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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

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Abstract

HMG-CoA lyase (HL) deficiency is a rare autosomal recessive genetic disorder that affects ketogenesis and leucine catabolism. We report a new Spanish patient who bears the frequent nonsense mutation G109T (Mediterranean mutation). This mutation can produce aberrant splicing with three mRNA variants: one of the expected size, the second with deletion of exon 2, and the third with deletion of exons 2 and 3. Recently our group proposed a 3D model for human HL containing a (beta-alpha)$_8$ (TIM) barrel structure. We have studied the effect of the deletions of exon 2 and exons 2 plus 3 on the proposed HL model. Exon 2 skipping led to the loss of beta-sheet 1, and the skipping of exons 2 and 3 caused the disappearance of alpha helix 1 and beta-sheets 1 and 2.

Keywords: HMG-CoA lyase (HL); (beta-alpha)$_8$ TIM Barrel; HMGCL; Exon skipping; Splicing variants; 3-Hydroxy-3-methylgluraric aciduria

1. Introduction

HMG-CoA lyase (HL) deficiency (Mckusick 24645) is a rare autosomal recessive genetic disorder that affects ketogenesis and L-leucine catabolism. The most prominent symptoms are vomiting, lethargy, coma, convulsions, metabolic acidosis, and hypoglycemia without ketoaciduria. The disease appears in the first year of life and is fatal in about 20% of cases [1,2]. To date, 28 allelic variants of the HL gene (HMGCL) have been reported in 53 patients (one case was an aborted foetus) [3–17]. Two allelic variants predominate: G122A, frequent in Saudi Arabia [11] and G109T, also called the Mediterranean mutation, common in Portugal and Spain [7,9,17].

The Mediterranean mutation can produce multiple aberrant splicing with three mRNA variants: one of the expected size that contains the premature stop codon TAA, the second with a deletion of 84 bp corresponding to the whole of exon 2, and the third with a deletion of 192 bp corresponding to skipping of exons 2 and 3 [7,9,15].

Recently, our group proposed a 3D model for human HL containing a (beta-alpha)$_8$ TIM barrel structure. The model is supported by the similarity with the analogous TIM barrel structure of functionally related proteins, by the localization

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-Co-enzyme A.

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of catalytic amino acids at the active site and by the coincidence between the shape of the substrate (HMG-CoA) and the predicted inner cavity [14].

Here we report a new Spanish patient with the Mediterranean mutation and we study the effect of splicing variants, with skipping of exon 2 and exon 2 plus 3, on the proposed HL model.

2. Experimental procedures

2.1. Case report

Spanish male patient P.J.G. was admitted to a local hospital at 1 month of age with: hypoglycemia without ketonuria, metabolic acidosis and hepatomegaly. At 5 months of age the patient suffered a second acute episode with 24 h of vomiting, lethargy, hepatomegaly, and respiratory disorders needing ventilatory support. Laboratory test revealed hypoglycemia without ketonuria, metabolic acidosis, hyperammonemia, hyperaminoacidemia (especially glutamine), cystinuria, and a slight increase of lactate and free carnitine. The analysis of organic acids in urine supported the diagnosis of HL deficiency and showed elevated excretion of the following acids: 3-hydroxy-3-methylglutaric (>40,000 mmol/mol creatinine), 3-methylglutaconic (7536 mmol/mol creatinine), 3-hydroxyisovaleric (6554 mmol/mol creatinine), glutaric (1868 mmol/mol creatinine), adipic (5824 mmol/mol creatinine), suberic (1017 mmol/mol creatinine), and sebacic (247 mmol/mol creatinine). The HMG-CoA lyase activity in fibroblasts was very low (0.027 nmol min^{-1} mg^{-1} of protein), less than 0.5% of control.

