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The p.T191M mutation of the *CBS* gene is highly prevalent among homocystinuric patients from Spain, Portugal and South America

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Abstract Classical homocystinuria is due to cystathionine β -synthase (CBS) deficiency. More than 130 mutations, which differ in prevalence and severity, have been described at the *CBS* gene. Mutation p.I278T is very prevalent, has been found in all European countries where it has been looked for with the exception of the Iberian peninsula, and is known to respond to vitamin B₆. On the other hand, mutation p.T191M is prevalent in Spain and Portugal and does not respond to B₆. We analysed 30 pedigrees from Spain, Portugal, Colombia and Argentina, segregating for homocystinuria. The p.T191M mutation was detected in patients from all

four countries and was particularly prevalent in Colombia. The number of p.T191M alleles described in this study, together with those previously published, is 71. The prevalence of p.T191M among *CBS* mutant alleles in the different countries was: 0.75 in Colombia, 0.52 in Spain, 0.33 in Portugal, 0.25 in Venezuela, 0.20 in Argentina and 0.14 in Brazil. Haplotype analyses suggested a double origin for this mutation. No genotype–phenotype correlation other than the B₆-nonresponsiveness could be established for the p.T191M mutation. Additionally, three new mutations, p.M173V, p.I429del and c.69_70+8del10, were found. The

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p.M173V was associated with a mild, B₆-responsive, phenotype.

Keywords Homocystinuria · CBS · T191M mutation · Latin-America · Spain · Portugal

Introduction

Homocystinuria due to cystathionine β -synthase (CBS) deficiency (MIM +236,200) is the most common in-born error of methionine metabolism. The worldwide incidence for this disease is highly variable and has been reported at 1 in 344,000 (Mudd et al. 1995), compared to a much higher incidence in Ireland of 1 in 65,000 (Naughten et al. 1998). However, estimates based on DNA screening of newborns are much higher: 1:6,000 (Refsum et al. 2004). CBS is a pyridoxal 5-phosphate (PLP)-dependent enzyme that condenses homocysteine and serine to form cystathionine, an irreversible step in the transsulfuration pathway (Mudd et al. 1995).

The first homocystinuric patient was described by Carson and Neill (1962) among mentally retarded individuals and, 2 years later, it was shown that the primary deficiency in homocystinuria was an enzymatic defect of CBS (Mudd et al. 1964) with an autosomal recessive mode of inheritance (Finkelstein et al. 1964). At the clinical level, symptoms are highly variable, affecting the eye, the skeleton, the vascular system and the central nervous system (CNS). Usually, these include ectopia lentis, osteoporosis, scoliosis, Marfanoid features, premature arteriosclerosis, thromboembolism and mental retardation. Biochemically, patients with CBS deficiency are characterized by severe hyperhomocysteinemia and homocystinuria, hypermethioninemia, and decreased plasma cysteine levels.

The human *CBS* gene, which has been mapped to 21q22.3 (Munke et al. 1988), encodes a CBS polypeptide of 63 kDa, which forms homotetramers (Kraus et al. 1978), binds heme and PLP, and is regulated by S-adenosylmethionine. More than 130 mutations have been detected in the *CBS* gene (Kraus 1998), and functional relevance has been tested for some of them in either bacterial (Kozich and Kraus 1992; de Franchis et al. 1994; Marble et al. 1994; Kluijtmans et al. 1996, among others) or a yeast (Kruger and Cox 1995) expression systems.

Although most mutations seem to be unique or restricted to only a few pedigrees, in some populations one mutation is relatively common. A c.833T > C transition (p.I278T) (Kozich and Kraus 1992) has been found in nearly 25% of all homocystinuric alleles from patients of different ethnic backgrounds. This common mutation has been reported to be generally associated with pyridoxine responsiveness and a relatively mild clinical phenotype when present in the homozygous state (Shih et al. 1995). On the other hand, a c.919G > A transition (p.G307S) is related to a more severe clinical phenotype

and has been detected mainly in alleles from homocystinuric patients of Celtic origin, representing nearly 70% of the homocystinuric alleles in Ireland (Gallagher et al. 1995). We previously reported the third most prevalent mutation, a c.572C > T transition (p.T191M), which is very common in the Iberian Peninsula, representing 50% of all Spanish homocystinuric alleles (Urreizti et al. 2003). Patients homozygous for the p.T191M mutation were found to be B₆-nonresponders. Recently, this mutation was also described in Venezuela (De Lucca and Casique 2004).

