Apolipoprotein CII-Padova (Tyr\(^{37}\)→stop) as a cause of chylomicronaemia in an Italian kindred from Siciliana

Suat Tuzgöll, Saskia M Bijvoet, Taco Bruin, Johannes P Kastelein, Michael R Hayden

Abstract

In this paper we report on the molecular defect underlying apolipoprotein CII (apoCII) deficiency in an Italian kindred. ApoCII serves as cofactor for lipoprotein lipase (LPL) in triglyceride hydrolysis of chylomicrons and very low density lipoproteins. Homozygous apoCII deficiency manifests with type I hyperlipoproteinemia and is a rare disorder of lipoprotein metabolism. Until now, only 10 kindreds with apoCII deficiency have been published and all underlying mutations were unique.

The proband was the offspring of a consanguineous mating. Sequencing of cloned DNA from the proband presented in this report showed homozygosity for a C→A substitution at position 3002 in the apoCII gene, resulting in the introduction of a premature stop codon at residue 37 of the mature apoCII protein. Therefore, a truncated apoCII is synthesised, lacking the part of the apolipoprotein that activates LPL.

This mutation has previously been described in another Italian family and is known as apoCII\(_{\text{Padova}}\). We propose that apoCII\(_{\text{Padova}}\) is a frequent cause of apoCII deficiency in persons of Italian descent.

The hydrolysis of the triacylglycerol core of circulating chylomicrons and very low density lipoproteins (VLDL) requires the presence of the plasma enzyme lipoprotein lipase (LPL) and its cofactor apolipoprotein (apo) CII.\(^1\)

LPL is synthesised in adipose, muscle, and several other tissues and is transported to the luminal surface of capillary endothelial cells where it can interact with circulating triacyl-glycerol rich lipoproteins.

ApoCII is an apolipoprotein, necessary for efficient LPL mediated triglyceride hydrolysis by binding, and thereby activating, LPL. Chylomicrons acquire apoCII from high density lipoproteins (HDL) upon entering the circulation.\(^2\) This apolipoprotein is initially synthesised in the liver as a preproapoprotein of 101 amino acids length, and undergoes subsequent cotranslational cleavage of a 22 amino acid signal peptide. The major form of apoCII in plasma (apoCII\(_{\text{L}}\)) contains 79 amino acids and is not glycosylated. Sialic acid containing isoforms (apoCII\(_{\text{H}}\) and CHI\(_{\text{L}}\)) and a 73 amino acid protein, lacking the six amino-terminal residues, constitute the remainder of apoCII mass in plasma.\(^3\)

Studies using either proteolytic fragments or synthetic peptides are suggestive of the presence of different functional domains in the mature protein. The N-terminal part of the protein seems to mediate lipoprotein binding, probably through amphipathic helix interaction with phospholipids. The C-terminal domain of apoCII has been suggested as the main site for LPL activation (residue 54 to 63) and for binding to LPL (residue 64 to 79).\(^4\) Not all studies, however, support these conclusions.\(^5\)

The gene encoding for apoCII maps to the long arm of chromosome 19 (19q13) and the complete nucleic acid sequence and genomic organisation have been elucidated.\(^6\)

ApoCII deficiency is a rare hereditary disorder, inherited as an autosomal recessive trait. The clinical manifestations of apoCII deficiency are identical to those of LDL deficiency and include upper abdominal pain, eruptive xanthomata, lipaemia retinai, and recurrent pancreatitis. ApoCII deficiency was first described in 1978.\(^8\) Since then 10 different genetic defects underlying apoCII deficiency in different parts of the world have been determined.\(^9\)

Here we report the molecular basis of functional apoCII deficiency in an Italian kindred from Sicily. We show a C→A transition, resulting in the introduction of a stop codon for Tyr\(^{37}\), which is identical to a previously reported mutation from another family with origins in a distant region. We suggest therefore that this mutation might be a frequent cause of apoCII deficiency in patients of Italian descent.

