Mitochondrial HMG–CoA Synthase Deficiency

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1. Introduction

The mitochondrial 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase deficiency (MIM 600234) is an autosomic recessive inborn error of metabolism, hard to characterize and probably underdiagnosed (Thompson et al., 1997). The enzyme failure is caused by mutations in the gene HMGCS2, located in chromosome 1. The illness was first diagnosed in 1997 (Thompson et al., 1997), in a six-year old boy, who presented a semicomatose state after three days with gastroenteritis and diet (Bouchard et al., 2001). Up to date, only eight patients have been reported with an estimated incidence of <1/1,000,000, although it could be higher because some patients probably have been misdiagnosed as Reye syndrome.

The mitochondrial HMG-CoA synthase enzyme (mHS, EC 4.1.3.5) has a main role in the synthesis of the ketone bodies and in the HMG-CoA formation. Ketone bodies act as an alternative glucose fuel in a number of tissues as heart, muscle and brain, and have a critical role during metabolic stress and starving, situations where the symptoms of the disease appear.

2. Ketogenesis

The ketogenesis is a metabolic process that takes place inside the mitochondria. The acetyl-CoA, originated during the fatty acids β-oxidation, is converted to acetoacetate, β-hidroxybutyrate and acetone. These metabolites are known as ketone bodies and act as a glucose alternative fuel in many tissues as heart, skeletal muscle and brain. In brain, ketone bodies play an essential role in situations of hypoglycemia (Edmond, 1992; Zammit & Moir, 1994). Besides their alternative fuel role, ketone bodies are lipogenic precursors too. They play a role during the myelinating process in the neonates’ brain (Nehlig & Pereira de Vasconcelos, 1993), and in a minor degree in the mammary gland during suckling (Zammit, 1981). Moreover, they seem to have some functions in metabolism regulation (Robinson & Williamson, 1980).

The main tissue where ketogenesis takes place is the liver (Zammit & Moir, 1994). However, it has been also found in kidney, adipose tissue (Thumelin et al., 1993), and intestine of...
suckling rats (Hahn & Taller, 1987) and, in a minor degree, in cortical astrocytes of newborn rats (Cullingford et al., 1998; Blázquez et al., 1998).

Ketone bodies can be elevated in physiological situations as: fasting, prolonged exercise, high-fat diet or pregnancy (Felig & Lynch, 1970), in the neonate (Hawkins et al., 1971); and in pathologic situations as: diabetes (Bates et al., 1968), obesity and disease of the glucose or glycogen metabolism (Mitchell et al., 1997).

![Ketone bodies synthesis pathway](image)

Fig. 1. Ketone bodies synthesis pathway

The main pathway of ketone bodies from acetyl-CoA includes the following reactions (Figure 1):

- In the first reaction, two acetyl-CoA molecules join to form acetoacetyl-CoA. This reaction is catalyzed by the acetoacetyl-CoA thiolase enzyme.
- Then, acetoacetyl-CoA reacts with another acetyl-CoA to produce the HMG-CoA. This is a condensation reaction where the mHS enzyme is involved, and whose failure is responsible of the disease.
- In the third reaction, the HMG-CoA is cleaved into acetoacetate, the first ketone body produced, and acetyl-CoA. This step is catalyzed by the HMG-CoA lyase (HL).
- Finally, in the last reaction, the acetoacetate is reduced to β-hydroxybutyrate, the second ketone body generated. The enzyme involved in this reaction is the β-hydroxybutyrate dehydrogenase. Acetone is formed by the spontaneous decarboxylation of acetoacetate.

There is an alternative ketogenic pathway different from the previously described, which takes place with substrates of aminoacids catabolism. Both pathways converge in the mitochondrial HL enzyme.

3. mHS enzyme

The mHS catalyzes the second reaction of the main pathway of the synthesis of ketone bodies and it is considered as the key enzyme of pathway regulation.

