Available online at www.sciencedirect.com
ScienceDirect
Molecular Genetics and Metabolism 92 (2007) 198–209
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Minireview

Molecular genetics of HMG-CoA lyase deficiency

Juan Pié [a], Eduardo López-Viñas [b], Beatriz Puisac [a], Sebastián Menao [a], Angeles Pié [a], Cesar Casale [a,1], Feliciano J. Ramos [a], Fausto G. Hegardt [c], Paulino Gómez-Puertas [b], Núria Casals [d,*]

[a] *Laboratory of Clinical Genetics and Functional Genomics, University of Zaragoza Medical School, E-50009 Zaragoza, Spain*
[b] *Molecular Modeling Group, Centre for Molecular Biology "Severo Ochoa" (CSIC-UAM), Cantoblanco, E-28049 Madrid, Spain*
[c] *Department of Biochemistry and Molecular Biology, School of Pharmacy, University of Barcelona, E-08028 Barcelona, Spain*
[d] *Department of Biochemistry and Molecular Biology, School of Health Sciences, International University of Catalonia, E-08195 Sant Cugat, Barcelona, Spain*

Received 2 April 2007; received in revised form 29 June 2007; accepted 29 June 2007
Available online 9 August 2007

Abstract

3-Hydroxy-3-methylglutaryl-CoA lyase (HL) deficiency is a rare autosomal recessive genetic disorder that affects ketogenesis and L-leucine catabolism, which generally appears during the first year of life. Patients with HL deficiency have a reduced capacity to synthesize ketone bodies. The disease is caused by lethal mutations in the HL gene (*HMGCL*). To date, up to 30 variant alleles (28 mutations and 2 SNPs) in 93 patients have been reported, with a recognizable population-specific mutational spectrum. This disorder is frequent in Saudi Arabia and the Iberian Peninsula (Portugal and Spain), where two mutations (122G>A and 109G>A) have been identified in 87% and 94% of the cases, respectively. In most countries a few patients have a high level of allelic heterogeneity. The mutations are distributed along the gene sequences, although some clustering was observed in exon 2, conforming a possible hot spot. Recently, the crystal structures of the human and two bacterial HL have been published. These experimentally obtained structures confirmed the overall architecture, previously predicted by our group and others using bioinformatic approaches, which shows the $(\beta\alpha)8$-barrel structure of the enzyme. In addition, the crystals confirmed the presence of an additional COOH domain containing important structures and residues for enzyme functionality and oligomerization processes. Here, we review all HMGCL mis-sense mutations identified to date, and their implication in enzyme structure and function is discussed. We found that genotype–phenotype correlations are difficult to establish because the evolution of the disease seems more related to the causes of hypoglycaemia (fasting or acute illness) than to a particular genotype.

Keywords: 3-Hydroxy-3-methylglutaryl CoA lyase; HL; 3-Hydroxy-3-methyl glutaric aciduria; Ketone body metabolism; TIM-barrel structure; Enzyme activity; Mutations

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase (HL) deficiency or 3-hydroxy-3-methylglutaric aciduria (OMIM 246450) is a rare autosomal recessive genetic disorder that affects ketogenesis and L-leucine catabolism, which usually appears in the first year of life. It belongs to a group of 29 genetic conditions for which effective treatment is available, and the American College of Medical Genetics (ACMG) and the Health Council of the Netherlands have recommended that hospitals with neonate units should have the capacity to diagnose it [1]. Patients with HL deficiency have a reduced capacity to synthesize ketone bodies. Acetoacetate and 3-hydroxybutyrate are primary energy sources for the brain when metabolic needs are not met by glucose [2]. Throughout suckling, ketone bodies are also substrates for the synthesis of lipids such as cholesterol in

* Corresponding author. Address: Faculty of Health Sciences, Universitat Internacional de Catalunya, C/ Josep Trueta s/n, E-08195 Sant Cugat del Valles, Barcelona, Spain.
E-mail address: ncasals@csc.unica.edu (N. Casals).
[1] Present address: Department of Molecular Biology, National University of Rio Cuarto, 5800 Rio Cuarto, Cordoba, Argentina.

1096-7192/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2007.06.020

myelin [3,4]. Frequently, abnormal cerebral white matter foci are seen on brain magnetic resonance imaging (MRI) in asymptomatic HL-deficient patients [5–7].

Other organs like the heart obtain most of their energy by metabolizing ketone bodies of hepatic origin. Hence alterations to this metabolic pathway may be associated with cardiac myopathy and liver disease [8,9]. The cDNA of human HL was reported in 1993, which led to the first genetic study of this disease in two Acadian siblings [10]. Since then, fewer than 100 affected patients have been diagnosed worldwide at molecular level, and 31 variant alleles have been identified (Table 1) [11–28]. In some cases, reported mutations have helped us to understand the structure, function and mRNA processing of the enzyme [15,16,21,22]. Recently, the crystal structures of human and two bacterial HLs have been published, allowing more accurate location of mutations and a better understanding of their effects on enzyme activity [30,31]. Here we review current knowledge on enzyme structure, reported mutations with biological significance, and their population distribution. We also report on the pathological splicing variants and the uncertain genotype–phenotype correlations of this disease.

Table 1
Mutations and polymorphisms of the *HMGCL* gene

Allelic variant	Exon/intron	Aminoacid change/predicted effect	Patients	Mutant alleles	Origin	References
Missense mutations						
c.122G>A	E2	R41Q	43	81	1 Czech, 1 Italian (ht), 40 (4 ht) Saudi, 1 Turkish.	[11–13]
c.124C>G	E2	D42H	1	1	1 Cajun (ht)	[12]
c.125G>A	E2	D42G	1	2	1 German	[12]
c.126G>T	E2	D42E	1	2	1 Austrian	[12]
c.144 G>T	E2	K48N	1	1	—	[48]
c.208G>C	E3	V70L	1	1	1 French-Canadian (ht)	[14]
c.225C>G	E3	S75R	1	2	1 German	[15]
c.434A>T	E5	E145V	1	1	1 Japanese (ht)	[16]
c.602C>A	E7	S201Y	1	2	1 English	[15]
c.608G>A	E7	G203E	1	2	1 Italian	[17]
c.610G>A	E7	D204N	2	3	1 Argentinean, 1 Portuguese (ht)	[15,18]
c.698A>G	E7	H233R	3	4	1 French (ht), 2 Czech (1ht)	[19,20,11]
c.788T>C	E8	L263P	1	1	1 French (ht)	[20]
c.835G>A	E8	E279K	2	3	2 Japanese (1 ht)	[16]
Nonsense mutations						
c.109G>T	E2	E37X; Exon 2 skipping	18	33	1 Moroccan, 12 Portuguese (3ht) 4 Spanish, 1 Turkish	[21,22,18,23]
c.121C>T	E2	R41X	1	1	1 English/German (ht)	[12]
c.286C>T	E4	Q96X	1	2	1 Italian	[24]
c.922C>T	E9	Q308X	1	2	1 Japanese	[16]
Deletions/insertions						
c.27delG	E1	P9P/frameshift: stop codon 33	1	1	1 Czech (ht).	[11]
c.134–137insA	E2	N46K/frameshift: stop codon 47	2	4	2 Italian	[25]
c.61–561del	E2,E3,E4,E5, E6	Deletes V21-E187 in frame	1	1	1 English	[26]
c.202–207delCT	E3	S69C/frameshift: stop codon 79	2	4	2 Acadian French-Canadian	[10]
c.145–561del	E3,E4,E5,E6	Deletes E49–E187 in frame	1	1	1 Turkish	[26]
c.504–505delCT	E6	V168V/frameshift: stop codon 176; Exon 5 and 6, 6 skipping	2	3	1 Spanish, 1 Portuguese (1ht)	[27,18]
c.561–750del	E7	Deletes E187-Q250 in frame	1	2	1 Japanese	[16]
c.913–915delTT	E9	F305Y/frameshift: stop codon 314	3	6	3 Saudi	[12,13]
Intronic mutations						
IVS3+1Gdel	I3	Exon 3 deletion	1	2	1 Japanese	[16]
IVS6+1G>A	I6		1	2	1 Saudi	[13]
IVS8+1G>T	I8	Exon 8 deletion	1	2	1 Turkish	[28]
Polimorfism						
c.654A>G	E7	L218L	1	—	1 Spanish	[21]
c.727A>G	E7	T243A	4	8	1 Moroccan, 1 Portuguese, 2 Spanish	[21]

Position refer to the numbering of the HL cDNA sequence in[10]. All have been named according to the recommendations of the Nomenclature Working Group [29]; ht, heterozygous.

