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## HMG–CoA Lyase Deficiency

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### 1. Introduction

The HMG-CoA lyase (HL) deficiency or 3-hydroxy-3-methylglutaric aciduria (MIM 246450) is an inborn error of intermediary metabolism that was first described in 1976 by Faull et al (Faull et al., 1976). Because its clinical manifestations, it has been included within the Sudden Infant Death Syndrome (Wilson et al., 1984). At present, it is considered a rare disease (<1/100,000 live neonates) that should be diagnosed at early age because there is a simple and effective treatment (Watson et al., 2006).

HL is a mitochondrial enzyme that catalyzes the cleavage of HMG-CoA to acetyl-CoA and acetoacetate, which is the common final step of ketogenesis and leucine catabolism (Figure 1). Patients with this disease suffer on the one hand, the absence of ketone bodies as alternative energy source of glucose and on the other hand, the accumulation of toxic metabolites of leucine catabolism. The most frequently affected organs are the liver and the brain, but the pancreas and the heart can also be involved. This chapter discusses a recent study of differential expression of human HL in liver, pancreas, testis, heart, skeletal muscle and brain that can help us to understand the consequences of this deficiency (Puisac et al., 2010).

It is an autosomal recessive disease caused by mutations in the *HMGCL* gene. The study of these mutations and patients' origin helps to draw a map of incidence in which three countries stand out for their high frequency: Saudi Arabia (Ozand et al., 1992), Spain and Portugal (Menao et al., 2009).

At present, the functional study of missense mutations is possible thanks to the knowledge of the structure (Fu et al., 2006) and mechanism of the enzyme (Fu et al., 2010) and also by the development of a method of simple and efficient expression of the protein (Menao et al., 2009). Finally, despite the current knowledge of the disease, genotype-phenotype correlations are difficult to establish.

### 2. HL enzyme

HL is a 325-aminoacid enzyme that has been purified from a variety of organisms and tissues, including pig heart (Bachawat et al., 1955), chicken liver (Kramer et al., 1980) and *Pseudomonas mevalonii* (Scher et al., 1989). In addition to the isoform located in the

mitochondrial matrix, it has been described another in peroxisomes (Ashmarina et al., 1994). The native mitochondrial isoform contains a leader peptide of 27 aminoacids at the N-terminal end, which guides HL towards the mitochondrial matrix, where the leader peptide is removed. This final isoform has a molecular mass of 31.5 kDa and an isoelectric point of 6.2.

