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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.

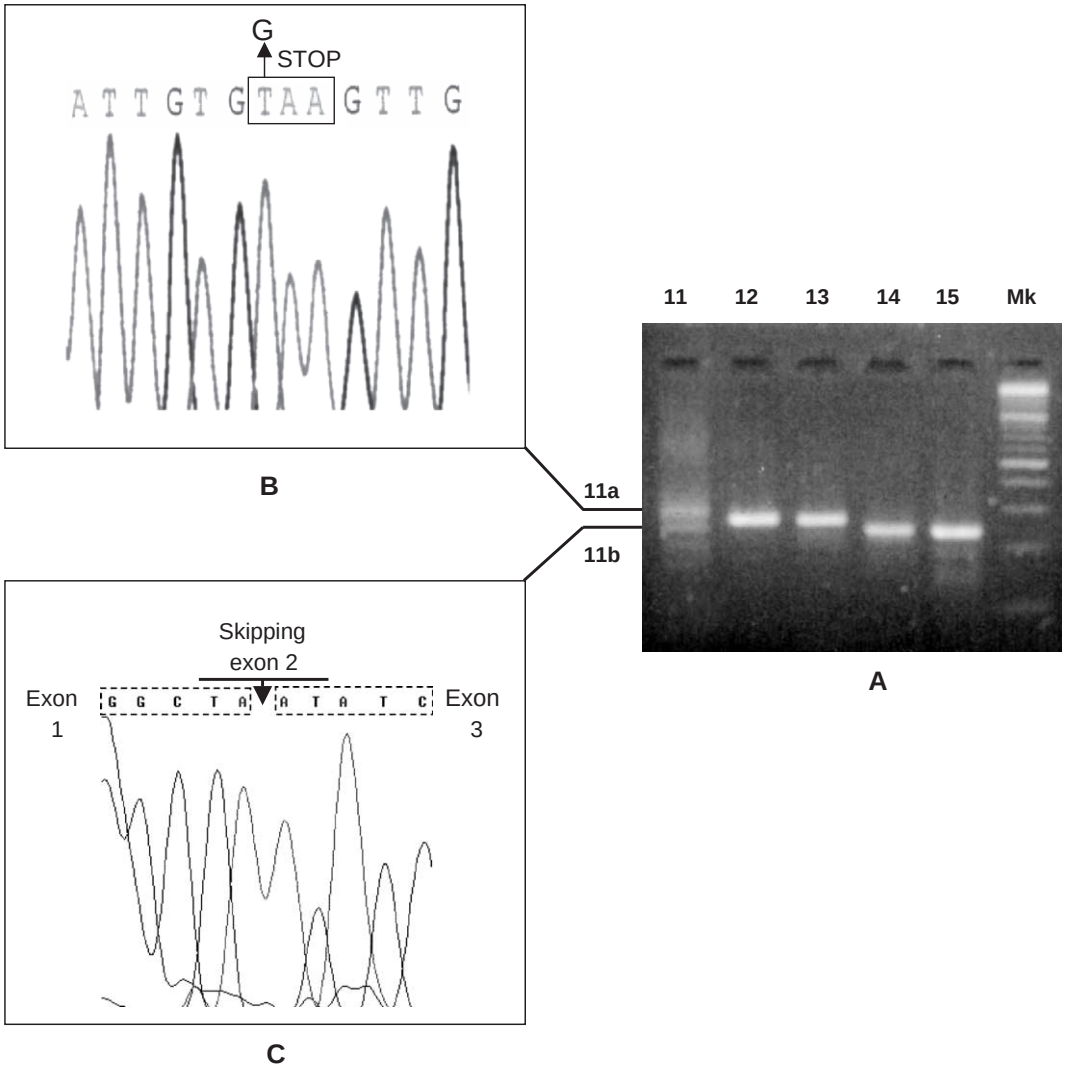

Fig. 1. (A) The different cDNA overlapping fragments 11, 12, 13, 14, and 15 from the patient were separated by electrophoresis in 2% agarose gels. The amplified fragment 11 shows two bands, one normal, of 247 bp (11a), and other of 163 bp with the skipping of exon 2 (11b). Mk, molecular marker. (B) The 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.