HL enzyme

HL (EC 4.1.3.4) is a mitochondrial enzyme that catalyzes the cleavage of HMG-CoA to acetyl-CoA and acetoacetic acid. This reaction is the common final step of the ketogenic pathway and leucine catabolism (Fig. 1). HL is a 325-amino-acid enzyme that has been partially purified from a variety of organisms and tissues, including pig heart [32] and bovine liver [33]. Pure forms of the avian, *Pseudomonas mevalonii* and recombinant human enzyme have been documented [34–36]. Human HL has 87% similarity with its mouse homologue, 82% with its chicken homologue, and 52% with *P. mevalonii*, and the sequence has been highly conserved throughout evolution (Fig. 2). The human enzyme is sensitive to oxidation, showing higher activity in reducing conditions (with highest activity at pH 9). In oxidative conditions, the enzyme can form a dimer of two identical monomers bound by a disulfide bridge, and its activity is lost [36]. The prokaryote enzyme is insensitive to oxidation because it lacks Cys^{323}. From a chemical point of view, the reaction is a retro-Claisen condensation, in which the enzyme acts as a base with a nucleophilic attack on C3 hydroxy group, and also as an acid taking the electrons liberated from the cleavage of the C2–C3 bond of HMG-CoA [19]. The enzyme needs divalent metals Mg^{2+} or Mn^{2+} for its activity [34]. The divalent cation forms a bridge between substrate and target protein [37].

Isoforms

Two isoforms of human HL have been found, which are classified as sorting enzymes. A single gene on chromosome 1 encodes these two proteins, which show differential location in mitochondria or peroxisomes. The native 298-aminoacid mitochondrial isoform contains a leader peptide of 27 aminoacids at the N-terminal end, which guides HL towards the mitochondrial matrix. There the leader peptide is removed, leaving the 298-aminoacid protein as the mature enzyme. This final isoform has a molecular mass of 31.5 kDa and an isoelectric point of 6.2. The function of HL in mitochondria is to catalyze the conversion of HMG-CoA into acetoacetate and acetyl-CoA [10]. This protein is also found in peroxisomes, where it is guided by the signal CKL tripeptide in the C-terminal end. In peroxisomes, the protein has 325 aminoacids, a molecular mass of 34.1 kDa and an isoelectric point of 7.6, which is much more basic than the mitochondrial protein [38]. Although both isoforms have lyase activity, the role of HMG-CoA in peroxisomes is still unknown. It may be involved in cholesterol synthesis or degradation of long-chain fatty acids

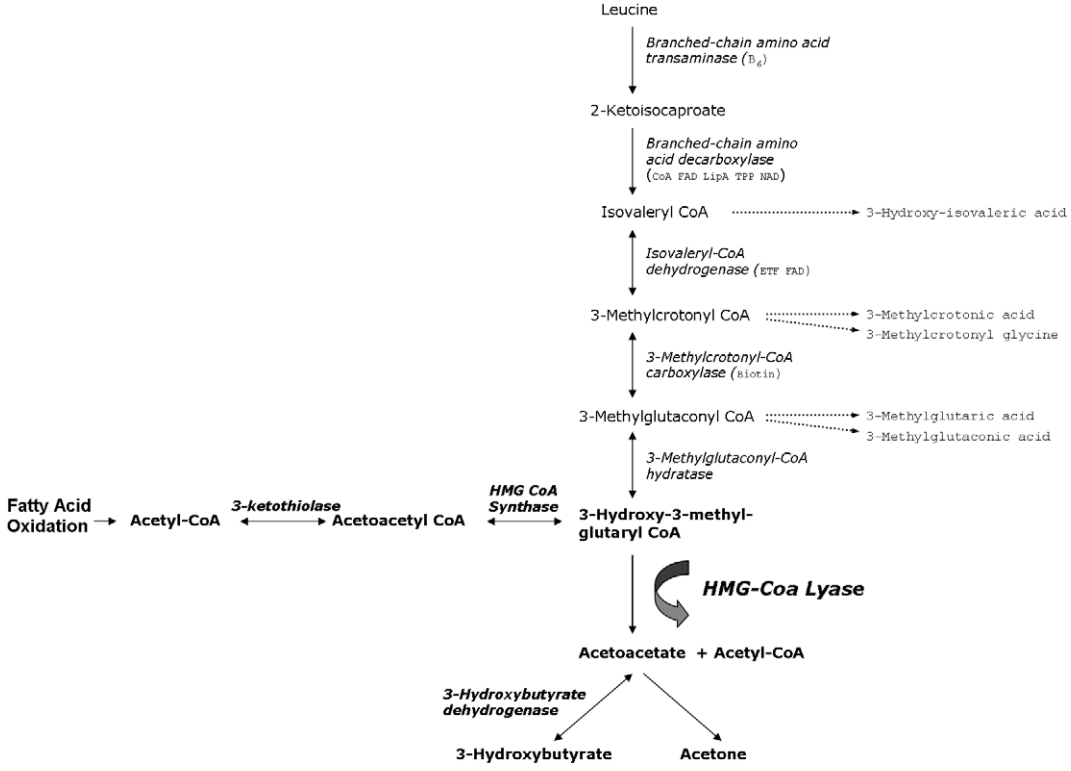

Fig. 1. Metabolic interrelationships of HL. HL plays an essential role in breaking down dietary proteins and fats for energy. Specifically, the enzyme is responsible for processing leucine, an amino acid that is part of many proteins. This enzyme also produces ketones during the breakdown of fats. If a mutation in the HMGCL gene reduces or abolishes the activity of this enzyme, the body is unable to process leucine or make ketone bodies. The organic acids (to the right of the scheme) which are formed as products of amino acid breakdown can cause the blood to become too acidic. Coenzymes: B6 (vitamin B6), CoA (Coenzyme A), FAD (flavin–adenin–dinucleotide), LipA (lipoic acid), TPP (thiamine pyrophosphate), NAD (nicotin-adenosin-dinucleotide, ETF (electron transfer flavoprotein), biotin.

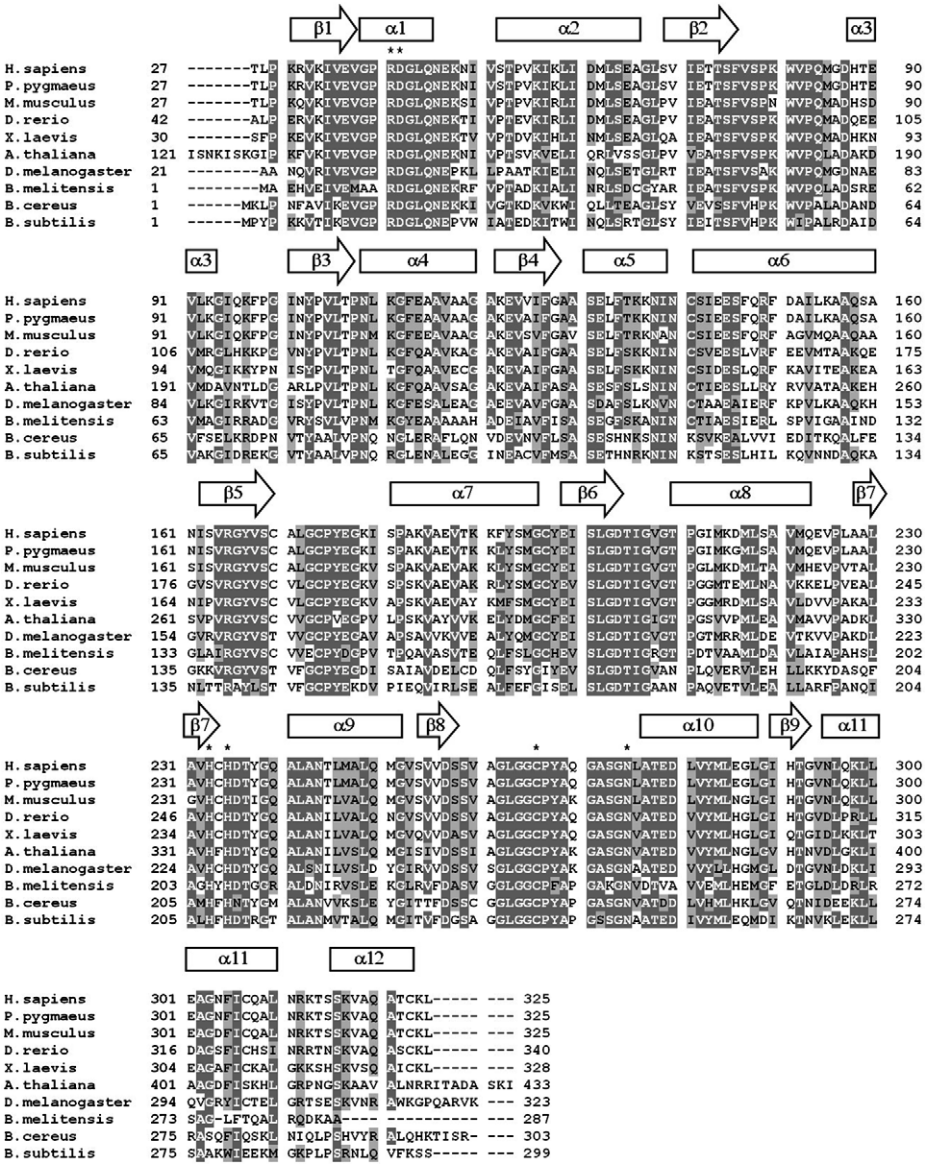

Fig. 2. Multiple alignment of representative sequences of 3-hydroxy-3-methylglutaryl-CoA lyase. Position of secondary structure elements is indicated. Residues involved in catalytic activity of the enzyme are marked (*). The species represented are: *Homo sapiens*, *Pongo pygmaeus* (Bornean orangutan), *Mus musculus*, *Danio rerio* (zebrafish), *Xenopus laevis*, *Arabidopsis thaliana*, *Drosophila melanogaster*, *Brucella melitensis*, *Bacillus cereus* and *Bacillus subtilis*.