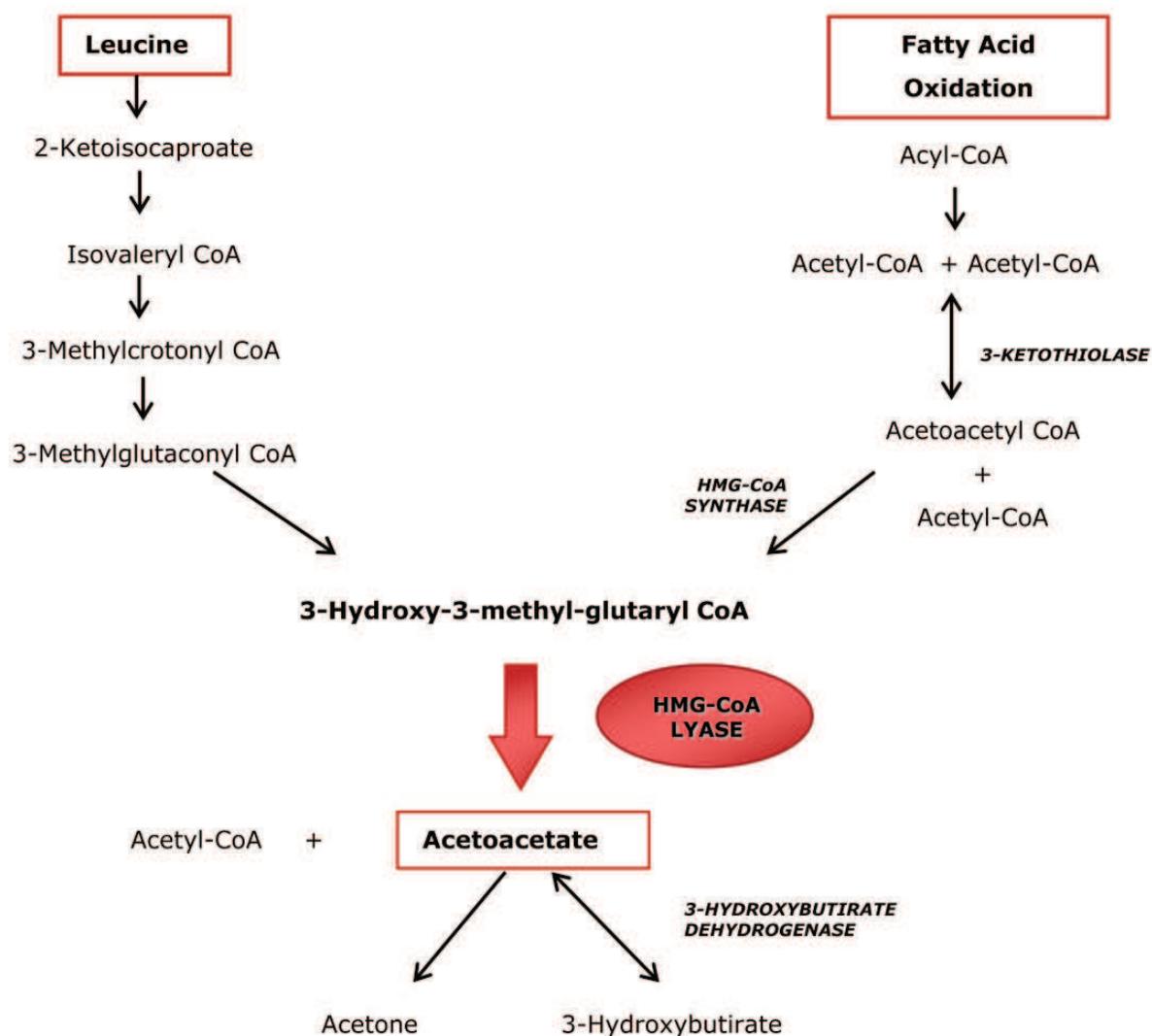


Fig. 1. Metabolic interrelationships of HL

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 (Pié et al., 2007). The catalytic active form is a homodimer of two identical monomers bound by a disulphide bridge (Roberts, 1994). The human enzyme is very sensitive to oxidation, showing higher activity in reductive conditions. It is also sensitive to the conditions of pH, showing an optimum activity at alkaline pH (pH=9). HL activity requires the presence of a divalent cation, such as  $Mg^{+2}$  or  $Mn^{+2}$ . The  $Mg^{+2}$  ion has an octahedral coordination with two water molecules, the imidazole nitrogens of catalytic residues His<sup>233</sup> and His<sup>235</sup>, the carboxylate group of Asp<sup>42</sup> and the amide oxygen of Asn<sup>275</sup>. Other catalytic residues in the vicinity include Arg<sup>41</sup> and Cys<sup>266</sup>.

## 2.1 Protein structure

The first attempt to build a 3D structural model of human 3-hydroxy-3-methylglutaryl-CoA lyase was based on a threading procedure using the crystallized structure of the TIM-barrel *hisA* gene from *Thermotoga maritima* as a template (Casals et al., 2003). The proposed model correspond to a  $(\alpha\beta)_8$  barrel with short loops on the  $\text{NH}_2$  terminal face and, in contrast, long and probably non-structured loops on the  $\text{COOH}$ -terminal face of the  $\beta$ -barrel. This model showed, for the first time, the structural proximity of the residues involved in the catalytic activity of the protein: Arg<sup>41</sup>, Asp<sup>42</sup>, Glu<sup>72</sup>, His<sup>233</sup> and His<sup>235</sup>, located near the cavity opened in the  $\text{COOH}$ -terminal face of the protein model (Figure 2).

This model was confirmed when the crystal structure of human HL was obtained (Fu et al., 2006). In addition to the basic TIM barrel structure, the monomer of human HL includes an additional polypeptide region made of residues 290-323 containing  $\beta$ -strand 9, and  $\alpha$ -helices 11 and 12. The active site is accessible only from the C-terminal side of the TIM barrel and the N-terminal barrel end is occluded. Crystal structures of the wild-type enzyme complex and inhibitor hydroxyglutaryl-CoA has demonstrated the interaction of Arg<sup>41</sup> and acyl-CoA's C1 carbonyl oxygen of substrate and explains why Arg<sup>41</sup> mutations cause drastic enzyme deficiency (Fu et al., 2010).

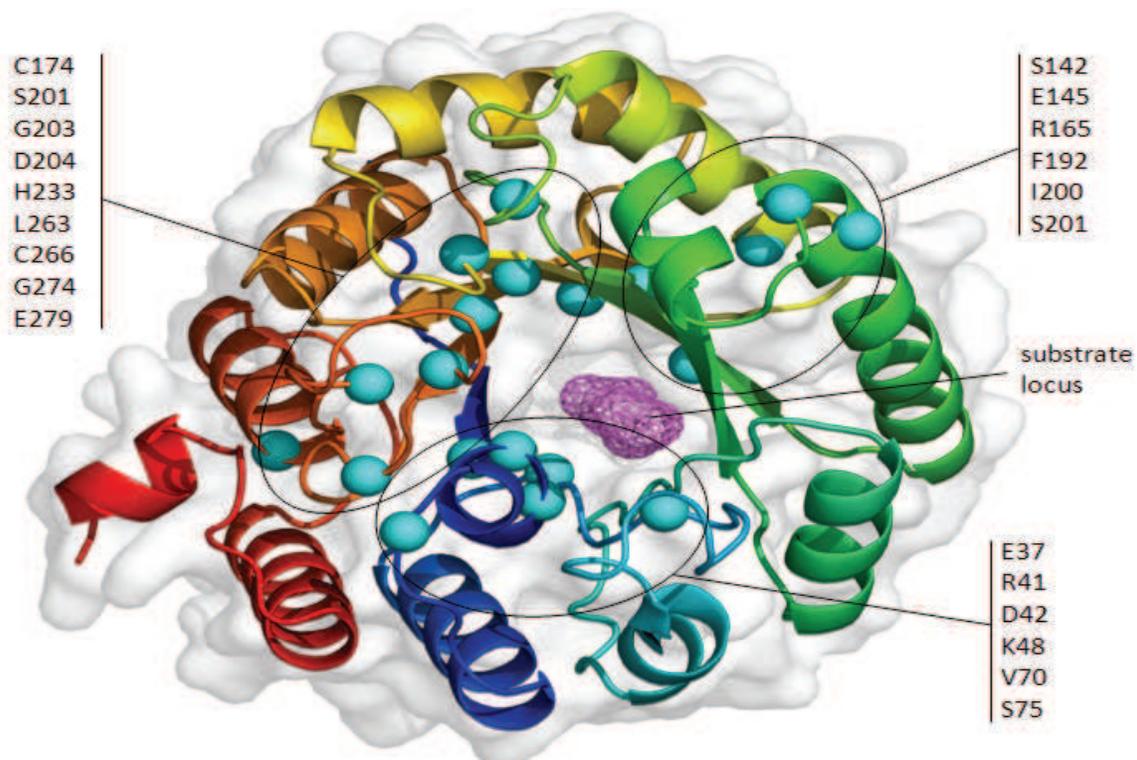


Fig. 2. Structural location of missense mutations in human HL. Blue spheres represent mutated residues

The native enzyme is a dimer in solution (Tuinstra et al., 2002) that was confirmed when the protein was crystallized. The area of contact between monomers is formed by additional secondary elements that are not part of the core TIM barrel structure,  $\beta$ -strand 9, and  $\alpha$ -helices 11 and 12. Recently it was suggested that multiple cysteine residues influence covalent adduct formation in HMG-CoA lyase as well as the dependence of enzyme activity on reducing agent (Montgomery & Mizioro, 2011).

