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DNA VARIANTS

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Two novel missense mutations in the cystathionine β-synthase gene in homocystinuric patients

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Abstract Direct sequencing of the coding region of the cystathionine β-synthase (CBS) gene in two homocystinuric patients revealed the presence of two novel missense mutations. The first mutation, a 1111G → A transition, resulted in the substitution of the evolutionary conserved valine-371 by a methionine residue (V371M) and created a new *Nla*III restriction site. The second mutation, a G→A transition at base-pair 494, resulted in an amino acid change from cysteine to tyrosine (C165Y) and abolished a *Bso*FI restriction site. Both mutations were found in a compound heterozygous state with the previously described 833T → C transition.

Introduction

Homocystinuria caused by cystathionine β-synthase (CBS) deficiency is an inborn error of methionine metabolism and is inherited as an autosomal recessive trait. The predominant clinical manifestations of the homozygous state are arteriosclerosis, thromboembolic complications, skeletal abnormalities, ectopia lentis and mental retardation (Boers 1986; Mudd et al. 1989). The large range in the severity of the clinical features and in the biochemical response to therapy suggests genetic heterogeneity of the CBS gene. CBS, a pyridoxal-phosphate-requiring enzyme, catalyzes the synthesis of cystathionine from homocysteine and serine (Finkelstein and Martin 1984). To date, 14 mutations have been reported in the coding region of the CBS gene (Kraus 1994), which has been mapped to 21q22.3 (Müncke et al. 1988). In this report, we describe the identification of two new missense mutations in this coding region.

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Materials and methods

Patient 1 was a 33-year-old man who suffered from ectopia lentis, osteoporosis, scoliosis and mental retardation. He also showed typically marfanoid features, such as arachnodactyly and dolichostenomelia. Therapy consisted of the daily administration of 750 mg pyridoxine, which reduced his total homocysteine levels to 35 μmol/l (control levels 8–18 μmol/l). Patient 2 was a 44-year-old male patient with many of the clinical features of homocystinuria: ectopia lentis, thrombosis, seizures, arachnodactyly and scoliosis. Family examination showed that a sister and a brother were also affected. All were pyridoxine-responsive.

For mutation detection, RNA was extracted from cultured fibroblasts (Chomczynski and Sacchi 1987) and first-strand cDNA was synthesized by standard methods. The cDNA product was amplified by the polymerase chain reaction (PCR) using three sets of oligonucleotides (Table 1). The generated PCR fragments covered the entire coding region and were subsequently subjected to direct sequencing according to the manufacturer's recommendations (Applied Biosystems, Forster City, CA, USA).

Table 1 Primers used for PCR amplification and sequence analysis of the cystathionine β-synthase cDNA

Primer	Nucleotide sequence (5′ → 3′)	Nucleotide position[a]
1 F	GTCAGCACCATCTGTCCGGTC	nt –26– –6
1 R	CTCGGAGCTCATCTTCTCTGGC	nt 528– 507
2 F	GATCGGGCTGGCCCTGGC	nt 453– 471
2 R	CCCGCCTCATCCACCACGG	nt 1358–1340
3 F	GACCAAGTTCCTGAGCGACAGGTG	nt 1146–1169
3 R	TCCTACCTGGCCGACTTCTCTC	nt 1720–1699

[a]The nucleotide position of the cystathionine-β-synthase cDNA is numbered according to Kraus et al. (1993)