Therapy in CBS-deficient patients usually consists of the administration of high doses of pyridoxine (vitamin B₆), the precursor of PLP. Nearly 50% of 1,600 patients with homocystinuria worldwide responded to pharmacological doses of pyridoxine with a substantial reduction in blood homocysteine concentrations (Mudd et al. 1985). Pyridoxine-nonresponsive patients are usually more severely affected than pyridoxine-responsive patients and, for the former, treatment consists usually of combinations of folic acid, hydroxycobalamin, and betaine to stimulate remethylation of homocysteine to methionine.

In this study, we analysed the presence of the p.T191M mutation in 35 homocystinuric patients from Spain, Colombia, Argentina and Portugal. Our data, together with those from previous studies, reveal the high frequency of this change both in the Iberian Peninsula and in several South American countries.

Materials and methods

Patients

This study involved 35 patients with homocystinuria due to CBS deficiency from 30 unrelated pedigrees of different geographical origins, including five sib-pairs. Sixteen of the families were from Spain, eight from Colombia, five from Argentina and one from Portugal. Patients were initially diagnosed on the basis of clinical manifestations suggestive of homozygous CBS deficiency, in combination with severe hyperhomocysteinaemia (typically above 150 μ mol/l), severe hypermethioninaemia (typically above 40 μ mol/l) and, in most cases, deficient CBS activity in cultured fibroblasts. Exceptionally, patient #63 was identified through a neonatal screening program. Enzyme activity was measured either in the laboratory of H.J. Blom (Nijmegen, The Netherlands), or in that of Dr. Rolland (Lyon, France). Clinical and biochemical data were provided by the patients' physicians. Disease severity and B₆ responsiveness were assessed by each physician according to the criteria described in Kraus et al. (1999). Our research was conducted in accordance with the tenets of the Declaration of Helsinki. The nature and possible consequences of the study were first explained to all patients and/or their parents, before their informed consent for inclusion in the research project was obtained.

DNA preparation

Genomic DNA was prepared from peripheral blood leukocytes, using a Wizard Genomic DNA purification Kit (Promega, Madison, WI).

Genotyping of mutation p.T191M and of the polymorphisms

Mutation p.T191M was typed by PCR amplification of exon 5 using a mismatched primer followed by digestion with the restriction enzyme *Hsp92II*. Primer sequences and PCR conditions are shown in Table 1. Analysis of the CBS 844ins68 polymorphism was carried out using primers CBS_8F and CBS_8R (Table 1) to yield a product of 596 bp (if wild-type) and of 664 bp (if the insertion was present). The analyses of the polymorphisms c.699C > T and c.1080C > T of *CBS* and c.677C > T of *MTHFR*, as well as the STR D21S1411 (GenBank L17803), were performed as previously described (Urreizti et al. 2003).

Scanning for unknown mutations of the CBS gene

A new collection of 13 primer pairs was designed to amplify the entire *CBS* coding exons together with intronic flanking sequences under three PCR programs (Table 1).

PCR reactions were performed in a volume of 50 µl containing 100 ng genomic DNA, 0.7 U *Taq* DNA polymerase (Promega, Wisconsin, MI) in the buffer supplied (including 1.5, 2 or 2.5 mM MgCl₂), 0.4 mM of each primer and 0.2 mM of each dNTP. The three PCR programs included a first denaturing step of 2 min at 95°C, followed by 35 cycles of 95°C for 30 s, an annealing step at 57, 59 or 65°C (Table 1) for 20 s, and an extension step at 72°C for 20 s. A final step of 5 min at 72°C was included in all three programs. PCR products were purified by GFX PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ). Subsequently, sequence reactions were performed using BigDye Terminator Cycle Sequencing v3.1, (ABI PRISM, Applied Biosystems, Foster City, CA). All mutations detected were confirmed by digestion of the PCR products with the appropriate restriction enzyme, and 50 control samples were analysed for all new mutations.

Haplotype analysis and linkage disequilibrium measurements

Haplotypes for the *CBS* single nucleotide polymorphisms (SNPs) c.699C > T and c.1080C > T were estimated from the genotypic data on 113 control individuals using PHASE (version 2.1.1) (Stephens et al. 2001). Linkage disequilibrium (LD) significance values were obtained by the Chi-square test.