## 2.2 Enzymatic reaction

The cleavage of HMG-CoA, catalyzed by the HL enzyme is the final step of ketogenesis, in which acetyl-CoA, mainly from the  $\beta$ -oxidation of fatty acids, is converted to acetoacetate,  $\beta$ -hydroxybutyrate and acetone (Figure 1). From a chemical point of view, the enzyme reaction is a retro-Claisen condensation, which requires an acid and a base for catalysis (Roberts et al., 1996). The base abstracts a proton from the C3 hydroxyl of HMG-CoA, which leads to the formation of a ketone (acetoacetate) and C2-C3 bond cleavage (Figure 3). In addition, a transient carbanion form of acetyl-CoA, is regenerated by the acid proton. However, the exact identity of molecules or residues that act as base or as acid was not precised. Recently a water molecule, positioned between D42 and the C3 hydroxyl of bound substrate has been proposed as a proton shuttle (Fu et al., 2010).

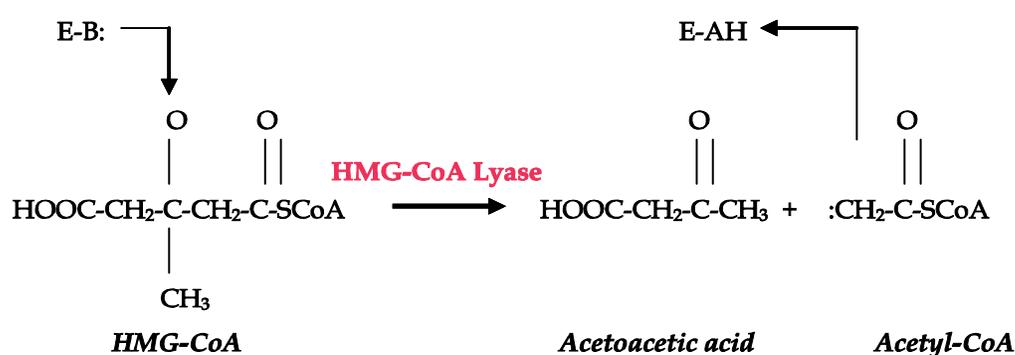


Fig. 3. Chemical reaction catalyzed by the enzyme HMG-CoA lyase. E-B: base, E-AH acid

## 2.3 Enzyme expression

HL is widely expressed in most tissues (Clikensbeard et al., 1975) mainly because it is necessary not only in tissues that synthesize ketone bodies, but for the catabolism of leucine as well. Activity levels of this enzyme have been reported in different eucaryotes organism tissues: pig heart (Bachawat et al., 1955), bovine liver (Stegink & Coon, 1968) and chicken liver, kidney, heart, brain, ileum and muscle (Clikensbeard et al., 1975). However, its distribution and activity in human tissues have been limited to enzyme assay in fibroblast (Wanders et al., 1988b) lymphoblast (Wysocki et al, 1976b) liver biopsy (Robinson et al,1980) amniocytes and chorionic villi (Wanders et al., 1988b) or pancreatic islets (MacDonald et al., 2007).

Recently, it has been reported the first study of mRNA levels, protein expression and enzyme activity of human HMG-CoA lyase in kidney, pancreas, testis, heart, skeletal muscle and brain (Puisac et al., 2010). The highest HL activity was found in liver and pancreas was the second with more activity (Figure 4c). This finding indicates that the pancreas has a high ketogenic capacity and suggests that if ketone bodies regulate the release of insulin (Biden & Taylor, 1983; Malaisse et al., 1990; Rhodes et al., 1985) some of them could be produced in the pancreas. HL activity in kidney was high and moderately high in testis and skeletal muscle. Surprisingly in muscle, although the mRNA levels were very low (Figure 4a), moderate HL activity was measured. Similar cases are reported in the literature (Lewin et al., 2001), which suggests that certain tissues may have a lower turnover of the HL protein versus an unstable mRNA. In testis, the low activity levels of HL compared with the high

enzyme expression (Figure 4b), suggest that HL activity could be regulated after translation in this tissue. However, very little HL is found in heart tissue and it is not present in the mitochondria from human brain.

## 2.4 Isoforms

Two different protein isoforms of HL have been found, which are codified by a single gene located in chromosome 1. While most HL is found in mitochondria, about 16-20% is located in peroxisomes (Ashmarina et al., 1999). To date, no satisfactory explanation has been found to explain this distribution. The protein found in peroxisomes is guided by the signal CKL tripeptide in the C-terminal end and it 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 (Ashmarina et al., 1994). As the mitochondrial isoform, it is a dimeric form and has lyase activity; however its role inside peroxisomes is still unknown. Probably, this is related to cholesterol synthesis or long chain fat acids degradation (Krisans et al., 1996).

## 3. HL deficiency

HMG-CoA lyase deficiency or 3-Hydroxy-3-methylglutaric aciduria (OMIM 246450) is a rare autosomal recessive genetic disorder that affects ketogenesis and L-leucine catabolism. For this reason, it is included within alterations of fatty acid metabolism and also within organic acidemias. This deficiency was first described by Faull et al in 1976 in a 7 month-old male with acidosis and hypoglycemia (Faull et al., 1976). Later, Wysocki et al showed that HL activity in the leukocytes of this patient was null (Wysocki et al., 1976). The gene knock-out in mice results in embryonic lethality (Wang et al., 1998) reflecting the physiological importance of this enzyme.