Material and methods

CASE 1

The index patient (II.5) is a 43 year old male of Sicilian origin. The patient was first seen when he was 24 years old at the Lipid Research Clinic at Shaugnessy Hospital (University of British Columbia) when he experienced his first episode of severe abdominal pain. On physical examination he had mild splenomegaly and laboratory tests showed hyperlipidaemia, while serum amylase was normal. Medication for hyperlipidaemia, including clofibrate and nicotinic acid, was not effective nor well tolerated. Between the ages of 24 and 34 the patient suffered from severe recurrent upper abdominal pain, two to three times per year.

No definite diagnosis was made at that time and an exploratory laparotomy was performed and an enlarged spleen was removed. There-
after he continued to suffer from numerous episodes of acute pancreatitis and abdominal pain. Lipoprotein electrophoresis and lipoprotein analysis at that time showed a type V lipoprotein pattern with the following lipoprotein levels: total cholesterol 5.57 mmol/l, HDL cholesterol 0.49 mmol/l, triglycerides 3.18 mmol/l, apolipoprotein B$_{	ext{apo}}$ 0.76 g/l, apolipoprotein AI 0.74 g/l. The apoE phenotype was E3/E3. When an optimal amount of normolipidaemic control plasma was used to activate the substrate emulsion, the patient showed LPL activity within the normal range. When the patient’s plasma was used as the source of activator, no LPL activity was detected. This confirmed the presence of normal LPL and the absence of LPL activator in the plasma of the index patient. Hepatic lipase activity was normal. Isoelectric focusing of chylomicron/ VLDL apoproteins showed absence of apoCII in the plasma of the index patient and intermediate levels in both parents. The patient was put on a fat restricted diet (less than 20% of total calories provided by fat), and bouts of abdominal pain did not recur.

The index patient comes from a sibship of six. His parents, three of his sibs, and his ancestors live in the small village of Siculiana on the island of Sicily. His parents are first cousins and there has been little migration into or from this area over the past 150 years.

**Lipoprotein analysis**

Lipoproteins were separated by ultracentrifugation as previously described and washed once at the upper density limit. Cholesterol and triglycerides were measured by standard enzymatic techniques, using reagents from Boehringer-Mannheim (Dorval, Quebec, Canada). The Corning ACI system was used for lipoprotein electrophoresis.

Apolipoprotein B was measured in isolated lipoproteins by tetramethyurea (TMU) treatment or by electrophoresis. Apolipoprotein AI and AII standards were purified by gel chromatography in the presence of 6 mol/l urea.

**DNA analysis**

*Polymerase chain reaction*  
Genomic DNA was extracted from the buffy-coat of 30 ml EDTA anticoagulated blood of the index patient and his available family members, for analysis by the polymerase chain reaction (PCR).

The reactions were performed in a DNA thermocycler (Perkin-Elmer Cetus, Norwalk, CT, USA) using the buffer recommended by the manufacturer in conjunction with 1-5 mmol/l Mg+++, 200 μmol/l deoxynucleotide triphosphates (dNTPs), 500 ng of each primer, 500 ng genomic DNA, and 2-5 units of Ampli- taq (Cetus) in 100 μl reactions. The primers were derived from intronic sequences of the apoCII gene and constructed to include intron-exon boundaries and complete exons in the amplified products.

The oligonucleotide primers were synthesised on the Gene Assembler Plus (Pharmacia, Uppsala, Sweden). The sequence of the primers and the annealing temperatures in the PCR reactions are given in table 1. Each exon of the apoCII gene of the index patient was individually amplified from 500 ng of genomic DNA. The samples were denatured at 94°C for one minute and annealed for one minute at the temperatures given in table 1 for the different oligomers. The reactions were extended at 72°C for one minute for a total of 30 cycles.

**Sequence analysis**  
The PCR products from the four exons of the apoCII gene of the index patient were precipitated with NH$_4$Ac/ethanol and size fractionated on a 2% agarose gel. The band, corresponding to the amplified exon size was excised and purified using Spinbind DNA Extraction Units (FMC Bioproducts, Denmark) and resuspended in 30 μl H$_2$O.

Taking advantage of the A base overhang added to all templates during the PCR reaction, each amplified and purified fragment was directionally ligated into the TA Cloning pCR vector (TA Cloning System, Invitrogen, San Diego, CA, USA).