[39]. It was initially hypothesized that the peroxisomal isoform was a monomer [40], but more recently a dimeric form has been identified [41]. While most HL is found in mitochondria, about 16–20% is located in peroxisomes [42]. To date, no satisfactory explanation has been found for this distribution.

Protein structure

The first attempt to build a 3D structural model of human 3-hydroxy-3-methylglutaryl-CoA lyase was performed by our group in 2003 [15], based on a threading procedure using the crystallized structure of the TIM-barrel hisA gene from *Thermotoga maritima* as a template (Protein Data Bank entry 1qo2) [43]. In the absence of direct evidence for structural relationships between these two genes, the model was based on the remote homology between their respective families of proteins [44]. Information extracted by multiple alignments of both families of sequences was used to build a 3D model of human HL. Analysis of the resulting data suggested that HL protein could adopt a (βα)8 barrel structure (also known as TIM barrel), consisting of an eightfold repetition of a β-sheet-loop-α-helix-loop motif, with the β-sheets forming the inner face of the barrel and the α-helices forming the external face. The structure contains short loops on the NH2-terminal face of β-barrel, and long, probably more-structured loops on the COOH-terminal face. This model

showed, for the first time, the structural proximity of the residues involved in the catalytic activity of the protein: Arg^{41}, Asp^{42}, Glu^{72}, His^{233} and His^{235}, located near the cavity on the COOH-terminal face of the model.

A second model was built one year later [45] using the published structure of a more homologous protein, 4-hydroxy-2-ketovalorate aldolase (PDB entry: 1nvm) [46], as a template. Interestingly, both models showed similar spatial arrangement of the secondary structural elements, as well as catalytic residues in the active center of the enzyme.

Recently, the crystal structures of human HL (PDB entry: 2cw6) [30] and bacterial HL (PDB entries 1ydo and 1ydn) [31] have been published, confirming the initial (βα)8-barrel structure proposed by our group. In addition to the basic TIM barrel structure, the monomer of human HL includes an additional COOH terminal region (residues 290–323) containing β-strand 9 and α-helices 11 and 12. Fig. 2 summarizes the position of secondary structure elements in a multiple alignment of a representative set of HL sequences ranging from bacteria to human. Fig. 3 shows the surface and backbone representation of crystallized human HL, with catalytic residues located at the active center, as well as some missense mutations in symptomatic probands, as commented below.

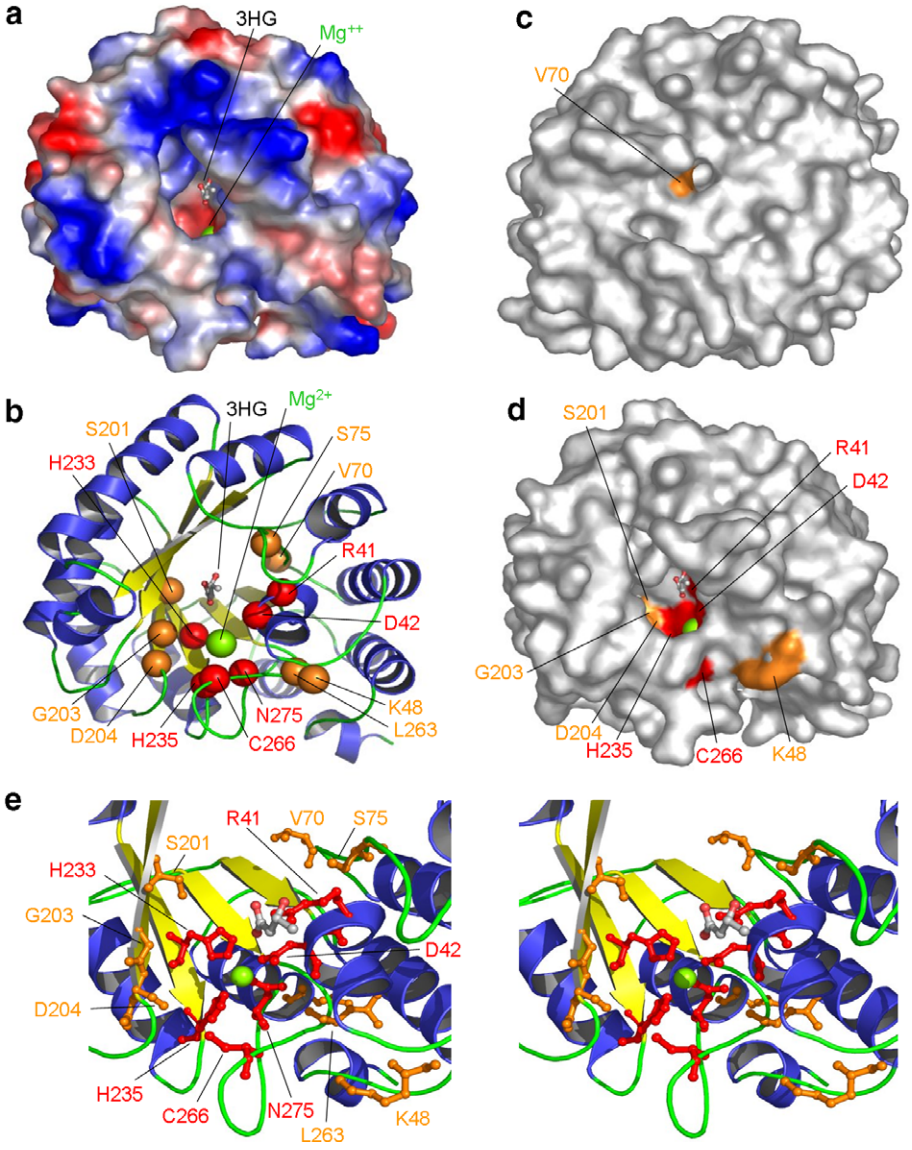

Fig. 3. Structural characteristics of human HL. (a) Surface of human HL (PDB entry: 2cw6; (Fu et al., 2006) indicating the position of co-crystallized 3-hydroxyglutaric acid -3HG- and Mg^{2+} ion in the active-center cavity. (b) Structure of HL showing the location of catalytic residues (red spheres) and missense mutations (orange spheres). Position of 3HG and Mg are also indicated. (c) Surface of the NH-face of the enzyme showing the presence of a small cavity in the central position of the barrel, and the close positioning of Val^{70} residue (orange). (d) Surface representation of the COOH-face of HL, indicating the position of residues in the active center deep hollow and in the surrounding area. Colors are as in B. (e) Stereo diagram showing the arrangement of important residues in the active center of the enzyme.

As predicted, the crystal structure of HL exhibited a COOH-end cavity formed by residues of the eight β-strands of the barrel and by the long loops on the COOH-terminal face. The crystal structure of HL contains a 10 Å deep funnel-shaped hollow, with a diameter of 9 Å. It houses an Mg^{2+} ion and one molecule of 3-hydroxyglutaric acid, the hydrolytic product of the inhibitor 3-hydroxyglutaryl-CoA. The Mg^{2+} ion has an octahedral coordination with two water molecules, the imidazole nitrogens of catalytic residues His^{233} and His^{235}, the carboxylate group of Asp^{42} and the amide oxygen of Asn^{275}. Other catalytic residues in the vicinity include Arg^{41} and Cys^{266}. The latter is located in the very flexible G-loop [31,30], apparently involved both in catalysis and enzyme multimerization processes. Human HL crystallizes as a dimer in which the contacts between monomers are formed by additional secondary elements that are not part of the core TIM barrel structure: β-strand 9 and α-helices 11 and 12. It has been postulated that an additional Cys^{323} is responsible for the HL dimer, through the formation of a reversible disulfide bond between the Cys^{323} of two monomers [47]. Analysis of crystallized human HL has shown that the distance between the two Cys residues (29 Å) is too great to allow disulfide linkage. The distance between Cys^{323} and Cys^{266} of adjacent monomers is shorter (14 Å), but still too long to allow the formation of the disulfide bond. Fu *et al*. suggested that the mobility of the G-loop, spanning residues 259 to 278, may facilitate the spatial approximation of Cys^{323} and Cys^{266}, allowing the formation of the disulfide bond [30].