3.1 Enzymatic reaction

The mHS enzyme catalyzes the condensation of acetyl-CoA and acetoacetyl-CoA into HMG-CoA. The HMG-CoA, produced inside the mitochondria, drives towards the formation of ketone bodies. The mHS catalyzed reaction follows a Bi Bi Ping-Pong substitution mechanism (Cleland, 1963; Miziorko et al., 1975). This reaction has three steps (Miziorko et al., 1977) (Figure 2):

- Firstly, acetyl-CoA reacts with the thiol group of the catalytic Cys\textsuperscript{166} in order to produce a covalent intermediate form of the acetyl-enzyme. This is the limiting reaction step (Miziorko et al., 1977).
- Then, acetoacetyl-CoA (second substrate) condenses with the intermediate product to give HMG-CoA, which will be attached to the enzyme by a covalent bond.
- In the final step, HMG-CoA is released from the enzyme by hydrolysis. High concentrations of acetoacetyl-CoA inhibit the first reaction step, because it competes with acetyl-CoA for the active site of the enzyme (Page & Tubbs, 1978). Magnesium can act as an inhibitor of the reaction too.

3.2 Protein structure

Human mHS has 508 amino acids and its structure is a homodimer formed by two identical monomers linked by a salt bridge (Figure 3). At the N-terminal end is the leader peptide, with 37 aa, which drives the protein to the mitochondria.

In 1985, the catalytic sequence of the enzyme was identified in a purified protein from chicken liver (Miziorko & Behnke, 1985). This sequence has 21 aa and a 100% homology with HMG-CoA synthases from other mammals. The catalytic aminoacid Cys\textsuperscript{120}, whose human homolog is the Cys\textsuperscript{166}, is located inside this region (Misra et al., 1995). The protein analysis shows three regions rich in proline, glutamine, serine and threonine, which could be PEST sequences (Pro-Glu-Ser-Thr) (Rogers et al., 1986). These regions are characterized by a quick turnover of proteins (Boukaftane et al., 1994; Aytè et al., 1990).

Recently, the human enzyme has been crystallized and its alpha/beta structure, with 17 beta sheets and 19 alpha helices, has been well characterized. The tunnel of substrate entry and
product exit is located outside of the molecule, at the opposite side of the interaction between the two monomers (Shafqat et al., 2010).

Fig. 2. A. Acetyl-enzyme intermediate formation. B. HMG-CoA formation
3.3 Enzyme expression

Studies of gene expression in human tissues in basal conditions showed a tissue-specific expression. High mRNA levels are detected in the liver, the organ with the highest ketogenic capacity. Expression is also high in colon, which is related to the fermentative processes that take place in it (Mascaró et al., 1995). A minor level of mRNA expression has been observed in heart, skeletal muscle, gonads, kidney and pancreas (Mascaró et al., 1995; Royo et al., 1993). Studies in rats have found mRNA expression in cortical astrocytes from newborns (Cullingford et al., 1998; Blazquez et al., 1998), and in the intestine of suckling animals (Bekesi & Williamson, 1990; Thumelin et al., 1993; Serra et al., 1993). Changes in the level of gene expression have been reported during development. In suckling rats, the highest levels of expression were detected at the third day of life, decreasing progressively until weaning, when mRNA levels are similar to those of adult well fed rats (Serra et al., 1993).

3.4 Enzyme regulation

Production of ketone bodies in hepatic mitochondria is a complex and highly regulated process (Guzmán & Geelen, 1993; Zammit & Moir, 1994). Their synthesis increases in starving situations and in high fat intake, while it decreases after feeding or insulin administration and in the suckling-weaning transition (Williamson & Whitelaw, 1978; McGarry & Foster, 1980; Robinson et al., 1980).
Initially, the acyl-CoA input to the mitochondria was considered the principal check-point of ketogenesis (Guzman & Geelen, 1993; McGarry & Brown, 1997). However, later studies showed that the mHS enzyme can regulate ketogenesis (Williamson et al., 1968). Therefore, metabolic conditions that imply an increase of ketone bodies are linked to an increase of the levels of \( \text{HMGCS2} \) gene expression (Casals et al., 1992) and of the mHS enzyme (Serrá et al., 1993). Meanwhile, conditions that cause a decrease of the synthesis of ketone bodies are related to a decrease in the mRNA levels of the \( \text{HMGCS2} \) gene (Casals et al., 1992) and of the mHS enzyme (Serrá et al., 1993).

Currently, two check-points are recognized in the control of the synthesis of ketone bodies, one at the same step that of the Carnitine Palmitoyl Transferase 1 reaction, which provides the substrate acetyl-CoA, and another in the ketogenic pathway, at the step of the mHS enzyme (Williamson et al., 1968; Dashti & Ontko, 1979). Regulation of mHS is the most important step and depends on two mechanisms: a long term regulation of the transcription of the \( \text{HMGCS2} \) gene (Casals et al., 1992; Hegardt, 1999), and a short term regulation of the protein. This one depends on succinyl and desuccinyl enzymes reactions (Lowe & Tubbs, 1985; Quant et al., 1990). All of these mechanisms are influenced by nutritional and hormonal factors.