Table 1 Primers used for cystathionine β-synthase gene (*CBS*) amplification

Name	Exons	Primers	Fragment size	Annealing temperature (°C)	[Mg ²⁺] (mM)
T191M-F ^a	5	CACTGGGGGCTGAGATTGTGAGCA	152	59	2.5
T191M-R		GGAATGCCTGTCTCCAGGCCCC			
CBS_1F	1	GGAACCCACAGCATCCGAG	302	59	2.5
CBS_1R		TCAGCCCTCTGTGATCAAAAGC			
CBS_2F	2	TTTCCAGTTCTTCGAGTGTG	346	57	2
CBS_2R		GGCCACTCATTAACCAGCGA			
CBS_3F	3	TTTTCAGAACCCACAGACC	438	65	1.5
CBS_3R		TCAAAGGAAGCTAGGTTGG			
CBS_4F	4–6	GGCCCCCTCTACCCCTCTG	626	59	1.5
CBS_6R		GGTTCTGAAGGGTGAATAGG			
CBS_7F	7	ACTTTTGGTTACCCACCG	342	57	2.5
CBS_7R		CCTTGTCTTCCCAAACACC			
CBS_8F	8	CTGAACATTAGGTCATTACC	598	57	2.5
CBS_8R		TTTCACACGTTTTCCCTGC			
CBS_9F	9	TGTCTGCAAAACGTGTTGG	527	59	2
CBS_9Rn		AGCTTCAGCCTTGTCTAG			
CBS_10F	10	CATGCTCCCATGCGTGACGTGC	244	59	1.5
CBS_10R		GCAGGATGGAGAGGAGCAGGG			
CBS_11F	11–12	CAGTGAGGTCCAGGAGAG	617	59	1.5
CBS_12R		AGACAGAACCCAGGACTG			
CBS_13F	13	CGCTGACCCCTGCCTGCCC	190	65	1.5
CBS_13R		GCCTGTAGGTGACTGCGCATC			
CBS_14F	14	ACTCCACAGAAAACCTCGTG	293	57	2
CBS_14R		TAAAGACTGGGTGTCACTG			
CBS_15F	15	CAAGTCTAACCCCATCTCC	217	57	2.5
CBS_15R		AAGTAAATGTGAGATTAAGAGG			
CBS_16F	16	AAAAAAAAAAGGAACACAGCC	323	57	2
CBS_16R		GAAAGCGAAGGAGAAGTGG			

^aMismatched primer

Results

p.T191M frequencies in the Iberian Peninsula and South America

The p.T191M mutation was typed in 35 homocystinuric patients from 30 unrelated pedigrees. The results are summarised in Table 2. Sixteen independent patients (and four sibs) were homozygous for the change, four patients were heterozygous, while the remaining ten did not carry this mutation. For an estimate of the frequency of p.T191M in different countries, these data were combined with those on genotypes of homocystinuric patients of the same geographic areas found in the literature (Table 2). In total, out of 156 alleles, 71 bore the p.T191M change. By country, the highest frequency was found in Colombia (75% of the *CBS* mutant alleles), followed by Spain (52%). In Argentina, only one homozygous T191M patient was found (20%).

Haplotype associated with p.T191M

To analyse the haplotypes associated with the p.T191M mutation, two internal polymorphisms (c.699C > T and c.1080C > T) and the microsatellite D21S1411, located 312.6 kb centromeric to the *CBS* gene, were typed in patients bearing the mutation. For this analysis, patients described in Urreizti et al. (2003) and M. Bermudez et al.

(2006) were included, making a total of 66 p.T191M alleles.

For the internal markers, phases could be established in 56 of the chromosomes (Table 3). Two different haplotypes, named I and II, were associated with the mutation. Neither of them carried the c.844ins68 variant (not shown). Haplotype I was present in 51/56 (0.91) p.T191M chromosomes. The frequency of haplotype I among Spanish p.T191M chromosomes was up to 0.96 (24/25) and among Colombian chromosomes was 100% (25/25). In order to calculate the LD between this haplotype and the mutation, the population frequency of haplotype I was established in 226 control chromosomes from Spain and was found to be 0.35 (Table 3). LD between the mutation and haplotype I was highly significant ($P = 4.2 \times 10^{-7}$).

Microsatellite D21S1411 was typed in 36 of the p.T191M bearing chromosomes (Table 3). The three alleles more commonly encountered were 299 (prevalent in Spanish p.T191M chromosomes), 303 (prevalent in Colombian p.T191M chromosomes) and 307 (prevalent in Portuguese p.T191M chromosomes). The analysis of this marker in 114 control chromosomes from Spain revealed that none of these alleles is frequent in the general population of this country (Fig. 1). LD between the mutation and the 299 allele of D21S1411 in the Spanish population was also highly significant ($P = 9.7 \times 10^{-8}$).