### 3.1 Clinical features

3-Hydroxy-3-methylglutaric aciduria is a severe condition in children, in fasting or in high glucose consumption, when ketone bodies are essential as alternative energy substrate. In approximately 30% of the cases the first symptoms appear between the second and fifth days of life or between 3 and 11 months. However, four patients with late onset (puberty and adult) have been reported (Sweetman et al., 1995; Vargas et al., 2007; Bischof et al., 2004; Reimao et al., 2009).

#### 3.1.1 Acute crisis

Acute crises tend to occur when there is no exogenous intake of glucose (starving cases) or when there is an excessive glucose metabolization (conditions of metabolic stress, febrile stress and exercise). Initial symptoms may include poor feeding, vomiting, diarrhea, followed by further complication as hypotonia, hypothermia, lethargy, cyanosis and apnea. (Schutgens et al., 1979; Gibson et al., 1988a; Gibson et al., 1988b). In some cases the progressive lowered state of consciousness leads to coma and subsequent death (Wysocki et al., 1986). Laboratory data that stand out are the metabolic acidosis and non-ketotic hypoglycemia (Table 1). Hypoglycemia can be explained by fasting or other intercurrent illness, while the hypoketonemia shows the inability of patients to synthesize ketone bodies. Metabolic acidosis and aciduria can be explained by the accumulation of acids metabolites from leucine catabolism: 3-hydroxy-isovaleric acid, 3-methylglutaconic acid, 3-methylglutaric acid and 3-hydroxy-3-methylglutaric acid. Occasionally, patients present with increased

bilirubin, liver transaminases and prothrombin time. It is also reported the appearance of hyperammonemia associated with increased proteolysis by deficiency of ketone bodies.

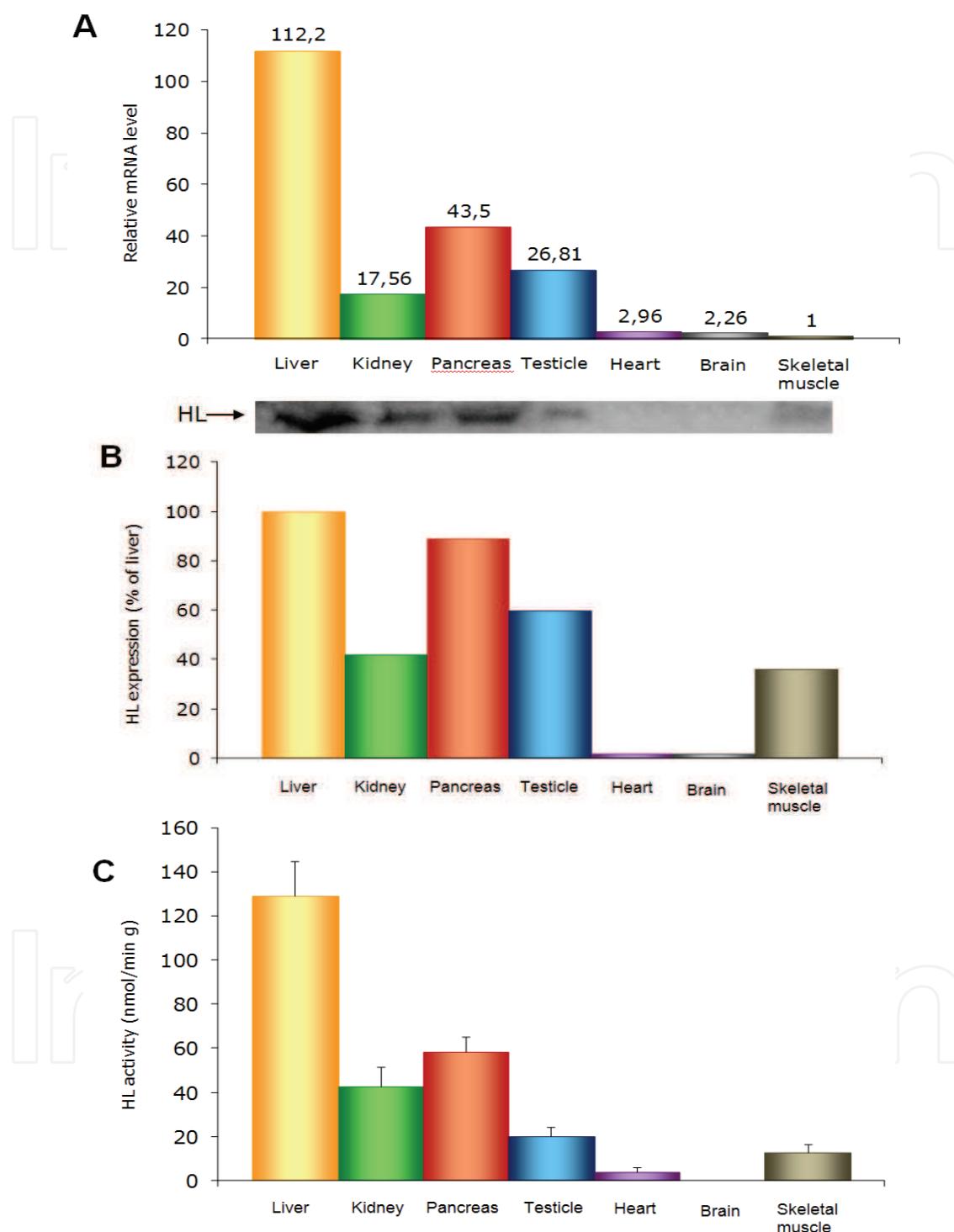


Fig. 4. Comparative analysis of mRNA levels, protein expression and enzymatic activity of HMG-CoA lyase in different human tissues. (A) Relative levels of mRNA HL expression in human tissues (B) HL protein expression measured in mitochondrial fraction from human tissues (C) HL activity was measured in the mitochondrial fraction of human tissues spectrophotometrically. Data are presented as mean  $\pm$ SEM

