12]. Genomic DNA mutation analysis of *CBS* gene (Table 1) was performed [13].

Clinical characteristics and molecular data of the 5 patients (4 males and 1 female) are shown in Table 2. In 3 cases (Pts #1,3,4) the diagnosis was made after the first thrombotic event, in the other 2 cases (Pts#1,5) upon detection of EL.

All values for biochemical and genetic factors turned out to be in a normal range except for the following: two individuals who were homozygous for *MTHFR*: c.677C>T(Pt#3) and c.1298A>G(Pt#1); 2 were heterozygous for: *MTR*c.2756A>G (Pt#5) and *MTHFR*c.1298A>G(Pt#4); only one was double heterozygote for *MTR*c.2756A>G and *MTHFR*c.677C>T (Pt#2).

We found 6 heterozygous missense mutations (p.Pro49-Leu, p.Arg125Gln, p.Ile278Thr, p.Ala157Pro, p.Gly307Ser,

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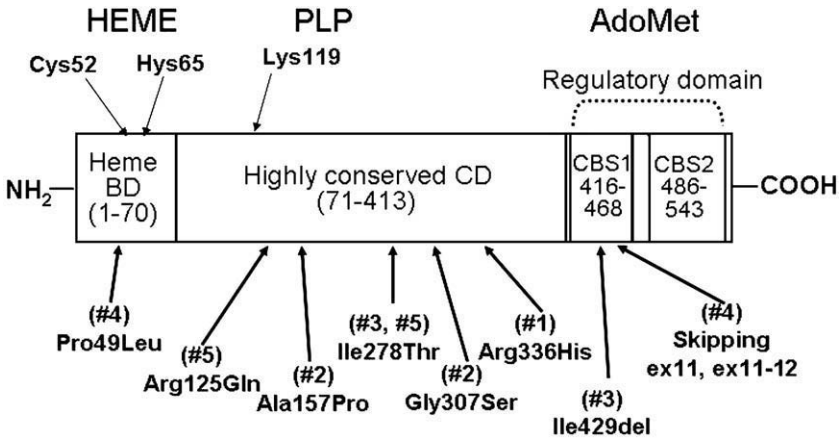

Fig. 1. The protein domains of human CBS. In the upper side are the binding sites for Heme, pyridoxal 5'-phosphate (PLP) and *S*-adenosyl-L-methionine (AdoMet). In the lower side the mutations described in this paper together with the identification number of patients are reported.(BD: Binding Domain; CD: Catalytic Domain).

p.Arg336His); 1 in frame deletion (p.Ile429del), 1 mRNA splicing mutation that alters intron 12 splice donor site (c.1357+1G>A).

Table 1
Primers used for mutation screening analysis of the human cbs gene.

Exon	name	Sequence (5'→3')	Size (bp)	Annealing temperature [°C]	Number of cycles
1	1S	ggAACCCCACAgCATCCgA	311	62	40
	1AS	ggTTATggATCAgCCCTCTT			
2	2S	TgTCTgCCAgggCTggTAC	272	60	35
	2AS	CCTTgCCTAAgggATCCATC			
3	3S	AAgCCCgTCCCCCACCCT	338	64	35
	3AS	ATgCggCTgCAgCTCAgC			
4+5	4S	AACCggCATCggggTgTgC	450	64	35
	5AS	CCgAgACCCTCCTAgggAAT			
6	6S	CACCTggggCCTggAgAC	232	66	35
	6AS	TACACTCCCAggCAgCCAg			
7	7S	gCAACgAgggCTgCTCCAA	309	62	40
	7AS	ACAgCTTTCAgCTCAgAAgC			
8	8S	AggCAgTTgTTAACggCggT	319	62	40
	8AS	TTggggCCCAgggTCAgC			
9	9S	TCTggggTCCTACCgCCTA	291	62	40
	9AS	TgCAggTCggTggCTgACT			
10	10S	gACATgCTCCCATgCgTgC	279	62	40
	10AS	Kruger at al 2003			
11	11S	AgCACTTgggggTCTCTgC	298	62	35
	11AS	AgggCCAgCACAggCCAC			
12	12S	ggTCCCTgTggCCTCCTg	297	64	40
	12AS	gTgACACTgACGgggTggA			
13	13S	CAgAgAgCgCTCCTCCCTg	267	66/64/62	3/3/32
	13AS	CCTgTTTgAgCTgCCTgTAgg			
14	14S	TCCCACgTTCggCTgCCAC	280	64	40
	14AS	CATggCAgAggCCAggCTT			
15	15S	gAgTTgggAggggCCCTg	258	62	35
	15AS	AggACCCTggAAggCCTC			
16	16S	CgCAgCAgCCCACCCAgC	236	62	40
	16AS	TAgggCTCAggAAAgCgAAG			
17	17S	gTCTCCCCTCgTTAACACAT	281	64	40
	17AS	CAAACTgCTCCCgCCAgC			

Patient *#2* leads the novel c.469G>C nucleotide CBS substitution in exon 4 corresponding to the p.Ala157Pro change localized in a highly conserved region (catalytic domain) at the dimer interface of the tetrameric protein structure. We found c.469G>C mutation in 3 out of 300 controls, so by definition this mutation has to be considered as a polymorphism. Moreover, this substitution would induce a bend in the protein structure but its pathogenic role remains to be defined for the relatively high frequency in controls (1%).Alternatively, a second mutation could be present in the promoter region not investigated.

Patient *#3* leads another mutation, carried by an Argentinean patient [14], recently published on-line that consists in a deletion of three nucleotides (c.1286_1288delTCA) causing a deletion of amino acid Ile429, the mutation being positioned in a non conserved domain. This mutation, however, is not present in 50 South American [14] and 300 Italian controls (this report).

The other 6 mutations are already reported in the database as pathogenic mutations.

The parents of patient #5 underwent hcy dosage and CBS mutation analysis. The father carried mutation p.Ile278Thr and mildly increased hcy level (23 μmol/L; n.v.<19) while the mother, presenting mutation p.Arg125Gln, had normal hcy levels (9.8 μmol/L; n.v.<13), both in heterozygous state.

Eight polymorphisms, 5 novel and 3 already published in the CBS database, were found (Table 2).

In our patients we detected heart and skin manifestations never reported in homocystinuric patients such as mild heart valve prolapse and/or regurgitation, and striae distensae suggesting, for the first time, that these patients can develop cardiovascular manifestations.

MacLean et al. [15] suggested that clinical homocystinuric manifestations related to the connective tissue may be correlated to the decrease of CBS production causing both an increase of hcy levels and a decrease of cysteines. The decrease of cysteines may affect the qualitative and quantitative synthesis of connective tissue proteins causing EL and other connective tissue manifestations. This hypothesis is