Analysis of the recently obtained crystal structures of *Bacillus subtilis* (Bs) and *Brucella melitensis* (Bm) [31] indicates that the former, BsHL, crystallizes as a dimer and tetramer, whereas the latter, BmHL, is only a monomer. The main difference between the structure and sequence of these two enzymes is the absence of the residues forming α-helix 12 in BmHL (Fig. 2), reinforcing the putative role of this element in dimerization. In BsHL, α-helix 12 maintains polar and non-polar interactions with the G-loop, with β-strand 7 and with the α-helix 12 of the adjacent monomer. Lys^{297}, located in this α-helix 12, is close (5.5 Å) to Lys^{297} of the second monomer. As the structural position of Lys^{297} BsHL is occupied by Cys^{323} in human HL, it has been suggested that a disulfide bond may form between the two Cys residues. Unfortunately, the crystal structure of human HL [30] does not support this hypothesis.

Comparison of the catalytic cavity of BsHL with the corresponding residues in human HL suggested a catalytic mechanism in which Asp^{42} stabilizes the position of the cation, while Arg^{41} stabilizes the intermediate enolate and Glu^{72} maintains the structural positioning of both Arg^{41} and Asp^{42}. Although Cys^{266} appears to be the putative catalytic base responsible for the nucleophilic attack, other residues, such as Asp^{42} or Tyr^{167}, cannot be ruled out.

Recently [48], a bioinformatic structural analysis of docking for HMG-CoA on the surface of human HL suggested a secondary position of the substrate in a region located close to Lys^{48} and defined by α-helix 12 surrounding residues The *in silico* docking model implicates Asn^{311} and Lys^{313} in substrate binding via polar contacts, and Lys^{48} via contact with the carboxyl group of the pantothenic acid moiety of HMG-CoA.

HL deficiency

The prevalence of 3-hydroxy 3-methylglutaric aciduria is less than 1/100,000 live neonates, although it is sometimes confused with sudden death and Reye syndrome [49]. It was first described by Faull et al. in 1976 in a 7-month-old male with acidosis and hypoglycemia [50]. Later, Wysocki and Hähnel showed that HL activity in the leukocytes of this patient was null [51]. The parents also showed a reduction of enzyme activity, although they were asymptomatic. A strain of HL-deficient mice has been created by gene targeting. Heterozygous HL-deficient mice are clinically normal but homozygous embryos have abnormal prenatal development and die at approximately 11.5 days *post-coitum* [52].

Clinical features

3-Hydroxy-3methylglutaric aciduria is an early onset disease. In approximately 30% of cases the first symptoms appear between the second and fifth days *post partum* or between 3 and 11 months. Two patients with late onset (puberty and adult) have been reported [53,5]. Acute clinical episodes include vomiting, diarrhea, dehydration, hypotonia, hypothermia, lethargy, cyanosis and apnea that sometimes progress to coma [54–57]. Other signs are hepatomegalia, macrocephalia, [58,56,59] or, less frequently, microcephalia [60], delayed development, acute pancreatitis [61] and dilated cardiomyopathy with arrhythmia [8]. Cerebral MRI findings have been reported only in a few patients. Cerebral white matter involvement is the most common finding [5–7]; more recently, one case of prominent corticospinal tract and pontine involvement has also been reported [62]. HL deficiency has been associated with Usher Syndrome type I, or VATER [63,64]. HL deficiency is fatal in approximately 20% of cases, although in those who survive the symptoms tend to remit after childhood [65]. In patients, low blood glucose levels are not followed by ketone body synthesis. Acute crises tend to occur when there is no exogenous intake of glucose (starvation) or when glucose metabolization is excessive (conditions of metabolic stress, febrile stress and exercise). Laboratory tests reveal metabolic acidosis with fasting non-ketotic hypoglycemia, which may impair the ketogenic pathway [53]. Abnormal liver-function tests, like prothrombin time, increased alanine and aspartate aminotransferases, and plasma bilirrubin levels have been observed [56]. Hyperammonemia has also been reported, attributed to increased proteolysis due to the lack of ketone bodies.

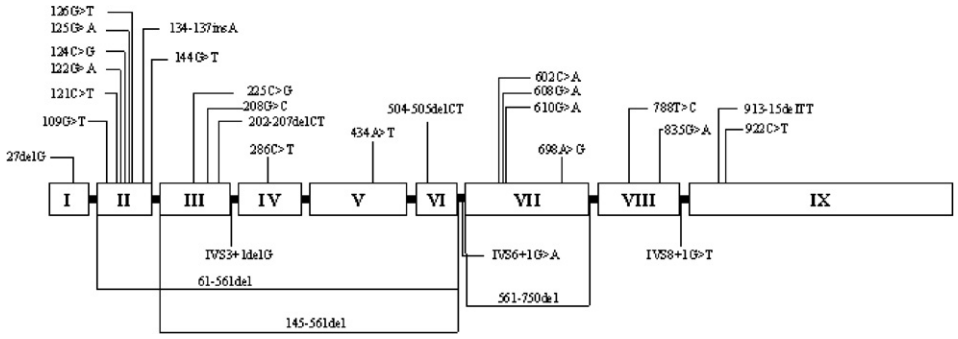

Fig. 4. Scheme of the mutations located in the human *HMGCL* gene. Seven allelic variants including c.122G>A and c.109G>T are clustered in the second half of exon 2, five of them within a 6-nt interval (121–126bp HL cDNA). Except c.134–137isnA, all these mutations affect aminoacids E37, R41, and D42, conserved in HL from mouse [78], chicken [10] and *P. mevalonii* [80].

Diagnosis

HL deficiency should be suspected in children with hypoketotic-hypoglycemia and metabolic acidosis. A preliminary diagnosis of HL deficiency may be indicated by the characteristic urinary organic acid profile, which includes high levels of 3-hydroxy-3-methylglutaric acid, 3-hydroxy-isovaleric acid, 3-methylglutaconic acid and 3-methylglutaric acid. In acute crises, glutaric acid, adipic acid, and 3-methylcrotonylglycine are sometimes increased, which can be detected in urine by GC/MS (gas chromatography linked to mass spectrometry). The characteristic 3-hydroxy-3-methylglutaric acid peak can also appear in carbamyl phosphate synthase deficiency and in the Leigh-like syndrome [66–71]. It is therefore necessary to perform a differential diagnosis. In prenatal diagnosis, the pattern of organic acids in amniotic liquid [72] and in maternal urine between the 23rd week of gestation and delivery has also been used as a diagnostic tool [73]. The confirmation of HL deficiency requires direct assay of the enzyme activity in leukocytes [51], fibroblasts [74] or liver biopsy [54,75]. HL activity has also been assayed in cultured amniocytes or chorionic villi [76,72,25]. Molecular diagnosis requires the analysis of the *HMGCL* gene.

Treatment and prognosis

During acute episodes, treatment is based on symptoms and consists of intravenous administration of glucose to control hypoglycemia, and bicarbonate to correct acidosis. Maintenance therapy is based on diets that restrict protein and fat, whose aim is to reduce the formation of toxic metabolites. However, the most important concern is to avoid metabolic stress such as intercurrent illnesses and starvation. Carnitine treatment has been proposed as a way to improve the patient's general condition by facilitating urinary excretion of toxic metabolites [77]. After the first years of life, in the absence of complications, the illness tends to remit, and in general, adults are free of symptoms.

Genetics

The HMGCL gene

The *HMGCL* gene (Genomic Accession No. NT_004610.17) encodes HMG-CoA human mitochondrial lyase and it is located on chromosome 1, at the 1p36.1-p35 position, between *FUCA1* and *TCEB3*. It has 9 exons and 8 introns and a total of 24,336 base pairs (bp) (Fig. 4). Its 5′-untranslated region presents the characteristic elements of a housekeeping gene, as well as a CpG isle that contains binding sites for SP1. There is no evidence of either a TATA box or a CAAT box [26]. Exon size ranges between 64 and 527 bp. Exon 1 and part of exon 2 encode a 27-aminoacid array that forms the signal peptide for mitochondrial entry. Exon 9 encodes 33 aminoacids and also contains 406 bp of 3′ untranslated region (3′UTR) [10]. The polyadenylation signal in humans and mouse is ATTAAA, while in the chicken it is AATAAA. The *HMGCL* gene is present in both eukaryotes and prokaryotes, and it has been cloned and studied in a variety of organisms, including humans [10], chicken [10], mouse [78], the *Rhodospilirrum rubrum* [79] and bacterial strains such as *Pseudomonas mevalonii* [80], *Brucella mellitensis* and *Bacillus subtilis* [31]. Human HMGCL is 75.6% similar to the chicken gene and 53.6% similar to that of *P. mevalonii*. The mRNA transcribed from human *HMGCL* (Genomic Accession No. NM_000191.1) has a size of 1.6 kb and it has been found in all tissues studied (liver, kidney, fibroblast, etc.…).

Mutational update

To date, 31 variant alleles in the *HMGCL* gene (29 mutations and 2 SNPs) in 93 patients have been reported (one case was an aborted fetus). (Table 1; Figs. 3 and 4). In the coding region, missense mutations are the most frequent (14), followed by nonsense mutations (4), frameshift deletions (4) or insertions (1), and 3 large deletions. Three mutations have been found in intron sequences that cause abnormal splicing. The mutations are uniformly distributed

along the gene sequences, although some clustering is observed in exon 2, suggesting that it could be a hot spot for mutations (Fig. 4).