Table 2 Frequencies of the T191M mutation in the Iberian Peninsula and in four Latin-American countries

	Patients by T191M genotype			Total number of patients	Allelic frequency
	T191M/T191M	T191M/other	Other/other		
Colombia					
This study	6	1	1	8	
M. Bermudez et al. (2006)	7	0	3	10	
Total	13	1	4	18	0.75
Spain					
This study	8 ^a	3	5	16	
Sperandeo et al. (1995)	0	0	1	1	
Urreizti et al. (2003)	5	2	6	13	
Total	13	5	12	30	0.52
Portugal					
This study	1	0	0	1	
Coudé et al. (1998)	0	0	1	1	
Castro et al. (2001)	0	0	3	3	
Gaustadnes et al. (2002)	0	0	1	1	
Urreizti et al. (2003)	2	0	1	3	
De Lucca (2004)	0	0	1	1	
Total	3	0	7	10	0.30
Venezuela					
De Lucca (2004)	1	0	3	4	0.25
Argentina					
This study	1	0	4	5	0.20
Brazil					
Porto et al. (2005)	1	1	9	11	0.14
All					
This study	16	4	10	30	
Other studies	16	3	29	48	
Total	32	7	39	78	

^aPatient #34, with genotype [T-191M;D444N]/[T191M;D444N] included

Table 3 Haplotypes associated with the p.T191M mutation in different populations

	699C > T	1080C > T	Number of alleles	Frequency	D21S1411 ^a	Number of alleles	Spain	Portugal	Colombia
56 chromosomes bearing p.T191M									
Haplotype I	C	T	51	0.91	Allele 299	16	13	2	1
					Allele 303	8	1	0	7
					Other allele	7	7	0	0
					ND ^b	20	3	0	17
Haplotype II	T	C	5	0.09	Allele 307	5	1	4	0
226 Spanish wild-type chromosomes									
Haplotype I	C	T	78	0.35					
Haplotype II	T	C	71	0.31					
Haplotype III	C	C	71	0.31					
Haplotype IV	T	T	6	0.03					

^aGenBank L17803^bNot determined

CBS mutant alleles other than p.T191M found in homocystinuric patients

To fully establish the *CBS* genotypes of the homocystinuric patients, the entire *CBS* coding region plus flanking sequences was scanned for mutations in all patients, including those previously found to be homozygous for the p.T191M mutation. The 24 alleles that remained unidentified were characterised, and were found to bear 14 different mutations, three of which were novel (Table 4). Additionally, patient #34, previously shown to be a p.T191M homozygote, was found to bear a second mutation, p.D444N, also in homozygosis.

Genotype–phenotype correlations

A wide variety of phenotypes, involving the skeleton, the eye and the CNS, was present among the 20 p.T191M homozygotes. The phenotypic features of some of these patients have been described previously (Urreizti et al. 2003). The lack of response to treatment with vitamin B₆ was the only feature present in all of these patients. Vascular disease was present in only two patients. Phenotypic characteristics of the four sib-pairs with this

genotype are listed in Table 5. Two of the pairs were discordant for several of the features.

Phenotypic characteristics of patients bearing other genotypes are listed in Table 4. Three patients, #22 (p.A226T/p.A226T), #38 (p.A114V/p.I429del) and #28 (p.M173V/p.T191M), were responsive to vitamin B₆ treatment. A marfanoid habitus, lens subluxation and skeletal anomalies were general among the patients, while vascular disease or CNS involvement were variable. The sib-pair 30a–30b was discordant for most of the traits. In patient #54 (p.D444N/ p.D444N), the main feature was a premature cerebral thrombosis. Patient #63 was diagnosed through a neonatal screening program. Her plasma total homocysteine value at diagnosis was 148.12 µmol/l, and after 6 months of treatment with pyridoxine (100 mg×2), cystine (400 mg×1) and a methionine-free diet it dropped to <20 µmol/l.