### 3.5 Isoenzymes

Two HMG-CoA synthase isoenzymes which catalyze the same reaction are known, however, they are located in different structures inside the cell. The mHS (Clinkenbeard et al., 1975) is involved in the synthesis of ketone bodies in the mitochondria, while the cytosolic HMG-CoA synthase (cHS) is critical for the synthesis of cholesterol (Clinkenbeard et al., 1975; Reed et al., 1975).

Expression pattern of cHS is different from the one described for mHS. The cytosolic enzyme is expressed in most of the tissues, and its gene is considered a house-keeping gene. High mRNA levels are observed in liver followed by heart, placenta and pancreas (Mascaró et al., 1995). Their transcriptional regulation is different from the mHS and it is negatively regulated by cholesterol and other isoprenoids (Hua et al., 1993).

The activity measures of the HMG-CoA synthase in a liver crude extract is divided between a 20-40% for the cHS and a 60-80% for the mHS (Clinkenbeard et al., 1975). Surprisingly, there is not reported any case with cHS deficiency.

### 4. mHS deficiency

Deficiency of mHS is an inborn error of metabolism that affects the synthesis of ketone bodies. It is a very rare autosomal recessive disorder reported in 1997 (Thompson et al., 1997), in a six year old boy. The patient had hypoglycemia, hypoketonemia and semicatamotic state, after three days with gastroenteritis and dieting. Symptoms quickly reversed after intravenous administration of glucose. Liver biopsy showed a decrease in the activity of mHS. Molecular study of the gene confirmed the diagnosis (Bouchard et al., 2001). Generally, the disease shows unspecific clinical symptoms and metabolites excretion profile, sometimes attributed to a fatty acid β-oxidation enzyme defect and that makes it to be underdiagnosed. Some authors pointed out that it can be misdiagnosed as Reye syndrome and be associated with the sudden infant death syndrome (Thompson et al., 1997). Symptoms usually appear after situations of starving and/or high energy expenditure.
(fever, stress, exercise). In normal conditions, metabolic pathways that provide alternative glucose sources, as the synthesis of ketone bodies, are activated. A defect of ketogenesis can trigger a coma in the individual with low glucose availability.

4.1 Clinical features
In all the cases reported, clinical symptoms have appeared in childhood, specially during the first year of life; however, two of the 8 cases were diagnosed at the age of four and six years, respectively.

Clinical features of mHS deficiency are unspecific (Table 1), which makes it hard to diagnose. Initial symptoms include vomiting and lethargy that can progress to coma. In most cases these symptoms are accompanied with hepatomegaly (Thompson et al., 1997) and in some cases with respiratory disease and encephalopathy. These symptoms quickly improve after glucose administration. Up to date, all the reported cases have had a favorable outcome, despite severe acute episodes. All the reported patients with mHS deficiency are still alive.

4.2 Diagnosis
4.2.1 Biochemical data
Hypoglycemia is the main biochemical anomaly detected during an acute episode. Increase of the levels of plasmatic free fatty acids and urine dicarboxylic acids is also found. Plasma levels of acylcarnitines, lactate and ammonium are within normal limits (Morris et al., 1998). In some cases, metabolic acidosis has been reported (Table 1).

This disease, as opposite to the HL deficiency, does not show a characteristic organic acids pattern in urine. The mHS substrate does not accumulate because it can be metabolized to acetyl-CoA during the β-oxidation.

4.2.2 Enzyme activity
An enzymatic assay in liver biopsy is needed to confirm the mHS deficiency, because liver is the tissue with the highest enzyme expression. This method has several limitations, being the main one that in liver homogenate we cannot distinguish between mHS and cHS enzyme activity. This problem impairs the interpretation of the results and explains that the assay has only been carried out in two patients (Thompson et al., 1997; Morris et al., 1998). The possibility to use other tissues or alternative cells as lymphocytes or fibroblasts has been proposed, but the low expression levels in these tissues did not allow investigators to obtain valid measurable levels (Thompson et al., 1997).

4.2.3 Molecular diagnosis
Molecular analysis is the method of choice to confirm the clinical diagnosis. It is done from genomic DNA of the patient, by PCR amplification and sequencing of the \textit{HMGCS2} gene.