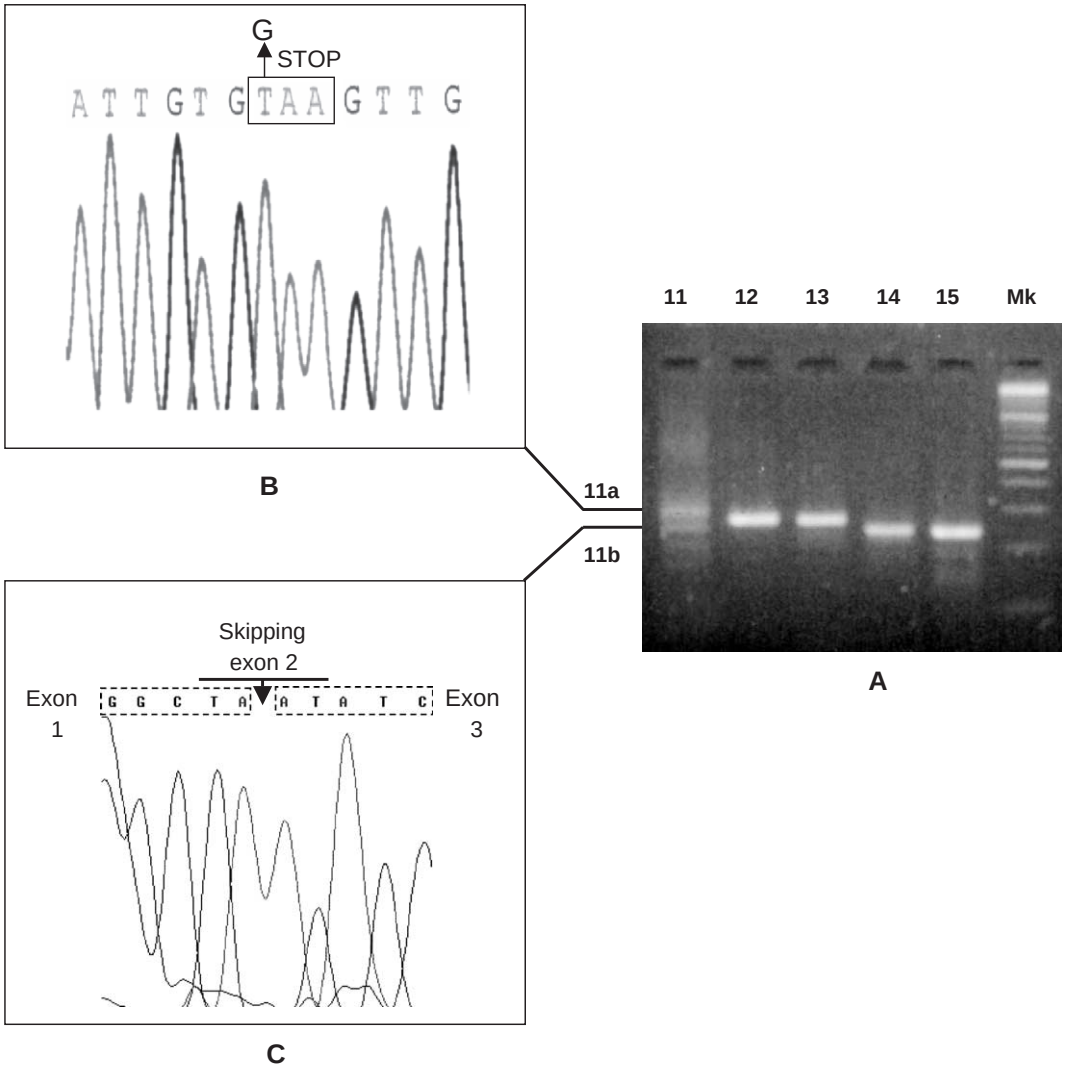

Fig. 1. (A) The different cDNA overlapping fragments 11, 12, 13, 14, and 15 from the patient were separated by electrophoresis in 2% agarose gels. The amplified fragment 11 shows two bands, one normal, of 247 bp (11a), and other of 163 bp with the skipping of exon 2 (11b). Mk, molecular marker. (B) The cDNA sequence from band -11a- shows the mutation G109T (indicated with arrow). The new codon stop appeared -TAA- is boxed. (C) The cDNA sequence from band -11b-shows the skipping of exon 2.

2.2. Mutational analysis

Mutational analysis was performed on DNA and RNA from cultured patient fibroblasts. DNA was isolated using the "DNAzol ® Reagent kit" from Invitrogen. RNA was obtained with "Ultraspec RNA" kit of BIOTECX. The "Ready-to-go T-primed First-Strand kit" of Pharmacia Biotech was used for the RT-PCR reaction. A fragment with an open reading frame (ORF: 1024 bp) was amplified and then reamplified in 5 consecutive overlapping fragments 11, 12, 13, 14, and 15. To explore the intron regions flanking exons, all HL exons were amplified. The amplification primers and PCR conditions were as previously described and supplied by Genosys [5–7,9]. The PCR products were purified with "QIAquick PCR Purification" and "QIAquick Gel Extraction" of QIAGEN, and sequenced with an Applied Biosystems 373 automated DNA sequencer, using the DNA sequencing kit of Perkin-Elmer.