Discussion

In this study, 35 homocystinuric patients from Spain, Colombia, Argentina and Portugal were genotyped for the p.T191M mutation of *CBS*; the mutation was detected in all four countries, illustrating its wide geographic distribution from South-Western Europe to

Fig. 1 Distribution of alleles (relative frequencies) of the D21S1411 STR (GenBank L17803) among 114 Spanish control chromosomes (*white*) and 25 Spanish chromosomes bearing the p.T191M mutation (*gray*). Allele names (numbers in the X-axis) correspond to the sizes of the PCR products

Table 4 Genotypes other than p.T191M/p.T191M. CNS Central nervous system, NSP neonatal screening program (patient #63 born on September 2004)

Patient number	Genotype	CBS c.699C > T	CBS c.1080C > T	CBS c.844ins68 > T	MTHFR c.677C > T	Origin	Clinical manifestation			Met ^b	Age at diagnosis	B ₆ responsive	Severity
							Eye	Skeleton	Vascular system	CNS	Marfanoid features		
22	p.A226T/p.A226T		C/C	wt/wt	C/T	Argentina	+	+	—	—	Yes	Yes	Mild
23	p.G148R/p.G148R	T/T	C/C	wt/wt	C/C	Argentina	+	—	—	+	Yes	No	Mild
38	p.A114V/p. I429del ^a	C/C	C/T	wt/wt	C/T	Argentina	+	—	+	—	No	Yes	Mild
64	p.c.69_70+8del10 ^a /c.828+1G>A	C/C	C/T	wt/wt	C/T	Argentina	+	+	—	+	Yes	No	Severe
48	p.R121H/p.R121H	C/C	T/T	ND ^c	T/T	Colombia	+	+	—	+	Yes	No	Severe
51	p.R121H/p.T191M	C/C	T/T	ND	T/T	Colombia	+	+	—	+	Yes	No	Severe
25	p.P145L/p.P145L	C/C	C/C	wt/wt	C/T	Spain	+	+	+	+	Yes	ND	Severe
28	p.M173V ^a /p.T191M	C/C	C/T	wt/ins	ND	Spain	+	+	—	—	Yes	Yes	Mild
30a	p.T191M/p.T353M	C/C	C/T	wt/wt	C/C	Spain	+	+	+	+	Yes	No	Severe
30b	p.T191M/p.T353M	C/C	C/T	wt/wt	C/C	Spain	+	+	—	—	Yes	No	Mild
34	p.[T191M; D444N] / p.[T191M; D444N]	C/C	T/T	wt/wt	C/T	Spain	+	+	—	—	Yes	No	Mild
40	p.T353M/p.T191M	C/C	C/T	wt/wt	C/T	Spain	+	+	+	—	Yes	ND	Mild
54	p.D444N/p.D444N	C/C	T/T	wt/wt	C/T	Spain	+	+	+	—	Yes	ND	Mild
63	p.T257M/p.T275M	C/C	C/C	ins/ins	C/C	Spain	+	+	+	+	1,087	1 month	NSP
65	p.R379Q/p.R379Q	C/C	C/C	wt/wt	C/T	Spain	+	+	+	+	Yes	ND	Severe
67	p.L338P/ p.L338P	T/T	C/C	wt/wt	ND	Spain	+	+	+	+	No	No	Severe

^aMutations not previously reported^bMethionine at diagnosis (μM), VH very high^cNot determined^dNot applicable

Table 5 Phenotypic features in sib-pairs bearing the p.T191M/p.T191M genotype. *Pyr* Pyridoxine, *Bet* betaine, *FA* folic acid, *MFD* methionine-free diet

Patient number	Age at diagnosis	Date diagnosis (month/year)	Genotype	<i>MTHFR</i> c.677C > T	Origin	Clinical manifestation				Marfanoid features	Met ^a	Treatment	B ₆ responsive	Severity
						Eye	Skeleton	Vascular system	CNS					
21a	10 years	10/98	p.T191M /p.T191M	C/C	Portugal	+	-	-	+	Yes	529	Pyr. Bet. ^b	ND ^c	Severe
21b	13 years	12/98	p.T191M /p.T191M	C/C	Portugal	+	-	-	-	No	61	no	ND	Mild
33a	6 years	02/90	p.T191M /p.T191M	C/T	Spain	+	+	-	-	Yes	112	Pyr.FA Bet.MFD	No	Moderate
33b	3 years	05/90	p.T191M /p.T191M	C/T	Spain	-	-	-	-	No	263	Pyr.FA Bet.MFD	No	Mild
41a	6 years	12/94	p.T191M /p.T191M	C/T	Spain	+	+	-	+	Yes	188	Pyr.FA	No	Severe
41b	19 years	02/95	p.T191M /p.T191M	C/T	Spain	+	+	-	+	Yes	239	Pyr.FA	ND	Severe
53a	-	-	p.T191M /p.T191M	C/T	Colombia	+	+	-	+	Yes	-	-	No	Severe
53b	-	-	p.T191M /p.T191M	C/T	Colombia	+	+	-	+	Yes	-	-	No	Severe