<b>Clinical manifestations in acute clinical episodes</b>	
Vomiting	Frequent all of them if the clinical picture worsens
Diarrhea	
Hipotonia	
Hypothermia	
Lethargy	
Apnea	
Coma	
<b>Laboratory test</b>	
<i>General biochemistry</i>	
Metabolic acidosis	Always present
Hypoglycemia	Always present
Hypoketonemia	Always present
Ketonuria	Absent
Hyperammonemia	Elevated frequently
Hepatic transaminases	Elevated frequently
Bilirubin	Elevated in some cases
Prothrombin time	Elevated in some cases
<i>Organic acids</i>	
3-hydroxy-3-methylglutaric	Elevated very frequently
3-methylglutaric	Elevated frequently
3-methylglutaconic	Elevated sometimes
3-hydroxyisovaleric	Elevated sometimes
<i>HL enzyme activity</i>	Less than 5%
<b>Affected organs</b>	
Brain	Macro o Microcephaly (infrequent) Alterations of the white matter (frequent) Epilepsy (infrequent) Cerebral infarction (infrequent)
Pancreas	Acute pancreatitis (infrequent)
Liver	Hepatomegaly (very frequent)
Heart	Dilated cardiomyopathy with arrhythmia (infrequent)

Table 1. Clinical and laboratory findings of the HMG-CoA lyase deficiency

### 3.1.2 Chronic complications

Chronic complications are uncommon but include: hepatomegalia, macrocephalia (Gibson et al., 1988b; Stacey et al., 1985) microcephalia (Lisson et al., 1981) and delayed development (Gibson et al., 1994). It has been reported that organs such as the brain, the liver and occasionally the pancreas and the heart are affected (Gibson et al., 1994; Leung et al., 2009; Muroi et al., 2000a; Urganici et al., 2001; Wilson et al., 1984; Zafeiriou et al., 2007; Zoghbi et

al., 1986). Recently, a study of mRNA levels, protein expression and enzyme activity of human HMG-CoA lyase in kidney, pancreas, testis, heart, skeletal muscle and brain has contributed to better understanding of the enzyme function and of the involvement of these organs in 3-hydroxy-3-methylglutaric aciduria (Puisac et al., 2010).

The liver is the organ most frequently affected in this deficiency, although involvement is usually mild, with elevated transaminases and hepatomegaly (Urganci et al., 2001; Wysocki & Hahnel, 1986). Ketogenesis is more active in the liver and the blockage of this pathway could result in an accumulation of toxic intermediate metabolites.

Pancreatitis is a potential complication in patients with organic acidemias, (Kahler et al., 1994) and some cases have been reported in 3-hydroxy-3-methylglutaric aciduria (Muroi et al., 2000a; Wilson et al., 1984). The finding of higher enzymatic activity in pancreas (Puisac et al., 2010) indicates that it may be more susceptible to toxic accumulation of metabolites. Among the brain abnormalities in these patients, cerebral white matter involvement is the most common reported finding (Lisson et al., 1981; Yalcinkaya et al., 1999; Zafeiriou et al., 2007) and also one case of prominent corticospinal tract and pontine involvement has also been reported (Yilmaz et al., 2006). HL is not found at different levels of mRNA, protein and enzymatic activity, in the mitochondria from human brain (Puisac et al., 2010). This suggests that the neurological alterations frequently associated with this deficiency, are related to hypoglycaemia and to the absence of the only alternative substrate to glucose for the brain, ketone bodies. Concomitantly, the organic acids would not be produced *in situ*, although they could cross the blood-brain barrier of an immature brain (Wajner et al., 2004) and cause damage.

Dilated cardiomyopathy has been described in two patients with 3-hydroxy-3-methylglutaric aciduria, one young male (Gibson et al., 1994) and one adult (Leung et al., 2009). In this last case, the authors suggest that the cardiomyopathy results from impaired ketogenesis, intracellular fatty acid accumulation and a secondary carnitine deficiency. However, very little HL was found in heart tissue (Puisac et al., 2010). This result does not support the hypothesis of local accumulation of organic acids or regulation the entry of fatty acids to the heart and thus prevent their accumulation as a cause of the cardiomyopathy. In HL deficiency heart disease could be caused by the lack of an alternative energy substrate. The heart is a continuously active muscle which uses various energy substrates depending on their availability (Kodde et al., 2007). Although ketone bodies are not an indispensable substrate, the added L-carnitine deficiency, which is caused by the HL deficiency, could alter the transport of fatty acids to the mitochondria for oxidation and the coupling between glycolysis and glucose oxidation (Allard et al., 2006).

### 3.2 Diagnosis

This deficiency should be suspected in children with hypoglycaemia, hypoketonemia and metabolic acidosis. A preliminary diagnosis is made from a characteristic pattern of organic acids in urine, with high levels of 3-hydroxy-3-methylglutaric acid, 3-hydroxy-isovaleric acid, 3-methylglutaric acid and 3-methylglutaconic acid (Table 1). The characteristic metabolite of this disease is the 3-hydroxy-3-methylglutaric acid, but can also occur in the deficiency of carbamyl phosphate synthetase or Leigh-like disease (Faull et al., 1976). The confirmation of HL deficiency requires direct assay of the enzyme activity in leukocytes (Wysocki et al., 1976a) fibroblasts (Wysocki et al., 1976b; Wanders et al., 1988a) or liver biopsy (Schutgens et al., 1979). In prenatal diagnosis the pattern of organic acids in amniotic liquid (Chalmers et al., 1979), in maternal urine (Duran et al., 1979) and measurements of HL activity in cultured amniocytes or chorionic villi (Mitchell et al., 1995; Chalmers et al., 1979)

could be used as diagnostic tools. The molecular characterization of mutations in the gene *HMGCL*, including alterations in the mRNA, helps to complete the diagnosis.