The mutational spectrum is clearly population specific. In Saudi Arabia and the Iberian Peninsula (Portugal and Spain) 3-hydroxy 3-methyl glutaric aciduria is frequent. Mutations c.122G>A and c.109G>A are prevalent in those countries, as they are found in 87% and in 94% of HL-deficiency patients, respectively. In most of the remaining countries, only a few patients are reported, with a high level of allelic heterogeneity. In Japan 4 mutations have been reported in 5 unrelated patients [16]; in Italy, 5 mutations in 5 patients [25,12,24]; in Turkey 4 mutations in 4 patients [26,28,21,12]. In the United Kingdom, 3 mutations in 3 patients, though one of them was of German origin (121C>T) [26,12,15]; in the Czech Republic, 3 mutations in 3 patients [11]; and in Germany, 2 mutations in 2 patients [12,15]. The French group, the Acadians (descendents of the 17th-century French colonists), the Cajuns (Acadians settled in Louisiana) and the French-Canadians, despite their common origin, present broad allelic heterogeneity: 5 mutations in 5 patients [14,10,20,12].

Two mutations are more common than the rest: one is the c.122G>A (81 alleles, 43 patients: 38 homozygous, 5 heterozygous with an allele unknown), prevalent in Saudi Arabia, where 40 patients carry it [12,13]. It has also been found in a patient in Italy, another in Turkey [12] and one in the Czech Republic, suggesting that this mutation may have arisen independently more than once [11]. The other common mutation is the c.109G>T (Mediterranean mutation) (33 alleles, 18 patients: 15 homozygous, 2 double heterozygous, 1 heterozygous with an allele unknown), found mostly in the Iberian Peninsula (12 patients in Portugal and 4 in Spain). It has also been described one case in Morocco and another in Turkey [21,22,18,23]. It has been hypothesized that in these countries the genetic hit was introduced during the Arabian invasions of the Iberian Peninsula in the eighth century. Genetic population studies using mtDNA haplotyping have already identified Arabian colonization in several regions of Portugal [18]. This mutation may have been transferred by the Magrebian invasion. Further studies would be necessary to elucidate whether the mutation originated in the Iberian Peninsula itself or the Magreb.

The high frequency of patients with HL deficiency in countries like Arabia Saudi, Portugal, and, in to a lesser extent, Spain, may justify a screening for the more common mutations (c.122G>A and c.109G>T) [13,81,18]. At a very long distance from this mutations there is the c.913-915delTT mutation, found in 3 patients of Saudi origin [12,13] and the c.698A>G mutation, found in 2 Czech patients and 1 French patient [20,11]. Another 5 mutations have been found, each of them in 2 patients and the great majority, 20 mutations, are found in one or two alleles of single patients of different origins. It is nevertheless surprising that a large number of patients are homozygous for any mutation, with no apparent consanguinity in their parents.

Two silent allelic variants (polymorphisms) have also been detected. One is c.727A>G, which produces the change of Thr^{243} to Ala^{243}. Interestingly, the mutated alanine coincides with the alanine conserved in chicken and *P. mevalonii* HL. This finding suggested that assignation of Thr in the HL cDNA sequence is a potential error. The other polymorphism is c.654A>G (L218L), which encodes the same amino acid, leucine [21].

Functional and structural analysis of the variant alleles

Missense mutations

One of the main advantages of our having access to the 3D structure of human HL is that it enables us to study the effects of clinical mutations within the enzyme active center. Unfortunately, none of the human or bacterial structures obtained for HL [30,31] has been solved in presence of natural substrate or inhibitor. Nevertheless, such structures have been used to generate bioinformatic models for putative substrate location and for the implication of residues in the active center in the enzymatic mechanism of the protein.

Mutant R41Q [13,12,11]. Arg^{41} is located in the active center of the HL structure [30], contacting a hydroxyglutaric acid molecule (Fig. 3e). Arg^{41} is probably involved in stabilizing the enolyzed form of acetyl-CoA [45], as supported by its position in the human HL structure. The mutated residue, Gln^{41}, lacks the electrostatic properties to maintain the same function.

Mutant D42H [12]. Asp^{42} is a magnesium (Mg++) ion ligand in human HL crystal structure. As suggested by Fu *et al.*, [30], mutation of this residue would impair the lyase reaction. In addition, positioning of the large His side chain in the locus occupied by Asp^{42}, would displace the substrate. *K48N* [48]. It has recently been proposed that Lys^{48} participates in a secondary putative positioning of substrate on the surface of human HL, located less than 4 Å away from three CO groups: one belonging to the proposed pantothenic acid moiety of HMG-CoA, and two corresponding to the backbone CO of residues Asn^{46} and Gly^{264}. As the side chain of Asn^{46} is oriented towards the active center, less than 4 Å from the catalytic residue Asp^{42}, lack of enzymatic activity of *K48N* was explained in terms of mis-positioning of the side chains of surrounding residues and weakening of substrate binding. Lys^{48} is almost completely conserved in all vertebrates (Fig. 2) as well as in plants and some bacteria, being substituted by other residues in insects and a few bacteria, such as *Bacillus subtilis*.

Mutant V70L [14]. Despite the crystallization of human HL, as well as BsHL and BmHL, there is no satisfactory explanation for the lack of activity in mutant Val^{70} to Leu. Val^{70}, a completely conserved residue in all organisms (Fig. 2), is located on the NH-side of HL structure (Fig. 3c), close to a central small hollow symmetric in

position to the large cavity located in the COOH-face of the barrel. Mutation to Leu would probably close this minor cavity, as the Leu side chain is somewhat longer than Val, but the implications of such an effect in lyase activity cannot solely be revealed by the crystal structural model.

Mutant S75R [15]. Ser^{75} is located in the central cavity of HL, bound to a water molecule (2.91 Å), which in turn forms a hydrogen bond with the co-crystallized 3-hydroxyglutaric acid, suggesting an important role for this residue in catalysis. Mutation to Arg, in addition to the modification of hydrogen-bonding to water, would probably disrupt the position of the nearby Arg^{41}, due to the larger size of the new Arg^{75} and to their respectively repulsive charges.

Mutant S201Y [15]. The gamma oxygen of Ser^{201} is positioned 3.34 Å from the beta-carbon of His^{233}, leaving no room for the large phenolic side chain of mutated Tyr, thus disturbing the active center of the enzyme through the displacement of the ion-ligating His^{233}.

G203E [17]. The alpha carbon of Gly^{203} is located 3.98 Å from the same beta carbon of His^{233}, as in the case of Ser^{201}. Similarly, substitution of the tiny Gly residue by a large, negatively charged Glu, perturbs the correct positioning of His^{233}, which also affects the whole charge in the catalytic cavity.

Mutant D204N [15,18]. Crystallized structures do not give clear a explanation for the decreased activity of the mutant D204N, as both residues could form the same hydrogen bonds with the surrounding aminoacids, maintaining the structure of β6-α8 loop, as indicated by [30].

MutantH233R [11,19,20]. His^{233}, like Asp^{42}, is an ion-ligand residue. Mutation to Arg would largely impair the lyase activity by altering the overall structure of the active center.

Mutan L263P [20]. Leu^{263} is located in the G-loop containing Cys^{266} (1). These two residues are believed to be involved in the multimerization that forms the catalytic basis of nucelophilic attack. Due to the characteristic steric properties of proline, the mutated residue could alter the flexibility of the loop.

Mutant E279K [16]. This mutant is probably a folding mutant [30], as Glu^{279} is not part of the (βα)8 barrel, and seems to be involved in the contact to the main chain of Val^{260}. The presence of Lys in the same position would impair the correct folding of local structures, resulting in protein instability [83].

Splicing mutations

Five variant alleles causing aberrant splicing have been described: two intron mutations (IVS3+1Gdel, IVS8+1G>T), one nonsense mutation (c.109G>A), one missense mutation (c.434A>T), and a frameshift deletion (c.504–505delCT). All of these produce seven new alternative mRNA transcripts, six with exon skipping : 2, 2–3, 3, 3′ half exon 5–6, 6 and 8, and one with insertion of the first 78 bp of intron 8 [28,21,27,22,16]. The association of the nonsense mutation c.109G>T (Mediterranean mutation) with the skipping of the constitutive exon 2 that contains it, has been attributed to the change caused by the nonsense mutation in the exon structure [21,82]. Moreover, other HL nonsense mutations, like c.286C>T affecting exon 4, did not originate alternatively spliced products [24]. It has been suggested that the Mediterranean mutation disrupts a positive exon splicing enhancer (ESE) in a purine-rich sequence (GAAGAAG) at the middle of exon 2, originating aberrant splicing with three transcripts: one of the expected size, with a premature stop codon 37, that generates a truncated mature protein with only nine residues; another with the skipping of exon 2; and a third with the skipping of exons 2 and 3 [21]. This suggests that, in some cases, a nonsense mutation could also hinder the splice site selection of the next exon downstream [22]. The deletion of exon 2 produces the loss of β-sheet 1, which contains the critical residue Asp^{42}. The skipping of exon 3 also caused the disappearance of α helix 1 and β-sheet 2, both of which contain Glu^{72}. These aminoacids are involved in the binding to the activator divalent cation, which explains the lack of activity of these mutants [45,83,23].