^aMethionine at diagnosis (μM)^bTreatment started at age 12 years^cNot determined

South America. Recently, the mutation was also reported in two other Latin American countries, namely Venezuela (De Lucca and Casique 2004) and Brazil (Porto et al. 2005). The highest prevalence was found in Colombia. In two different groups of patients, gathered in Medellín (this study) or Bogotá (M. Bermudez et al. 2006) the p.T191M homozygous genotype was above 70%. A homozygous patient was also detected among five Argentinian patients. This is the first mutation report on homocystinuric patients from Argentina.

The finding of the mutation associated with two different *CBS* haplotypes strongly suggests a double origin for the p.T191M mutation. While haplotype I was the most frequently encountered in the Spanish and Colombian chromosomes, haplotype II was found in 4/6 Portuguese chromosomes and in a Spanish chromosome. The mutation associated with haplotype I might have arisen in Spain and travelled to Colombia where it expanded. Both historical data and the limited information on the external STR D21S1411 would support this hypothesis.

The high prevalence of p.T191M makes the study of genotype-phenotype correlations an important issue. A wide spectrum of phenotypes, ranging from mild to severe, were observed. This highlights the relevant roles played by the genetic background and, probably more importantly, by nongenetic factors such as age at diagnosis, treatment and diet. The main common features for the p.T191M homozygotes seem to be the low prevalence of vascular disease and the lack of response to B₆ treatment. Because pyridoxine does not help these patients, additional treatments should be conducted. In this regard, some patients treated with betaine displayed a significant reduction of total plasma homocysteine. The p.T191M mutation was expressed heterologously in *Escherichia coli* and the resulting protein displayed neither detectable enzyme activity nor response to PLP (Urreizti et al. 2006).

Three new mutations were identified among the patients: p.M173V, p.I429del and c.69_70+8del10. The p.M173V change was present in compound heterozygosis with p.T191M in a Spanish homocystinuric patient (#28) whose phenotype was rather mild, and responsive to B₆. This mutation was also expressed in *E. coli* (Urreizti et al. 2006). In agreement with the mild patient's phenotype, the resulting protein retained 40% of the wild-type enzyme activity, and displayed a moderate response to PLP. The p.I429del mutation was present, in compound heterozygosis with p.A114V, in an Argentinian patient (#38) presenting a mild phenotype and a positive response to B₆. The fact that the accompanying mutation is known to be associated with a mild phenotype (Kozich et al. 1993; de Franchis et al. 1999) precludes our drawing conclusions on the effects of p.I429del. Regarding the third novel mutation identified, c.69_70+8del10, the fact that the 10-bp deletion included the donor site of intron 1 supports a pathogenic role for this change. According to reports on another mutation, IVS1+1G>A (g.210G>A, Gordon et al.

1998), in which the intron 1 splicing donor site was also affected, the probable consequences of this mutation are the deletion of exon 1 or a deletion of 157 bp starting at position c.153.

Patient #54 was homozygous for mutation p.D444N, and presented with a phenotype characterised by vascular involvement as the main feature, together with a Marfanoid habitus. This mutation lies in the C-terminal domain, where Maclean et al. (2002) described several patient-derived CBS mutations, including p.D444N, associated with a phenotype characterised by vascular involvement as the only feature. The first patient described with the p.D444N/p.D444N mutation (Kluijtmans et al. 1996) presented with a more complex phenotype, including psychomotor retardation and marfanoid features such as excessive height, dolichosternomelia, and arachnodactyly. It should be noted that two patients in our series bore p.D444N: patient #54 (described above) and patient #34. The latter was homozygous for the double-mutant allele p.[T191M; D444N]. Both patients originate from the same region of Spain.

Finally, it is also worth mentioning the potential usefulness of the neonatal screening program based on tandem-mass spectrometry, which allowed the identification of a homocystinuric patient and the immediate start of treatment. The follow-up of this patient will establish whether this early start is an efficient way to avoid the manifestation of clinical symptoms.

In summary, we have shown that the p.T191M mutation, previously reported as a prevalent CBS mutation in Spain and Portugal, is also highly prevalent in Latin American countries. Haplotype analyses suggest that the mutant allele had two independent origins. The lack of response to vitamin B₆ treatment is the main common phenotypic feature associated with this mutation.

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