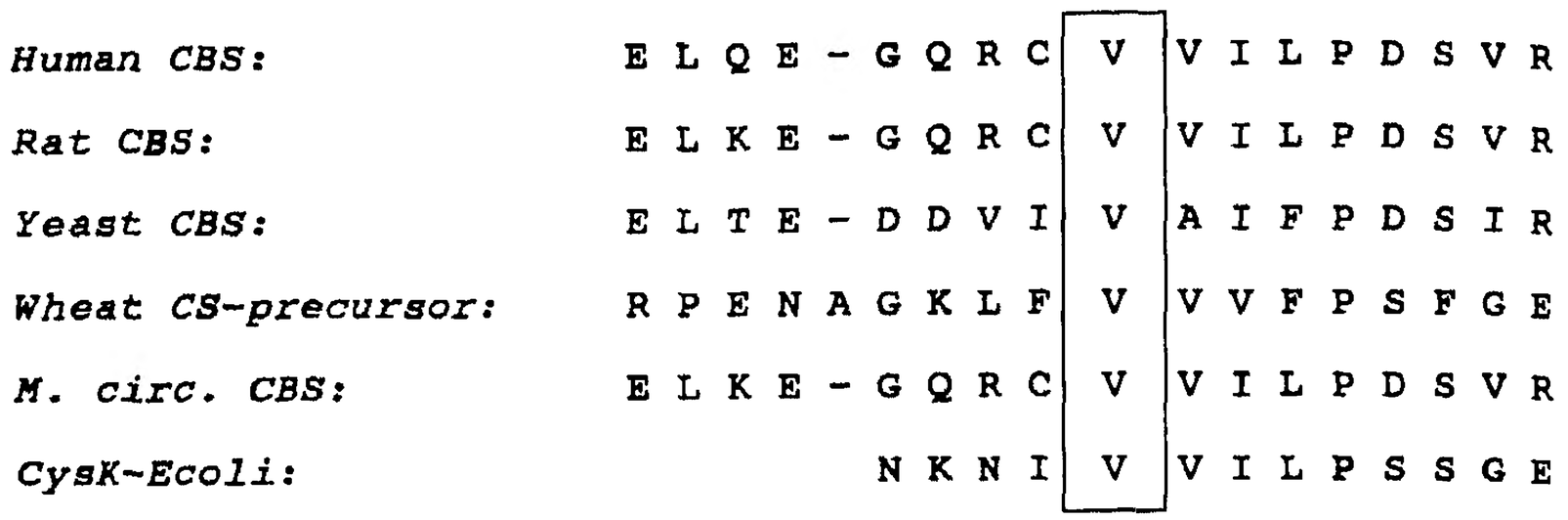

Fig. 1 Comparison of amino acid sequences of homologous proteins of different species. *CysK-Ecoli Escherichia coli* acetylserine lyase; *M. circ. CBS Mucor circinelloides* cystathionine β-synthase. The amino acids are indicated by *capital letters*. A gap (–) has been introduced to maintain alignment

Results and discussion

Direct sequence analysis of the entire coding region of the CBS gene in patient 1 showed a novel 1111G → A transition and the previously described 833T → C (I278T; Hu et al. 1993) missense mutation (data not shown). The observed G-to-A transition resulted in an amino acid exchange from valine to methionine at codon 371 of the mature protein (V371M) and introduced a new *Nla*III restriction site. Both transitions were confirmed at the genomic DNA level by *Nla*III and *Bsr*I restriction analysis, respectively. The substituted valine-371 residue appears to be highly conserved in homologous proteins in both prokaryotic and eukaryotic species (Fig. 1). Evolutionary conservation may indicate the functional relevance of an amino acid in the structure and function of the mature protein. Thus, this mutation is thought to interfere with the normal functioning of the CBS protein.

Patient 2 was found to be a compound heterozygote for the 833T → C missense mutation and a novel 494G → A transition. This latter transition resulted in a substitution of cysteine-165 by tyrosine (C165Y) in the CBS protein and abolished a *Bso*FI restriction site. The putative amine acid is located in the catalytic domain of the protein (J. P. Kraus, personal communications). This G-to-A transition may disrupt a disulphide bond in this catalytic domain. The disruption of a disulphide bond could interfere with intermolecular interactions and consequently with the catalytic activity of the protein. However, the present data provide no indications whether these two new missence mutations are compatible with pyridoxine responsiveness in vivo, because the other mutation (833T → C) is associated with responsiveness both in the homozygous and in the compound heterozygous states (Hu et al. 1993).

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