The frameshift deletion c.504-5delCT increased the constitutive transcript (with the skipping of exons 5 and 6) but the resulting protein was inactive [16,27]. In addition, two transcripts, one with a deletion CT and the other with a deletion of exon 6, both produced a frameshift with a loss of protein that explained the lack of activity. It has been suggested that the CT deletion may affect the interaction between the small nuclear ribonucleoproteins (snRNPs) and exon 6 [27].

The intron mutation IVS8+1G>T allowed us to characterize the size and limits of exon 8 and the first 78 bp of intron 8 before establishing the overall genomic organization of HL gene [28]. This mutation also produced two alternative splicings: one which led to the skipping of the whole of exon 8 (the catalytic site was completely removed), and other which led the activation of one cryptic donor splice site in the same intron, causing a 17 missense amino acid stray that preceded a stop codon. The proportion of the inserted mRNA with respect to the deleted mRNA was only 1.2%, as determined by quantitative RT-PCR [28].

Genotype–phenotype correlations

Clinical symptoms of affected patients are variable. The genotype–phenotype correlation is difficult to establish because the progress of the disease seems to be more related to the causes of hypoglycaemia (fasting or acute illness) than to a specific genotype. For example, the effects in patients carrying the same mutation, for instance the so-called Mediterranean mutation (c.109G>T), may range from moderate symptoms to severe crises with lethal consequences. This is why in clinical practice it is fundamental to avoid situations that may cause hypoglycemia in these patients [21,22,18,23].

Acknowledgements

This study was supported by *the Ayuda a los Grupos Consolidados de Investigación de Aragón (B20) from the Diputación General de Aragón*, Spain; by Grant SAF2004-06843-C03 from the *Ministerio de Educación y Ciencia*, Spain; by Grant C3/08 from the *Fondo de Investigación Sanitaria of the Instituto de Salud Carlos III,* and by the *Ajut de Suport als Grups de Recerca de Catalunya* (2005SGR-00733), Spain. CBM-SO receives financial support from Fundación Ramón Areces. We also thank to "Biomol-Informatics SL –www.bioinfo.es–" for bioinformatic support.

References

[1] P.A. Bolhuis, G.C. Page-Christiaens, The advisory report 'Neonatal screening' from the Health Council of The Netherlands, Ned. Tijdschr. Geneeskd. 149 (2005) 2857–2860.
[2] M.A. Kayer, Disorders of ketone production and utilization, Mol. Genet. Metab. 87 (2006) 281–283.
[3] F.G. Hegardt, Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: a control enzyme in ketogenesis, Biochem. J. 338 (1999) 569–582.
[4] A.A. Morris, Cerebral ketone body metabolism, J. Inherit. Metab. Dis. 28 (2005) 109–121.
[5] F. Bischof, T. Nagele, R.J. Wanders, F.K. Trefz, A. Melms, 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency in an adult with leukoencephalopathy, Ann. Neurol. 56 (2004) 727–730.
[6] M.S. van der Knaap, H.D. Bakker, J. Valk, MR imaging and proton spectroscopy in 3-hydroxy-3-methylglutaryl coenzyme A lyase deficiency, Am. J. Neuroradiol. 19 (1998) 378–382.
[7] C. Yalcinkaya, A. Dincer, E. Gunduz, C. Ficicioglu, N. Kocer, A.C. Aydin, MRI and MRS in HMG-CoA lyase deficiency, Pediatr. Neurol. 20 (1999) 375–380.
[8] K.M. Gibson, S.B. Cassidy, L.H. Seaver, R.J.A. Wanders, N.G. Kennaway, G.A. Mitchell, R.P. Spark, Fatal cardiomyopathy associated with Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency, J. Inher. Metab. Dis. 17 (1994) 291–294.
[9] R.J. Pollitt, Disorders of mitochondrial long-chain fatty acid oxidation, J. Inherit. Metab. Dis. 18 (1995) 473–490.
[10] G.A. Mitchell, M.F. Robert, P.W. Hruz, S. Wang, G. Fontaine, C.E. Behnke, L.M. Mende-Mueller, K. Schappert, C. Lee, K.M. Gibson, Cloning of human and chicken liver HL cDNAs and characterization of a mutation causing human HL deficiency, J. Biol. Chem. 268 (1993) 4376–4381.
[11] E. Pospisilova, L. Mrazova, J. Hrda, O. Martincova, J. Zeman, Biochemical and molecular analyses in three patients with 3-hydroxy-3-methylglutaric aciduria, J. Inherit. Metab. Dis. 26 (2003) 433–441.
[12] G.A. Mitchell, P.T. Ozand, M.F. Robert, L. Ashmarina, J. Roberts, K.M. Gibson, R.J. Wanders, S. Wang, I. Chevalier, E. Plochl, H. Miziorko, HMG CoA lyase deficiency: Identification of five causal point mutations in codons 41 and 42, including a frequent Saudi Arabian Mutation, R41Q, Am. J. Hum. Genet. 62 (1998) 295–300.
[13] M. Al-Sayed, F. Imtiaz, O.A. Alsmadi, M.S. Rashed, B.F. Meyer, Mutations underlying 3-hydroxy-3-methylglutaryl CoA lyase deficiency in the Saudi population, BMC Med. Genet. 16 (2006) 86.
[14] G.A. Mitchell, M.F. Robert, G. Fontaine, S. Wang, M. Lambert, D. Cole, C. Lee, M. Gibson, M.H. Miziorko, HMG CoA lyase (HL) deficiency: detection of a causal mutation in an affected French-Canadian sibship, Am. J. Hum. Genet. 51 (suppl.) (1992) A173.
[15] N. Casals, P. Gomez-Puertas, J. Pie, C. Mir, R. Roca, B. Puisac, R. Aledo, J. Clotet, S. Menao, D. Serra, G. Asins, J. Till, A.C. Elias-Jones, J.C. Cresto, N.A. Chamoles, J.E. Abdenur, E. Mayatepek, G. Besley, A. Valencia, F.G. Hegardt, Structural $(\beta\alpha)_8$ TIM Barrel Model of 3-hydroxy-3-methylglutaryl Coenzyme A Lyase, J. Biol. Chem. 278 (2003) 29016–29023.
[16] J. Muroi, T. Yorifuji, A. Uematsu, T. Nakahata, Molecular and clinical analysis of Japanese patients with 3-hydroxy-3-methylglutaryl-CoA lyase (HL) deficiency, J. Inherit. Metab. Dis. 23 (2000) 636–637.
[17] C. Mir, E. Lopez-Vinas, R. Aledo, B. Puisac, C. Rizzo, C. Dionisi-Vici, F. Deodato, J. Pie, P. Gomez-Puertas, F.G. Hegardt, N. Casals, A single-residue mutation, G203E, causes 3-hydroxy-3-methylglutaric aciduria by occluding the substrate channel in the 3D structural model of HMG-CoA lyase, J. Inherit. Metab. Dis. 29 (2006) 64–70.
[18] M.L. Cardoso, M.R. Rodrigues, E. Leao, E. Martins, L. Diogo, E. Rodrigues, P. Garcia, M.O. Rolland, L. Vilarinho, The E37X is a common HMGCL mutation in Portuguese patients with 3-hydroxy-3-methylglutaric CoA lyase deficiency, Mol. Genet. Metab. 82 (2004) 334–338.
[19] J.R. Roberts, G.A. Mitchell, H.M. Miziorko, Modeling of a mutation responsible for human 3-hydroxy-3-methylglutaryl coenzyme A lyase deficiency implicates histidine 233 as an active site residue, J. Biol. Chem. 271 (1996) 24604–24609.
[20] N. Zapater, J. Pie, J. Lloberas, M.O. Rolland, B. Leroux, M. Vidailhet, P. Divry, F.G. Hegardt, N. Casals, Two missense point mutations in different alleles in the 3-hydroxy-3-methylglutaryl coenzyme A lyase gene produce 3-hydroxy-3-methylglutaric aciduria in a French patient, Arch. Biochem. Biophys. 358 (1998) 197–203.
[21] J. Pie, N. Casals, C.H. Casale, C. Buesa, C. Mascaro, A. Barcelo, M.O. Rolland, T. Zabot, D. Haro, F. Eyskens, P. Divry, F.G. Hegardt, A nonsense mutation in the 3-hydroxy-3-methylglutaryl-CoA lyase gene produces exon skipping in two patients of different origin with 3-hydroxy-3-methylglutaryl-CoA lyase deficiency, Biochem. J. 323 (Pt 2) (1997) 329–335.
[22] C.H. Casale, N. Casals, J. Pie, N. Zapater, C. Perez-Cerda, B. Merinero, M. Martinez-Pardo, J.J. Garcia-Penas, J.M. Garcia-Gonzalez, R. Lama, B.T. Poll-The, J.A. Smeitink, R.J. Wanders, M. Ugarte, F.G. Hegardt, A nonsense mutation in the exon 2 of the 3-hydroxy-3-methylglutaryl coenzyme A lyase (HL) gene producing three mature mRNAs is the main cause of 3-hydroxy-3-methylglutaric aciduria in European Mediterranean patients, Arch. Biochem. Biophys. 349 (1998) 129–137.
[23] B. Puisac, E. Lopez-Vinas, S. Moreno, C. Mir, C. Perez-Cerda, S. Menao, D. Lluch, A. Pie, P. Gomez-Puertas, N. Casals, M. Ugarte, F. Hegardt, J. Pie, Skipping of exon 2 and exons 2 plus 3 of HMG-CoA lyase (HL) gene produces the loss of beta sheets 1 and 2 in the recently proposed (beta-alpha)8 TIM barrel model of HL, Biophys. Chem. 115 (2005) 241–245.
[24] S. Funghini, E. Pasquini, M. Cappellini, M.A. Donati, A. Morrone, C. Fonda, E. Zammarchi, 3-Hydroxy-3-methylglutaric aciduria in an Italian patient is caused by a new nonsense mutation in the HMGCL gene, Mol. Genet. Metab. 73 (2001) 268–275.
[25] G.A. Mitchell, C. Jakobs, K.M. Gibson, M.F. Robert, A. Burlina, C. Dionisi-Vici, L. Dallaire, Molecular prenatal diagnosis of 3-Hydroxy-3-methylglutaryl-coenzime A lyase deficiency, Prenat. Diagn. 15 (1995) 725–729.
[26] S.P. Wang, M.F. Robert, K.M. Gibson, R.J. Wanders, G.A. Mitchell, 3-Hydroxy-3-methylglutaryl CoA lyase (HL): mouse and human HL gene (HMGCL) cloning and detection of large gene deletion in two unrelated HL-deficient patients, Genomics 33 (1996) 99–104.
[27] N. Casals, J. Pie, C.H. Casale, N. Zapater, A. Ribes, M. Castro-Gago, S. Rodriguez-Segade, R.J. Wanders, F.G. Hegardt, A two-base deletion in exon 6 of the 3-hydroxy-3-methylglutaryl coenzyme A lyase (HL) gene producing the skipping of exons 5 and 6 determines 3-hydroxy-3-methylglutaric aciduria, J. Lipid Res. 38 (1997) 2303–2313.
[28] C. Buesa, J. Pie, A. Barcelo, N. Casals, C. Mascaro, C.H. Casale, D. Haro, M. Duran, J.A. Smeitink, F.G. Hegardt, Aberrantly spliced mRNAs of the 3-hydroxy-3-methylglutaryl coenzyme A lyase (HL) gene with a donor splice-site point mutation produce hereditary HL deficiency, J. Lipid Res. 37 (1996) 2420–2432.