### 3.3 Treatment

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 restrictive protein and fat diet, 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 to improve the patient's general state by facilitating urinary excretion of toxic metabolites (Dasouki et al., 1979). Moreover, L-carnitine, can be essential to prevent the development of cardiomyopathy (Puisac et al., 2010).

### 3.4 Prognosis

Despite this disease belongs to a group of 29 genetic conditions for which effective treatment is currently available (Watson et al., 2006), HMG-CoA lyase deficiency is fatal in approximately 20% of the cases. Nevertheless, early and careful treatment may result in a good prognosis with normal growth and development. Besides, in absence of complications, illness tends to improve with time and adults are usually free of symptoms.

## 4. *HMGCL* gene

The *HMGCL* gene (Gen Bank NM\_000191.2) located in the short arm of chromosome 1 (1p36.1-p35), between *FUCA1* and *TCEB3* encodes human HL. It has 9 exons and 8 introns (Figure 5) and a total of 24,336 base pairs. Its 5'-untranslated region bears the characteristic elements of a housekeeping gene, as well as a CpG island that contains binding sites for SP1. There is no evidence of the existence neither of a TATA box nor a CAAT box (Wang et al., 1996). Exons size varies between 64 and 527 base pairs (bp) and the introns range between 600 and 3400 bp. Exon 1 and part of exon 2 codify a 27 aminoacids array that forms the signal peptide for mitochondrial entering. Exon 9 codifies for 33 codons at the C-terminal ending and also has 406 bp from the 3'-untranslated region (Mitchell et al., 1993). The polyadenylation signal in humans and mouse is ATTAAA. This gene is present in both eukaryotes and prokaryotes and it has been cloned and studied in a variety of organisms, including humans (Mitchell et al., 1993), chicken (Mitchell et al., 1993), mouse (Wang et al., 1993), the *Rhodospirillum rubrum* (Baltscheffsky et al., 1997) and bacterial strains such as *Pseudomonas mevalonii* (Anderson et al., 1989), *Brucella mellitensis* and *Bacillus subtilis* (Forouhar et al., 2006).

The mRNA transcribed from *HMGCL* human gene has a size of 1.6 Kb and it has been found in all tissues studied albeit in widely differing amounts (Puisac et al., 2010) (Figure 4a) Tissues with the highest expression are liver 112.2 arbitrary units (100%) pancreas 43.5 (39%), kidney 17.56 (16%), testis 26.81 (24%), heart 2.96 (2,6%) brain 2.26 (2%) and skeletal muscle 1 (0.89%).

This gene presents a physiological splicing with three variants, one with all exons encoding the active protein and two with deletion of exons 5 and 6 and deletion of exons 5, 6 and 7 that encode inactive proteins (Muroi et al., 2000; Beatriz Puisac PhD thesis). These last two transcripts appear in tissues such as heart, brain and skeletal muscle which have little or no ketogenic potential.

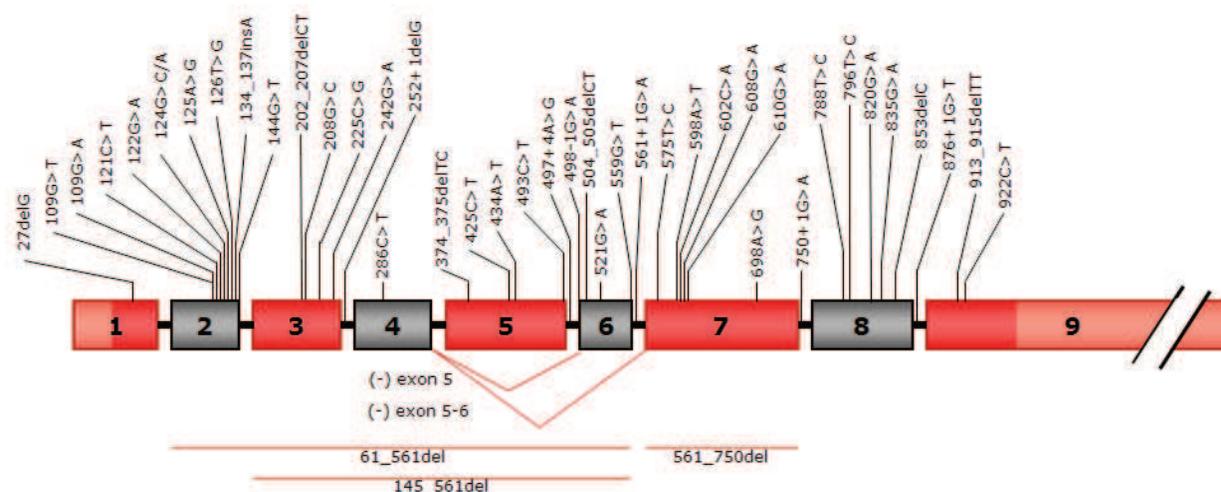


Fig. 5. Scheme of mutations located in the human *HMGCL* gene

#### 4.1 Mutational update

To date 50 variant alleles in the *HMGCL* gene (48 mutations and 2 SNPs) in more than 100 patients have been reported (Table 2). The missense mutations are the most frequent (25) followed by intronic mutations (7), frameshift deletions (6), nonsense mutations (6), large deletions (3) and insertions (1). The mutations are uniformly distributed along the gene sequences, although some clustering is observed in exon 2 and exon 7, suggesting that they could be hot spots for mutations (Figure 5).

Three 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 (Mitchell et al., 1998; Al-Sayed et al., 2006) and that has also been found in a patient in Italy, another in Turkey (Mitchell et al., 1998) and one in the Tcheck Republic suggesting that this mutation may have arisen independently more than once (Pospisilova et al., 2003).