2.3. Generating a structural model for HMG-CoA lyase

A structural 3D model for human HMG-CoA lyase was constructed by aligning the protein sequence with the available structures of the *Thermotoga maritima* HisA protein (Protein Data Bank entry: 1QO2) and the *Pseudomonas mevalonii* 4-hydroxy-2-oxovalerate aldolase (PDB entry:1NVM). Both structures belong to the superfamily of the (beta-alpha)$_8$ barrels or TIM barrels. To obtain the three-dimensional model of HMG-CoA lyase, the program DeepView and the SWISS-MODEL [18–21] server facilities (http://www.expasy.ch/swissmod/SWISS-MODEL.html) were used. The atomic coordinates of the HMG-CoA molecule were obtained from the PDB entry 1qax, and the docking calculations to obtain a molecular model of the interaction of the substrate and the modelled enzyme were performed using the program Hex [22] based on spherical polar Fourier correlations. The structural quality was checked using the program ProsaII [23]; the WHATCHECK routines from the WHAT IF program and the PROCHECK validation program from the SWISSMODEL server facilities [24] were also used. In brief, the model quality was within the expected range for homology modelling-obtained protein models. Recently, a second 3D model for HL has been published [25], using as template the first hit-1NVM-provided by the 3D-PSSM threading web server (http://www.sbg.bio.ic.ac.uk/3dpssm). To compare the two structural models, one constructed as indicated above, and the second following the steps described by Tuinstra et al. [25], the coordinates of the backbone atoms of the structures were extracted and aligned using the Dali algorithm for structural comparison [26]. Root-mean-square deviation (RMSD) was measured between the corresponding backbone atoms of the two models. This gave a value of 1.3 angstroms, which is in the range of close structural similarity and thus provides stronger confidence for both 3D structures.

3. Results and discussion

The analysis of overlapping fragment 11 revealed two bands, one of the expected size (247 bp; 11a) with the Mediterranean mutation (G109T), and the other abnormal (163 bp; 11b) with a deletion of the whole of exon 2 (84 bp). This transversion (G109T) changed the triplet 37 GAA to the stop codon UAA (Fig. 1). A minor transcript associated with the Mediterranean mutation with skipping of exons 2 and 3 (192 bp) has also been reported [9]. Sequencing of the amplified exon 2 confirmed that patient P.J.G. was homozygous for the allelic variant G109T. Mutations in donor, acceptor and pyrimidine rich sequences flanking exon 2 were not observed. It has been suggested that the