[29] S.E. Antonarakis, the Nomenclature Working Group, Recommendations for a Nomenclature System for Human Gene Mutations, Hum. Mutat. 11 (1998) 1–3.
[30] Z. Fu, J.A. Runquist, F. Forouhar, M. Hussain, J.F. Hunt, H.M. Miziorko, J.J. Kim, Crystal structure of human 3-hydroxy-3-methylglutaryl-CoA Lyase: insights into catalysis and the molecular basis for hydroxymethylglutaric aciduria, J. Biol. Chem. 281 (2006) 7526–7532.
[31] F. Forouhar, M. Hussain, R. Farid, J. Benach, M. Abashidze, W.C. Edstrom, S.M. Vorobiev, R. Xiao, T.B. Acton, Z. Fu, J.J. Kim, H.M. Miziorko, G.T. Montelione, J.F. Hunt, Crystal structures of two bacterial 3-hydroxy-3-methylglutaryl-CoA lyases suggest a common catalytic mechanism among a family of TIM barrel metalloenzymes cleaving carbon-carbon bonds, J. Biol. Chem. 281 (2006) 7533–7545.
[32] B.K. Bachhawat, W.G. Robinson, M.J. Coon, The enzymatic cleavage of beta-hydroxy-beta-methylglutaryl coenzyme A to acetoacetate and acetyl coenzyme A, J. Biol. Chem. 216 (1955) 727–736.
[33] L.D. Stegink, M.J. Coon, Stereospecificity and other properties of highly purified beta-hydroxy-beta-methylglutaryl coenzyme A cleavage enzyme from bovine liver, J. Biol. Chem. 243 (1968) 5272–5279.
[34] P.R. Kramer, H.M. Miziorko, Purification and characterization of avian liver 3-hydroxy-3-methylglutaryl coenzyme A lyase, J. Biol. Chem. 255 (1980) 11023–11028.
[35] C. Narasimhan, H.M. Miziorko, *Pseudomonas mevalonii* 3-hydroxy-3-methylglutaryl-CoA lyase: characterization of the isolated recombinant protein and investigation of the exnzyme's cation requirements, Biochemistry 31 (1992) 11224–11230.
[36] J.R. Roberts, C. Narasimhan, P.W. Hruz, G.A. Mitchell, H.M. Miziorko, 3-Hydroxy-3-methylglutaryl coenzyme A lyase: Expression and isolation of the recombinant human enzyme and investigation of a mechanism for regulation of enzyme activity, J. Biol. Chem. 269 (1994) 17841–17846.
[37] P.W. Hruz, V.E. Anderson, H.M. Miziorko, 3-Hydroxy-3-methylglutaryldithio-CoA: utility of an alternative substrate in elucidation of a role for HMG-CoA lyase's cation activator, Biochim. Biophys. Acta 1162 (1993) 149–154.
[38] L.I. Ashmarina, N. Rusnak, H.M. Miziorko, G.A. Mitchell, 3-Hydroxy-3-methylglutaryl-coenzyme A lyase is present in mouse and human liver peroxisomes, J. Biol. Chem. 269 (1994) 31929–31932.
[39] S.K. Krisans, Cell compartmentalization of cholesterol biosynthesis, Ann. N.Y. Acad. Sci. 804 (1996) 142–164.
[40] L.I. Ashmarina, M.F. Robert, M.A. Elsliger, G.A. Mitchell, Characterization of the hydroxymethylglutaryl-CoA lyase precursor, a protein targeted to peroxisomes and mitochondria, Biochem. J. 315 (1996) 71–75.
[41] R.L. Tuinstra, J.W. Burgner, H.M. Miziorko, Investigation of the oligomeric status of the peroxisomal isoform of human 3-hydroxy-3-methylglutaryl-CoA lyase, Arch. Biochem. Biophys. 408 (2002) 286–294.
[42] L.I. Ashmarina, A.V. Pshezhetsky, S.S. Branda, G. Isaya, G.A. Mitchell, 3-Hydroxy-3-methylglutaryl coenzyme A lyase: targeting and processing in peroxisomes and mitochondria, J. Lipid. Res. 40 (1999) 70–75.
[43] D. Lang, R. Thoma, M. Henn-Sax, R. Sterner, M. Wilmanns, Structural evidence for evolution of the beta/alpha barrel scaffold by gene duplication and fusion, Science 289 (2000) 1546–1550.
[44] R.R. Copley, P. Bork, Homology among (betaalpha)(8) barrels: implications for the evolution of metabolic pathways, J. Mol. Biol. 303 (2000) 627–641.
[45] R.L. Tuinstra, C.Z. Wang, G.A. Mitchell, H.M. Miziorko, Evaluation of 3-hydroxy-3-methylglutaryl-coenzyme A lyase arginine-41 as a catalytic residue: use of acetyldithio-coenzyme A to monitor product enolization, Biochemistry 43 (2004) 5287–5295.
[46] B.A. Manjasetty, J. Powlowski, A. Vrielink, Crystal structure of a bifunctional aldolase-dehydrogenase: sequestering a reactive and volatile intermediate, Proc. Natl. Acad. Sci. USA 100 (2003) 6992–6997.
[47] J.R. Roberts, C. Narasimhan, H.M. Miziorko, Evaluation of cysteine 266 of human 3-hydroxy-3-methylglutaryl coenzyme A lyase as a catalytic residue, J. Biol. Chem. 270 (1995) 17311–17316.
[48] P. Carrasco, S. Menao, E. López-Viñas, G. Santpere, J. Clotet, A.Y. Sierra, E. Gratacos, B. Puisac, P. Gómez-Puertas, F.G. Hegardt, J. Pié, N. Casals, C-Terminal end and aminoacid Lys48 in HMG-CoA Lyase are involved in substrate binding and enzyme activity, Mol. Genet. Metab. 91 (2007) 120–127.
[49] W.G. Wilson, M.B. Cass, O. Sovik, K.M. Gibson, L. Sweetman, A child with acute pancreatitis and recurrent hypoglycemia due to 3-hydroxy-3-methylglutaryl-CoA lyase deficiency, Eur. J. Pediatr. 142 (1984) 289–291.
[50] K. Faull, P. Bolton, B. Halpern, J. Hammond, D.M. Danks, R. Hahnel, S.P. Wilkinson, S.J. Wysocki, P.L. Masters, Letter: Patient with defect in leucine metabolism, New Eng. J. Med. 294 (1976) 1013.
[51] S.J. Wysocki, R. Hähnel, 3-Hydroxy-3-methylglutaric aciduria: 3-Hydroxy-3-methylglutaryl-CoA lyase levels in leukocytes, Clin. Chim. Acta 73 (1976) 373–375.
[52] S.P. Wang, J.D. Marth, L.L. Oligny, M. Vachon, M.F. Robert, L. Ashmarina, G.A. Mitchell, 3-Hydroxy-3-methylglutaryl-CoA lyase (HL): gene targeting causes prenatal lethality in HL-deficient mice, Hum. Mol. Genet. 13 (1998) 2057–2062.
[53] L. Sweetman, J.C. William, in: C.R. Schreiver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Basis of Inherited Disease, vol. I, seventh ed., Mc Graw-Hill Inc., New York, 1995, pp. 1400–1402.
[54] R.B.H. Schutgens, H. Heymans, A. Ketel, H.A. Veder, M. Duran, D. Ketting, S.K. Wadman, Lethal hypoglycemia in a child with a deficiency of 3-hydroxy-3-methylglutaryl coenzyme A lyase, J. Pediatr. 94 (1979) 89–91.
[55] S.J. Wysocki, R. Hähnel, 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency: A review, J. Inher. Metab. Dis. 9 (1986) 225–233.
[56] K.M. Gibson, J. Breuer, W.L. Nyhan, 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency, Review of 18 reported patients, Eur. J. Pediatr. 148 (1988) 180–186.
[57] K.M. Gibson, J. Breuer, K. Kaiser, W.L. Nyhan, E.E. McCoy, P. Ferreira, C.L. Greene, M.G. Blitzer, E. Shapira, F. Reverte, C. Conde, P. Bagnell, D.E.C. Cole, 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: Report of five new patients, J. Inher. Metab. Dis. 11 (1988) 76–87.
[58] T.E. Stacey, C. De Sousa, B.M. Tracey, A. Whitelaw, J. Mistry, P. Timbrell, R.A. Chalmers, Dizygotic twins with 3-hydroxy-3-methylglutaric aciduria; unusual presentation, family studies and dietary management, Eur. J. Pediatr. 144 (1985) 177–181.
[59] N. Urganci, M. Arapoglu, M. Evruke, A. Aydin, A rare cause of hepatomegaly: 3-hydroxy-3-methylglutaryl coenzyme-a lyase deficiency, J. Pediatr. Gastroenterol. Nutr. 33 (2001) 339–341.
[60] G. Lisson, D. Leupold, D. Bechinger, C. Wallesch, CT findings in a case of deficiency of 3-hydroxy-3-methylglutaril-coenzyme A lyase, Neuroradiology 22 (1981) 99–101.
[61] J.H. Walter, P.T. Clayton, J.V. Leonard, 3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) lyase deficiency, J. Inher. Metab. Dis. 9 (1986) 287–288.
[62] Y. Yylmaz, N. Ozdemir, G. Ekinci, T. Baykal, C. Kocaman, Corticospinal tract involvement in a patient with 3-HMG coenzyme A lyase deficiency, Pediatr. Neurol. 35 (2006) 139–141.
[63] M. Jones, B. Wilcken, H. Kilham, The long-term evolution of a case of 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency associated with deafness and retinitis pigmentosa, J. Inherit. Metab. Dis. 20 (1997) 833–834.
[64] M. Al-Essa, M. Rashed, P.T. Ozand, 3-Hydroxy-3-methylglutaryl-CoA lyase deficiency in a boy with VATER association, J. Inherit. Metab. Dis. 21 (1998) 443–444.
[65] G.N. Thompson, R.A. Chalmers, D. Halliday, The contribution of protein catabolism to metabolic decompensation in 3-Hydroxy-3-methylglutaric aciduria, Eur. J. Pediatr. 149 (1990) 346–350.
[66] K.F. Faull, P.D. Bolton, B. Halpern, J. Hammond, D.M. Danks, The urinary organic acid profile associated with 3-Hydroxy-3-methylglutaric aciduria, Clin. Chim. Acta 73 (1976) 553–559.