Allelic variant	Exon/ Intron	Aminoacid change/ Predicted effect	Patients	Mutant Alleles	Origin	References
<b>Missense mutations</b>						
c.109G>A	E2	E37K	2	4	2 Pakistani	Menao 2009
c.122G>A	E2	R41Q	43	81	1 Czech, 1 Italian (ht), 40 Saudi (4 ht), 1 Turkish	Al-Sayed 2006; Mitchell 1998; Pospisilova 2003
c.124G>A	E2	D42N	1	1	1 Brazilian (ht)	Vargas 2007
c.124G>C	E2	D42H	1	1	1 Cajun (ht)	Mitchell 1998
c.125A>G	E2	D42G	2	4	1 German, 1 Palestinian	Menao 2009; Mitchell 1998
c.126T>G	E2	D42E	1	2	1 Austrian	Mitchell 1998

Allelic variant	Exon/ Intron	Aminoacid change/ Predicted effect	Patients	Mutant Aleles	Origin	References
c.144G>T	E2	K48N	1	1	1 Spanish	Carrasco 2007
c.208G>C	E3	V70L	1	1	1 French-Canadian (ht)	Mitchell 1992
c.225C>G	E3	S75R	1	2	1 German	Casals 2003
c.425C>T	E5	S142F	1	1	1 Spanish (ht)	Menao 2009
c.434A>T	E5	E145V	1	1	1 Japanese (ht)	Muroi 2000
c.493C>T	E5	R165W	1	2	1 Turkish	Koling 2000
c.494G>A	E5	R165Q	1	1	1 French? (ht)	Pierron 2009
c.494G>T	E5	R165Q	1	1	1 Tawianese (ht)	Lin 2009
c.521G>A	E6	C174Y	1	2	1 Palestinian	Menao 2009
c.575T>C	E7	F192S	1	2	1 Spanish	Menao 2009
c.598A>T	E7	I200F	1	2	1 French	Menao 2009
c.602C>A	E7	S201Y	1	2	1 English	Casals 2003
c.608G>A	E7	G203E	1	2	1 Italian	Mir 2006
c.610G>A	E7	D204N	2	3	1 Argentinean, 1 Portuguese (ht)	Casals 2003; Cardoso 2004
c.698A>G	E7	H233R	4	5	2 Czech (1ht), 1 French (ht), 1 English (ht)	Menao 2009; Pospisilova 2003; Roberts 1996; Zapater 1998
c.788T>C	E8	L263P	1	1	1 French (ht)	Zapater 1998
c.796T>C	E8	C266R	1	1	1 Greek (ht)	Zafeiriou 2007
c.820G>A	E8	G274R	1	1	1 French? (ht)	Pierron 2009
c.835G>A	E8	E279K	2	3	2 Japanese (1 ht)	Muroi 2000
<b>Nonsense mutations</b>						
c.109G>T	E2	E37X; Exon 2 skipping	31	55	1 Argentinian, 2 Moroccan, 13 Portuguese (4ht), 11 Spanish (1ht), 1 Turkish, 3 portuguese-brazilian (2ht)	Cardoso 2004; Casale 1998; Menao 2009; Pié 1997; Puisac 2005
c.121C>T	E2	R41X	1	1	1 English/German (ht)	Mitchell 1998
c.242G>A	E3	W81X	1	2	1 Ecuatorian	Menao 2009
c.286C>T	E4	Q96X	1	2	1 Italian	Funghini 2001
c.559G>T	E6	E187X	1	1	1 Spanish (ht)	Menao 2009
c.922C>T	E9	Q308X	1	2	1 Japanese	Muroi 2000

Allelic variant	Exon/ Intron	Aminoacid change/ Predicted effect	Patients	Mutant Aleles	Origin	References
<b>Deletions/ insertions</b>						
c.27delG	E1	P9P/ frameshift: stop codon 33	1	1	1 Czech (ht)	Pospisilova 2003
c.134-137insA	E2	N46K/frameshift: stop codon 47	2	4	2 Italian	Mitchell 1995
c.61-561del	E2,E3,E4,E5,E6	Deletes V21-E187 in frame	1	1	1 English	Wang 1996
c.202-207delCT	E3	S69C/ frameshift: stop codon 79	3	6	2 Acadian French-Canadian, 1 Spanish	Mitchell 1993
c.145-561del	E3,E4,E5,E6	Deletes E49-E187 in frame	1	1	1 Turkish	Wang 1996
c.374-375delTC	E5	V125D/frameshift: stop codon 150	1	1	1 Greek (ht)	Zafeiriou 2007
c.504-505delCT	E6	V168V/frameshift: stop codon 176; Exon 5 and 6, 6 skipping	10	11	3 Portuguese-brazilian (3ht), 2 Portuguese (2ht), 5 Spanish (4 ht)	Cardoso 2004; Casals 1997; Menao 2009; Vargas 2007
c.561-750del	E7	Deletes E187-Q250 in frame	1	2	1 Japanese	Muroi 2000
c.853delC	E8	Frameshift: stop codon 258	1	1	1 English (ht)	Menao 2009
c.913-915delTT	E9	F305Y/ frameshift: stop codon 314	3	6	3 Saudi	Al-Sayed 2006; Mitchell 1998
<b>Intronic mutations</b>						
IVS3+1Gdel	I3	Exon 3 deletion	1	2	1 Japanese	Muroi 2000
IVS3+1G>A	I3	Exon 3 deletion	1	1	1 Tawianese (ht)	Lin 2009
IVS5+4A>G	I5	Exon 5 deletion/ exon 5-6 deletion	1	1	1 English (ht)	Menao 2009
IVS6-1G>A	I5		1	1	1 Taiwanese (ht)	Lin 2009
IVS6+1G>A	I6		1	2	1 Saudi	Al-Sayed 2006
IVS7+1G>A	I7	r.slp	2	2	2 Spanish (2 ht)	Menao 2009
IVS8+1G>T	I8	Exon 8 deletion	1	2	1 Turkish	Buesa 1996