The ligation products were then transformed into the recommended INVαE’ cells and incubated for 12 to 14 hours at 37°C on LB agar plates containing kanamycine and X-gal. The following day, six independent white colonies were isolated and grown overnight in LB medium for DNA isolation purposes. Small amounts of the extracted plasmid DNA were digested with HindIII and EcoRI to determine the size of the inserted fragments.

Positive samples were subjected to double stranded DNA sequencing using the Sequenase version 2.0 (US Biochemicals, Cleveland, OH) after annealing of 1 pmol of the required “nested” PCR primer according to the ligated exon. Annealing was performed by heating for two minutes at 65°C and cooling down until room temperature was reached. The labelling reaction took place at 4°C for five minutes followed by termination for five minutes at 39°C. These samples were loaded on a 6% polyacrylamide 5 mol/l urea sequencing gel and electrophoresed at 55°C for two hours at 1900 V.

**Mutation detection using RsaI restriction endonuclease**

Exon 3 of all family members was separately

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sequence of PCR primers for exons 1, 2, 3, and 4 of the apoCII gene</th>
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<tbody>
<tr>
<td>Exon Sequence of PCR primers</td>
<td>Annealing temp</td>
</tr>
<tr>
<td>4' primer: 5'-GTCCTGCGTTGAGAGGAG-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>3' primer: 5'-TCTCAAGCGATCTTGAG-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>4' primer: 5'-GAGAGAGGAGGAGGTG-3'</td>
<td>55°C</td>
</tr>
<tr>
<td>3' primer: 5'-TCTCAAGCGATCTTGAG-3'</td>
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<td>55°C</td>
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amplified. PCR amplified exon 3 DNA (50 ng) was subsequently mixed with 2 μl of recommended buffer and 20 units of RsaI restriction enzyme (Boehringer Mannheim) and incubated at 37°C for three hours. The DNA was then subjected to electrophoresis on 2% agarose gels.

Results

LIPIDS AND LIPOPROTEINS

Values for lipids and lipoproteins for the index patient and two sisters, II.2 and II.6, are summarised in table 2.

In the proband, TG levels are raised, mainly owing to high VLDL concentrations. LDL and HDL cholesterol are slightly decreased. Lipoprotein profiles in the two sisters exhibit no hypertriglyceridaemia; however, VLDL, LDL, and HDL plasma concentrations are decreased in both sisters.

MUTATION ANALYSIS

DNA sequence analysis was performed for all four exons from the index patient. A substitution of A for C was detected at position 3002 in exon 3 (fig 1). This mutation leads to the introduction of a premature stop codon (TAA) at the position corresponding to amino acid 37 of mature apoCII. The index patient represents a true homozygote for this nucleotide substitution, previously reported in another apoCII deficient kindred from Italy as apoCII*mut19,20.

As previously described, this mutation leads to the loss of an RsaI restriction enzyme site (GTAC→GTA) in the normal apoCII gene.21 Amplification of exons from all family members was performed by PCR. Digestion of amplified normal exon 3 with RsaI should result in the formation of two fragments of 180 and 73 bp, respectively. However, digestion of amplified exon 3 from the proband homozygous for this mutation or his heterozygous sibs should yield a single band of 253 bp or a combination of the 253, 180, and 73 bp bands, respectively, as indicated in fig 2.

Fig 3 illustrates the restriction endonuclease analysis of amplified exon 3 of all family members, indicating true homozygosity for the index patient (II.5), heterozygosity for both parents (I.1, I.2), and for two sisters and a brother (II.1, II.2, and II.6). One brother (II.3) did not carry the nucleotide substitution in exon 3. No other DNA alterations were detected in any other exons or in exon-intron boundaries of the apoCII gene.

Discussion

In this study we have identified a nonsense mutation in the apoCII gene, underlying apoCII deficiency and chylomicronaemia in a patient of Italian descent.

Sequence analysis of the apoCII gene of the proband showed, on both alleles, a substitution of cytosine for adenosine (C→A) at nucleotide 3002 in exon 3, resulting in the introduction of a premature stop codon at the position corresponding to residue 37 of the mature apoCII protein. As a consequence, a truncated 36
Apolipoprotein CII-Padova (Tyr111→stop) as a cause of chylomicronaemia

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S Tuzgöl, S M Bijvoet, T Bruin, J J Kastelein and M R Hayden

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