A deletion of one nucleotide results in functional deficiency of apolipoprotein CII (apo CII Toronto)

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SUMMARY Apolipoprotein CII Toronto is a mutant non-functional apo CII resulting in apo CII deficiency. A portion of the mutant apo CII gene was cloned into lambda gt10 and subclones were sequenced. A deletion of one base was found in the codon for amino acid Thr68, resulting in alteration of six amino acids and premature termination of the protein at amino acid 74.

Apolipoprotein CII (apo CII), a major apolipoprotein of chylomicrons and VLDL, and a component of HDL, is the cofactor for the activation of lipoprotein lipase. Lipoprotein lipase, found mainly in adipose tissue and muscle, hydrolyses plasma triglycerides at the surface of capillary endothelium allowing their further transport. The requirement for apo CII in this lipolysis was shown in a patient with no functional apo CII, who had marked fasting chylomicronaemia, hypertriglyceridaemia, and chronic pancreatitis.¹ The deficiency, which we have identified in 14 subjects in a single kindred, is inherited as a autosomal recessive trait, in which homozygotes have 10 to 20 times the normal amount of plasma triglycerides, a lower ratio than normal of apo CII/CIII in VLDL, and minimal lipoprotein lipase activation.^{2,3} The mutant apo CII, apo CII Toronto, has an isoelectric point of 5.54 for the major component, compared with 4.88 for normal apo CII.⁴

The normal apo CII gene has been cloned and its genomic structure determined.⁵ We report here the cloning and sequencing of exons IV and V of the mutant apo CII Toronto gene. A single base pair

deletion results in a frame shift, with an alteration of six terminal amino acids and premature termination of the protein.

Materials and methods

DNA was isolated from the buffy coat of blood collected in EDTA⁶ from an affected homozygote (VI.II), described previously.² The cloning and sequencing strategy was based on the structure of the normal apo CII gene, as described by Wei *et al*⁵ (fig 1). The 3.5 kb *Eco*RI fragment contains the complete gene, except for a small portion of the 3' untranslated region. DNA (200 µg) was digested with *Eco*RI and subjected to electrophoresis in low melt agarose. DNA of fragment size 2.5 to 5 kb was recovered from the gel, passed through a NACS (BRL) column, ethanol precipitated, and ligated into *Eco*RI digested λgt10. Ligated DNA was packaged with an in vitro packaging kit (Gigapak), and plated in *E. coli* C600. The resulting genomic library of 6×10⁵ pfu was screened⁷ using an apo CII cDNA clone, A12,⁸ kindly provided by O Mykelbost and S E Humphries. This cDNA clone contained 320 base pairs of the apo CII gene, beginning within exon III (fig 1). The apo CII insert was nick

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FIG 1 Structure of the normal apo CII gene, based on sequence data.⁶ Untranslated regions are cross hatched, exons are solid. The region included in clone A12⁸ is shown. R=*Eco*RI, P=*Pst*I, V=*Pvu*II.

The DNA sequence was obtained from the region 26 bp 5' to exon IV to 230 bp 5' of the *EcoRI* site at the end of the non-translated region in exon IV, and confirmed on the opposite strand. A portion of the sequencing gel is shown in fig 2. In the mutant gene, a thymine in the third position of the ACT codon for Thr68 has been deleted, causing a frame shift. Results of this frame shift are shown in table 1. Amino acid 68 is unaltered; however, all amino acids after the frame shift are changed, with a premature termination at amino acid 74 instead of at the normal amino acid 79. A hydropathy plot of the mutant protein compared with the normal, using the Pustell sequence analysis program (IBI) (data not shown), showed that the absence of the five terminal amino acids produces a mutant protein with a more hydrophobic terminus.

Blot hybridisation of *TaqI* digested DNA indicated that all five affected homozygotes were homozygous for the 3.8 kb fragment.

Discussion

Deletion of a single base results in a marked alteration of apo CII Toronto from the normal apo CII. The amino acid sequence predicted from the mutant DNA sequence confirms the recent report of the protein sequence of apo CII Toronto, determined after our sequencing studies were in progress.¹⁴ However, the base pair deletion is in the codon for amino acid 68, and not in codon 69 as was predicted from the protein sequence.¹⁴

The additional terminal glutamic acid residues in the normal apo CII account for the lower pI (4.88) in comparison with that of apo CII Toronto (5.54).

Three functional domains of apo CII have been postulated through the study of peptide fragments.¹⁵ These studies indicate that the possible lipid binding domain consists of residues 13 to 22, 29 to 40, and 43 to 52. The lipoprotein lipase activation domain includes residues 56 to 67. Residues 65 to 75 are

predicted to constitute a lipoprotein lipase binding domain. The last three residues, 77 to 79, are apparently required for lipase activation, and their deletion has previously been reported to lead to complete inactivation of apo CII.¹⁵ Apo CII Toronto lacks residues 75 to 79 and is completely inactive, as predicted. The differences at the COOH terminus between the mutant and normal apo CII would be predicted to alter the tertiary conformation of the protein, and perhaps prevent the binding of apo CII to lipoprotein lipase, thus preventing activation. An alteration in the physical conformation is compatible with the observation that antibody to apo CII apparently binds less firmly to apo CII Toronto than to the normal apo CII, at least when the complex is subjected to electrophoresis (W C Breckenridge, 1987, personal communication). However, antigen-antibody binding takes place in solid medium, as in two dimension electrophoresis.⁴ Premature termination of the protein may interfere with binding of both lipoprotein lipase and antibody.

Since this mutation accounts for charge and functional changes, any other nucleotide alterations present in exons not sequenced are unlikely, if present, to be functionally important.

At least eight other families with apo CII deficiency have been described (table 2). No mutants for which the pI of the mutant apo CII was reported or could be estimated from a published photo appeared to be identical to apo CII Toronto. Because of linkage disequilibrium throughout the region of the apo CII gene and the flanking polymorphism, the presence of different sizes of *TaqI* fragments could indicate the presence of different mutant alleles. Alleles in cases 1 and 6 are associated with the 3.8 kb *Taq* fragment and in case 5 with the 3.5 kb fragment, the only cases in which the associated polymorphism has been reported. The use of haplotypes of both *TaqI* and *BglIII* polymorphisms flanking the apo CII gene²⁶ would be helpful in identifying potential different mutations. Case num-

TABLE 2 Patients (probands) with apolipoprotein CII deficiency.

Case No	CII name	Sex	Age of proband at ascertainment	Ethnic origin	Mutant protein by IEF*	Refs
1	Toronto	M	59	British	+(pI 5.5)	1, 2
2		F	13	Japanese	?	16
3		F	2	Italian (?)	+	17
4		F	30	British (?)	+	18
5	Padova	M	33	Dutch	+(↓ pI)†	19, 20
6		M	41	Italian	+(pI~4.95)†	21, 22
7		F	11	American	+(pI<4.9)	22, 23
8	Bethesda	M	5	Puerto Rican	—	24
9	St Michaels	F	54	Anglo-Saxon	+(forms dimers)	25

*Normal pI of major band 4.88.

†Based on appearance of gel in publication; +=protein present.

bers 1, 5, 6, 8, and 9 appear to result from different mutations.

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