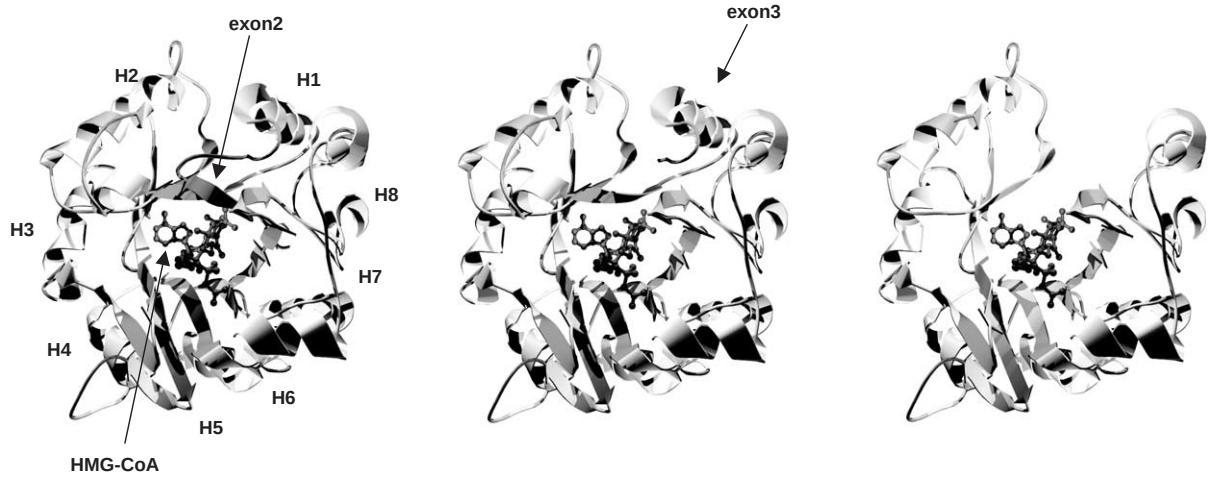

Fig. 2. Homology model for HMG-CoA lyase. Left: model for the wt enzyme; a view from the C-terminal edge of the (alpha-beta)$_8$ barrel. Positions of the eight alpha helices of the structure and the putative location of the substrate are indicated. The position of the beta sheet corresponding to the sequence of the exon 2 of the protein is also indicated. Centre: a hypothetical model illustrating the lack of exon 2. Position of sequence corresponding to exon 3 is indicated. Right: hypothetical model for HMG-CoA lyase lacking both exons 2 and 3, assuming conservation of the rest of the protein structure.

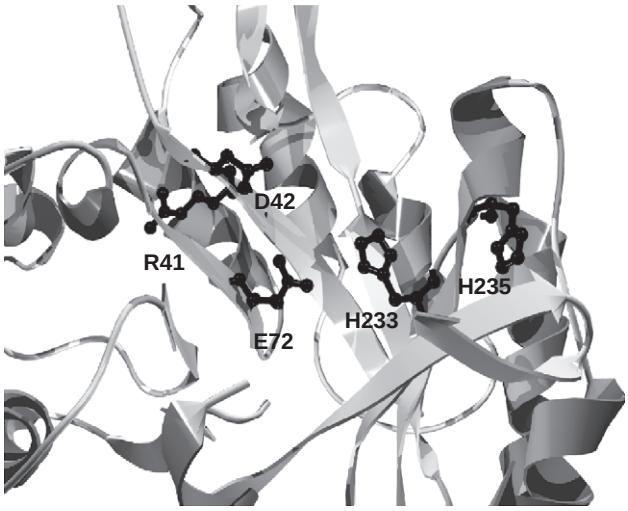

Fig. 3. A view of the active site of the HMG-CoA lyase model indicating the location of residues R41, D42, E72, H233, and H235. The positions of these aminoacids in the structure are similar to the corresponding positions in the model proposed in [24].

Mediterranean mutation disrupts a positive exon-splicing enhancer (ESE) in a purine-rich sequence (GAAGAAG) in the middle of exon 2 and causes aberrant splicing with three transcripts [7,15]. The first, normal-sized transcript with the stop codon 37 generates a truncated mature protein with only nine residues, thus losing the key aminoacids of the enzyme [7]. We studied the effect of the second transcript with deletions of exon 2 and the third transcript with deletion of the exons 2 plus 3 on the proposed HL $(beta\text{-}alpha)_8$ TIM barrel model [14]. Exon 2 skipping produced the loss of beta-sheet 1, which contains the critical residue aspartate-42. The transcript with skipping of exons 2 and 3 caused the disappearance of alpha helix 1 and beta-sheets 1 and 2, which contain glutamate-72 and aspartate-42 (Fig. 2). These aminoacids are involved in the binding to the activator divalent cation, which explains the lack of activity of these mutants [25,27]. Moreover, it has been proposed that aspartate-42 and histidine-233 are the active site residues that are candidates to deprotonate the substrate C3 hydroxyl group and to protonate the carbanion form of acetyl-CoA prior to product release [25]. The spatial relationships of these residues (R41, D42, E72, H233, and H235) are shown in the picture of the active site of the HMG-CoA lyase model proposed (Fig. 3). In addition, beta-sheets play an important role in the stability of proteins and form the inner core of the TIM barrel structures [14,28]. Recently, an independent 3D model for HMG-CoA lyase, obtained using the 3D-PSSM web server facilities, was described by Tuinstra et al. [25]. The similarity of the two 3D structural models was deduced from the root-mean-square deviation (RMSD) between the corresponding backbone atoms. The value obtained (1.3 angstroms) indicates satisfactory superimposition, which reinforces both 3D models and increases confidence in our conclusions based on the structures.

The HL deficiency has a heterogeneous origin (28 allelic variants in 54 probands). The new case reported here raises the number of patients to 18 and the number of alleles with the Mediterranean mutation to 33 worldwide [7,9,17]. In Portugal and Spain 16 out of 17 patients have this mutation. The low diversity suggests the mutation originated in this region. It is difficult to draw conclusions about genotype/phenotype correlations, because the patients received different treatment, and the onset of an acute episode frequently depends on external factors such as fasting or acute illness [9].

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