4.2.4 Differential diagnosis with HMG-CoA lyase deficiency
The HL is the immediately posterior to mHS enzyme in the pathway of the ketone bodies synthesis. HL deficiency (3-hydroxy-3-methylglutaric aciduria) has very similar clinical manifestations to mHS deficiency, although there are significant differences that the clinicians should know in order to differentiate them.
<table>
<thead>
<tr>
<th>Signs and laboratory data</th>
<th>mHS deficiency</th>
<th>HL deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIGNS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Lethargy</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Abnormal Breathing</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Coma</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Normal development</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Death</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypoketonemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hyperammonemia</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Transaminase</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>High urine dicarboxylic acid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>High 3-hydroxy-3-methylglutaric, 3-hydroxyisovaleric, 3-methylglutaconic and 3-methylglutaric acids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Normal level of carnitine</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

+Always; -Never; +/- Sometimes.

Table 1. Signs and Biochemical data of mHS and HL deficiency

Patients with HL deficiency may present acute crisis as well, during the neonatal or early infancy periods. Both diseases appear when exogenous supply of glucose fails (fasting periods), or when excessive glucose consumption exists (stress, fever or exercise). In the majority of patients with mHS deficiency, the acute crisis begins with hepatomegaly and superficial coma. However, in HL deficiency, or other β-oxidation enzymes, symptoms of the deficiency usually involve several tissues (Thompson et al., 1997). In the latter, most of the patients show muscular weakness and myopathy (Ribes et al., 2003). In the HL deficiency symptoms as dehydration, hypotonia, hypothermia, tachypnea, lethargy, coma and even death, have been reported (Wysoki & Hahnel, 1986; Menao et al., 2009). Up to date, no patient with mHS deficiency has died due to this disease.

Complications of 3-hydroxy-3-methylglutaric aciduria include, macrocephaly (Stacey et al., 1985), development delay and dilated cardiomyopathy with arrhythmia (Gibson et al., 1994). Microcephaly has been reported in one patient (Lisson et al., 1981). Up to date, no complications have been reported in patients with mHS deficiency.

The most important difference between both diseases is found in the urine profile of the organic acids, which is characteristic only in HL deficiency. Metabolites that accumulate in urine are the 3-hydroxy-isovaleric acid, the 3-methylcrotonyl glycine, the 3-methylglutaric...
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acid, the 3-methylglutaconic acid and the 3-hydroxy-3-methylglutaric acid. Neither of these metabolites is increased in mHS deficiency. Clinical and biochemical data of both deficiencies are included in Table 1.

4.3 Treatment
Treatment is symptomatic during acute episodes and consists of intravenous administration of glucose to correct hypoglycemia. Long-term maintenance therapy includes low-fat diet and avoidance of fasting periods of more than 12 hours. It is also important to avoid situations of metabolic stress, mainly produced by recurrent illness that can be prevented by extra caloric intake (Morris et al., 1998).

5. HMGCS2 gene
Although mHS is codified by a gene (Ayte et al., 1990) different from the cHS gene (Gil et al., 1986), their high homology suggests a common origin about 500 millions years ago, when vertebrates appeared (Boukaftane et al., 1994).

Human HMGCS2 gene is located in chromosome 1 (1p12-p13), between the markers WI-7519 and D1S514. It has 10 exons and 9 introns, with a total size of 21,708 bp (Figure 4). Exons length oscillates between 107 and 846 bp, and the intron’s size varies between 0.4 and 4.1 kDa. cDNA has a total size of 2,082 bp.

So far, no splice variants of the gene have been experimentally confirmed, although the current Expressed Sequence Tag database includes a variant with a deletion of exon 4.

5.1 Mutation update
Up to date, 8 patients have been diagnosed by molecular analysis. Among them nine allelic variants have been identified: seven missense variants, one nonsense and one intronic mutation (Figures 3 and 4). One of these variants, the Y167C, affects to a closed Cys166 amino acid, which is considered the catabolic site of the enzyme (Hegard, 1999). The nonsense variant R424X (Bouchard et al., 2001; Morris et al., 1998), produced a truncated protein of 424 amino acids that probably cannot be incorporated into the active protein dimer. The intronic mutation is located in the first nucleotide of the intron 5 (c.1016+1G>A). In general, this type of mutations produces the deletion of the affected exon (exon 5 in this case), although in this disease the mechanism has not been confirmed (Zschocke et al., 2002).