Allelic variant	Exon/ Intron	Aminoacid change/ Predicted effect	Patients	Mutant Aleles	Origin	References
<b>Polimorfism</b>						
c.252+34C>T	I3		1	1	1 Palestinian (ht)	Menao 2009
c.654A>G	E7	L218L	1	-	1 Spanish	Pié 1997
c.727A>G	E7	T243A	4	8	1 Moroccan, 1 Portuguese, 2 Spanish	Pié 1997

Table 2. Mutations and polymorphisms in the *HMGCL* gene. Position refers to the numbering of the HL cDNA sequence in Mitchell et al., 1993. ht, heterozygous

The second most frequent mutation is the c.109G>T (Mediterranean mutation) (55 alleles, 31 patients: 24 homozygous, 6 double heterozygous, 1 heterozygous with an allele unknown), found mostly in the Iberian Peninsula (13 patients in Portugal, 11 in Spain and 3 in brazilian-portugueses). It has also been described two cases in Morocco and another in Turkey (Pié et al., 1997; Casale et al., 1998; Cardoso et al., 2004; Puisac et al., 2005; Menao et al., 2009). It has been hypothesized that in Portugal and Spain the genetic hit was introduced during the Arabian invasions of the Iberian Peninsula in the eighth century. Further studies should be necessary to dillucidate if the mutation origin is the Iberian Peninsula itself or the Magreb (Pié et al., 2007).

The third most frequent mutation is c.504\_505delCT (11 alleles, 10 patients: 1 homozygous, 9 double heterozygous), although its incidente is much lower than the first two. It seems to be exclusively located in the Iberian Peninsula, where 15% of Portuguese (2 cases) (Cardoso et al., 2004) and 27% of Spanish patients have it (Menao et al., 2009). This mutation is also present in 3 of the 4 molecularly diagnosed Brazilians patients, though they were of Portuguese origin (Vargas et al., 2007).

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 (Muroi et al., 2000b), in Taiwan 3 mutations in 2 patients (Lin et al., 2009) in Italy, 5 mutations in 5 patients (Mitchell et al., 1995; Mitchell et al., 1998; Funghini et al., 2001) in Turkey 4 mutations in 4 patients (Wang et al., 1996; Buesa et al., 1996; Pié et al., 1997; Mitchell et al., 1998) . In the United Kingdom 6 mutations in 5 patients, though one of them was of German origin (c.121C>T) (Wang et al., 1996; Mitchell et al., 1998; Casals et al., 2003; Menao et al., 2009), 3 mutations in 3 patients in the Tcheck Republic (Pospisilova et al., 2003) and in Germany 2 mutations in 2 patients (Mitchell et al., 1998; Casals et al., 2003). The French group, the Acadians (descendents of the 17th-century French colonists), the Cajuns (Acadians settled in Louisiana) and the French-Canadians, despite of their common origin, present a great allelic heterogeneity: 6 mutations in 6 patients (Mitchell et al., 1992; Mitchell et al., 1993; Zapater et al., 1998; Mitchell et al., 1998; Menao et al., 2009).

#### 4.2 Genotype-phenotype correlations

The genotype-phenotype correlation is difficult to establish because the progress of the disease seems to be more related to the causes that produce hypoglycaemia (fasting or acute illness) than to a specific genotype. For example, patients carrying the same mutation, for

instance the so-called Mediterranean mutation (c.109G>T) may have from moderate to severe crises of lethal consequences. This is why in clinical practice it is fundamental to avoid situations that may cause hypoglycemia in these patients (Pié et al., 2007).

Several studies agree that studied missense mutations cause a loss of enzyme activity greater than 95% although these mutations often produce mild phenotypes (Mitchell et al., 1998; Carrasco et al., 2007; Menao et al., 2009). This suggests that the illness appears only in very severe genotypes, and that partial disruption of the enzyme is probably compatible with normal function. This adds more difficulties to establish genotype-phenotype correlations because we only see the effects of very severe genotypes.

## 5. Conclusion

Although 3-hydroxy-3-methylglutaric aciduria is a very rare disease, given the availability of an effective treatment, we recommend the screening of this disease in any child with hypoglycemia and metabolic acidosis. Moreover, in countries with a greater number of diagnosed cases (Saudi Arabia, Portugal and Spain), we also recommend to screen for the following mutations: c.122G>A, c.109G>T and c.504\_505delCT. The high percentage of splicing mutations justifies including the measurement of the mRNA.

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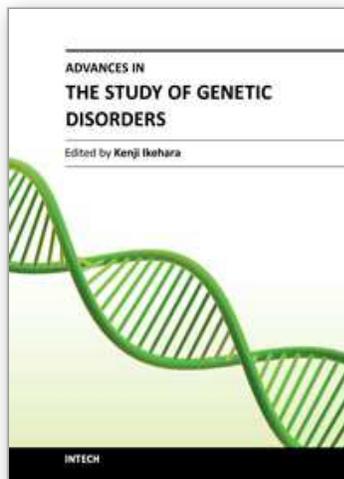
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## **Advances in the Study of Genetic Disorders**

Edited by Dr. Kenji Ikehara

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The studies on genetic disorders have been rapidly advancing in recent years as to be able to understand the reasons why genetic disorders are caused. The first Section of this volume provides readers with background and several methodologies for understanding genetic disorders. Genetic defects, diagnoses and treatments of the respective unifactorial and multifactorial genetic disorders are reviewed in the second and third Sections. Certainly, it is quite difficult or almost impossible to cure a genetic disorder fundamentally at the present time. However, our knowledge of genetic functions has rapidly accumulated since the double-stranded structure of DNA was discovered by Watson and Crick in 1956. Therefore, nowadays it is possible to understand the reasons why genetic disorders are caused. It is probable that the knowledge of genetic disorders described in this book will lead to the discovery of an epoch of new medical treatment and relieve human beings from the genetic disorders of the future.

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