[67] S.J. Wysocki, S.P. Wilkinson, R. Hähnel, C.Y.B. Wong, P.K. Panegyres, 3-Hydroxy-3-methylglutaric aciduria, combined with 3-methylglutaconic aciduria, Clin. Chim. Acta 70 (1976) 399–406.
[68] S.J. Wysocki, R. Hähnel, Methylcrotonylglycine excretion in 3-Hydroxy-3-methylglutaric aciduria, Clin. Chim. Acta 86 (1978) 101–108.
[69] M. Duran, D. Ketting, S.K. Wadman, C. Jakobs, R.B.D. Schutgens, H.A. Veder, Organic acid excretion in a patient with 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency, Clin. Chim. Acta 90 (1978) 187–193.
[70] C. Jakobs, M. Bojasch, M. Duran, D. Ketting, S.K. Wadman, D. Leupold, 3-Methyl-3-buteneoic acid: An artifact in the urinary metabolic pattern of patients with 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency, Clin. Chim. Acta 106 (1980) 85–89.
[71] P. Divry, M.O. Rolland, J. Teyssier, J. Cotte, M.C. Formosinho Fernandes, I. Tavares de Almeida, C. Da Siveira, 3-Hydroxy-3-methylglutaric aciduria combined with 3-methylglutaconic aciduria: A new case, J. Inher. Metab. Dis. 4 (1981) 173–174.
[72] R.A. Chalmers, B.M. Tracey, J. Mistry, T.E. Stacey, I.R. Mc Fadyen, Prenatal diagnosis of 3-Hydroxy-3-methylglutaric aciduria by GC–MS and enzymology on cultured amniocytes and chorionic villi, J. Inher. Metab. Dis. 12 (1989) 286–292.
[73] M. Duran, R.B.H. Schutgens, A. Ketel, H. Heymans, M.W.J. Bernstssen, D. Ketting, S.K. Wadman, 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: Postnatal management following prenatal diagnosis by analysis of maternal urine, J. Pediatr. 95 (1979) 1004–1007.
[74] S.J. Wysocki, R. Hähnel, 3-Hydroxy-3-methylglutaric aciduria: deficiency of 3-hydroxy-3-methylglutaryl-CoA lyase, Clin. Chim. Acta 71 (1976) 349–351.
[75] A.L. Robinson, D.H. Williamson, Physiological roles of ketone bodies as substrates and signals in mammalian tissues, Physiol. Rev. 60 (1980) 143–187.
[76] R.J. Wanders, P.H. Zoeters, R.B. Schutgens, J.B. de Klerk, M. Duran, S.K. Wadman, F.J. van Sprang, A.M. Hemmes, B.S. Voorbrood, Rapid diagnosis of 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency via enzyme activity measurements in leukocytes or platelets using a simple spectrophotometric method, Clin. Chim. Acta 189 (1990) 327–334.
[77] M. Dasouki, D. Buchanan, N. Mercer, K.M. Gibson, J. Thoene, 3-Hydroxy-3-methylglutaric aciduria: response to carnitine therapy and fat and leucine restriction, J. Inherit. Metab. Dis. 10 (1987) 142–146.
[78] S. Wang, J.H. Nadeau, A. Duncan, M.F. Robert, G. Fontaine, K. Schappert, K.R. Johnson, E. Zietkiewicz, P. Hruz, H. Miziorko, 3-Hydroxy-3-methylglutaryl coenzyme A lyase (HL): cloning characterization of a mouse liver HL cDNA and subchromosomal mapping of the human and mouse HL gene, Mamm. Genome. 4 (1993) 382–387.
[79] M. Baltscheffsky, M. Brosche, T. Hultman, L. Lundvik, P. Nyren, Y. Sakai-Nore, A. Severin, A. Strid, A 3-hydroxy-3-methylglutaryl–CoA lyase gene in the photosynthetic bacterium *Rhodospirillum rubrum*, Biochim. Biophys. Acta 1337 (1997) 113–122.
[80] D.H. Anderson, V.W. Rodwell, Nucleotide sequence and expression in Escherichia coli of the 3-hydroxy-3-methylglutaryl coenzyme A lyase gene of *Pseudomonas mevalonii*, J. Bacteriol. 171 (1989) 6468–6472.
[81] O.A. Alsmadi, F. Al-Kayal, M. Al-Hamed, B.F. Meyer, Frequency of common HFE variants in the Saudi population: a high throughput molecular beacon-based study, BMC Med. Genet. 3 (2006) 43.
[82] P. Belgrader, L.E. Maquat, Nonsense but not missense mutations can decrease the abundance of nuclear mRNA for the mouse major urinary protein, while both types of mutations can facilitate exon skipping, Mol. Cell. Biol. 14 (1994) 6326–6336.
[83] R.L. Tuinstra, H.M. Miziorko, Investigation of conserved acidic residues in 3-hydroxy-3-methylglutaryl-CoA lyase: implications for human disease and for functional roles in a family of related proteins, J. Biol. Chem. 278 (2003) 37092–37098.