In order to prove the effect of the mutation in the enzyme activity, protein over-expression has been tried with recombinant DNA techniques although with no success (Bouchard et al., 2001).

Fig. 4. Scheme of the mutations located in the human gene HMGCS2
Table 2. Clinical findings and biochemical data from patients with mHS deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease debut</th>
<th>Genotype</th>
<th>Symptoms and signs</th>
<th>Organic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>6 years</td>
<td>F174L/F174L</td>
<td>Gastroenteritis, hypoglycemia, hipoketonemia, superficial coma</td>
<td>Normal</td>
</tr>
<tr>
<td>Patient 2</td>
<td>1 year and 4 months</td>
<td>R424X/unknown</td>
<td>Diarrhea, vomiting, hepatomegaly, hypoglycemia, hipoketonemia, coma</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 3</td>
<td>11 months</td>
<td>G212R/R500H</td>
<td>Gastroenteritis, vomiting, hepatomegaly, hypoglycemia, hipoketonemia, coma</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 4</td>
<td>9 months</td>
<td>G212R/IVS5+1G&gt;A</td>
<td>Gastroenteritis, diarrhea, vomiting, hepatomegaly, hypoglycemia, hipoketonemia, coma</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 5</td>
<td>4 years and 6 months</td>
<td>V54M/Y167C</td>
<td>Gastroenteritis, diarrhea, vomiting, hypoglycemia, hipoketonemia, coma</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 6</td>
<td>1 year and 7 months</td>
<td>V54M/Y167C</td>
<td>Hepatomegaly, normal hepatic function</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 7</td>
<td>7 months</td>
<td>M307T/R188H</td>
<td>Vomiting, hepatomegaly, hypoglycemia, hipoketonemia, encephalopathy</td>
<td>Dicarboxylic aciduria</td>
</tr>
<tr>
<td>Patient 8</td>
<td>1 year</td>
<td>M307T/R188H</td>
<td>Vomiting, lethargy</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Mutations G169D, T233A, K243E, L266S, I407T, R505Q, R84X and exon 1 deletion were reported by Pitt et al., at the 11th International Congress on Inborn Errors of Metabolism, San Diego CA and by Shafqat et al., 2010. Mutations I56N, T233A, K243E and R84X were reported by Shafqat et al., 2010. No clinical data were available from the patients who carried these mutations.
Another approach consisted in studying, with an indirect method, the mutation effect in MEV-1 cell cultures (Aledo et al., 2001). These studies were based in the cell mevalonate auxotrophy correction when they are transfected with cDNA of the HMGCS2 gene. The expression of the mHS gene gives to MEV-1 cells the capacity to synthesize HMG-CoA inside the mitochondria, which is transformed in mevalonate and cholesterol (Ortiz et al., 1994). As expected, the mutated mHS cDNA transfection does not correct the MEV-1 cells auxotrophy, which proves the deficiency of HMG-CoA synthesis.

Recently, new mutations in mHS deficiency patients have been reported, but no clinical information was given. There are 8 missense mutations and one nonsense (Shafqat et al., 2010).

In Table 2 an updated list of mutations reported in the HMGCS2 gene is displayed, together with the patients’ clinical data.

6. Genotype-phenotype correlations

With the current knowledge available, it is difficult to establish strong genotype-phenotype correlations, among other reasons, for instance, because we ignore relevant information such as the levels of enzyme activity in affected patients. The seriousness of the disease may be more related to the agent that triggered hypoglycemia and to the time without treatment than with the mutation itself.

7. Conclusion

Although mHS deficiency is an extremely rare disease, it is likely underdiagnosed and its prevalence is higher than estimated. Pediatricians may suspect this disorder in infants with vomiting, mild hepatomegaly, hypoglycemia, metabolic acidosis, increased levels of plasmatic free fatty acids and dicarboxylic aciduria, specially if symptoms appeared after a situation of metabolic stress, usually due to dieting in a gastroenteritis or during an infection process. Currently, the only reliable diagnostic –confirmatory- test is the molecular analysis of the gene, since measurements of the levels of enzymatic activity are masked by the activity of isoenzyme cHS.

8. Acknowledgment

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Zammit VA. (1981). Regulation of hepatic fatty acid metabolism. The activities of mitochondrial and microsomal acyl-CoA:sn-glycerol 3-phosphate O-acyltransferase and the concentrations of malonyl-CoA, non-esterified and esterified carnitine, glycerol 3-phosphate, ketone bodies and long-chain acyl-CoA


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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