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ANALYSIS OF THE ApoC-II GENE IN ApoC-II DEFICIENT PATIENTS

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Apolipoprotein C-II (apoC-II), a 79 amino acid protein, is a cofactor for lipoprotein lipase, the enzyme which catalyzes the lipolysis of triglycerides on plasma chylomicrons and VLDL. Patients with apoC-II deficiency have marked elevations in plasma triglycerides, chylomicrons, VLDL, and a type I hyperlipoproteinemia. In order to evaluate the molecular defect in apoC-II deficiency, genomic DNA was analyzed using Southern Blot from 2 independent apoC-II deficient patients and compared to normal controls. Restriction digests of genomic DNA were performed with five different enzymes and the restriction fragments analyzed utilizing a 354 base pair nick-translated apoC-II probe for hybridization following Southern blotting. The restriction fragments varied from 0.8 to 21 Kb, and the pattern with normal DNA was identical to that of the two apoC-II deficient patients. The present study reveals that the apoC-II gene is present in patients with apoC-II deficiency. In addition, no insertional or deletional polymorphism was detected in the apoC-II gene of apoC-II deficient patients.

Human plasma apolipoprotein[†] C-II, a 79 amino acid protein, plays a pivotal role in lipid metabolism as a cofactor for lipoprotein lipase, the enzyme which hydrolyzes the triglycerides in plasma chylomicrons and VLDL (1-3). Recently we have reported the complete nucleic acid sequence of preapoC-II (4). The derived amino acid sequence contains a 22 residue prepeptide which is co-translationally cleaved prior to secretion, as well as a 79 amino acid mature protein with a sequence identical to that previously described (5). The apoC-II gene has now been localized to chromosome 19 (6,7) which also contains the apoE (8) and LDL receptor (9) genes.

[†] Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoprotein; Kb, kilobase; TBE, Tris borate EDTA, pH 8.3; SSC, standard saline citrate, pH 7.1; $NaDodSO_4$, sodium dodecylsulfate; BRL, Bethesda Research Laboratories.

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ApoC-II is synthesized primarily by the liver (10) and secreted into plasma where it is reversibly bound principally to chylomicrons, VLDL, and HDL (11). ApoC-II in HDL rapidly associates with newly secreted triglyceride rich lipoproteins synthesized by the liver and intestine. Following lipolysis, triglyceride rich lipoproteins are converted to remnants and apoC-II dissociates from these particles and reassociates with HDL (12). The distribution of apoC-II on plasma lipoproteins is continually changing between HDL and triglyceride rich lipoproteins as a result of the secretion, metabolic conversion, and catabolism of the plasma lipoproteins (11).

The importance of apoC-II as an activator of lipoprotein lipase has been established by the absence of lipoprotein lipase activity in patients with apoC-II deficiency. This syndrome, which is inherited as an autosomal recessive trait, has been described in various independent families (13-18). Clinical presentation in homozygous individuals may include xanthelasma, hepatosplenomegaly and pancreatitis. Because the clearance of chylomicrons is greatly impaired, these patients have marked elevation of plasma triglycerides, chylomicrons, decreased LDL and HDL concentrations, and a type I lipoprotein phenotype. Infusions of normal plasma (13,16,18), or isolated apoC-II fractions (18) result in transient normalization of plasma triglycerides and lipoproteins. ApoC-II deficiency is documented by the absence of apoC-II by radioimmunoassay or immunoelectrophoresis, and indirectly by an absence of lipoprotein lipase activity corrected by the addition of apoC-II containing plasma. At the present time, the molecular defect that results in apoC-II deficiency is unknown. In order to study the nature of the underlying defect in apoC-II deficiency, we have utilized Southern blot techniques to determine the presence of major insertional or deletional polymorphisms in the DNA of 2 patients with apoC-II deficiency.

MATERIALS AND METHODS

Apolipoprotein C-II cDNA probe: The cDNA probe used in this study was obtained from an apoC-II cDNA clone which contained the nucleic acid sequence encoding for the entire mature apoC-II protein and the 22 amino acid containing prepeptide (4). A 354 base pair long cDNA insert which encoded for most of the structural sequence of apoC-II as well as part of the 3' untrans-

lated end (see Fig. 1) was obtained by cleavage with Alu 1 (BRL, 3U of enzyme/μg DNA for 2 hours at 37°C) and purified by agarose gel electrophoresis. Four hundred nanograms of purified DNA was radiolabeled to a specific activity of 1 x 10^9 cpm/μg by nick-translation (19).

DNA preparation: Blood for DNA isolation was collected from normolipidemic volunteers into tubes containing a final concentration of 3mM Na EDTA. ApoC-II deficient blood was obtained from an 11 yo American white female and a 41 yo Italian white male previously documented to have apoC-II deficiency (15,20). DNA was isolated from whole blood cell nuclei as described by Bell et al. (21), and yields of DNA were 200-500 μg/ml per 10 ml of blood.

Southern blot analysis: Ten micrograms of DNA were digested with 40 units of BamHI (BRL), Bgl I (BRL), EcoRI (BRL), Hind III (BRL) and SST 1 (BRL) for 4 hrs at 37°C. The incubation was terminated by the addition of 20 mM EDTA, and the reaction mixture was heated in a 65°C bath for 10 min. The restriction fragments were separated by 0.7% agarose slab gel electrophoresis (0.7 x 11 x 14 cm) at 35 V for 16 hr in TBE. DNA was transferred to nitrocellulose membrane filter paper (Schleicher and Schuell) bidirectionally as described by Southern (22) and baked for 2 hr at 80°C. Filters were incubated for at least 6 hr in 20 ml of pre-hybridization solution containing 5X SSC, 5X Denhardt's solution, 0.1% $NaDodSO_4$ and 100 μg/ml salmon sperm DNA at 65°C in a sealed bag. Hybridization was performed for 20 hr at 65°C in 20 ml of buffer containing 5X SSC, 5X Denhardt's solution, 0.1% $NaDodSO_4$, and 1 X 10^7 cpm nick-translated apoC-II insert DNA.

RESULTS

Figure 1 illustrates the restriction endonuclease cleavage map of the apoC-II cDNA insert in the recombinant plasmid clone. The 354 base pair long Alu 1 fragment used to prepare the nick-translated probe contained 15 amino

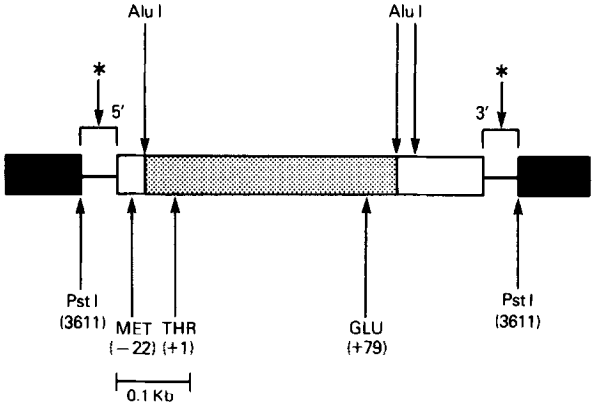

Figure 1. Map of the apoC-II cDNA insert of the recombinant plasmid clone. The insertion of apoC-II cDNA (open bar) and its orientation in pBR322 plasmid DNA (solid bar) are indicated. The mRNA sequence is represented by the upper DNA strand with the 5' and 3' termini as indicated. Asterisks indicate the sites of the dG-dC tails. Sites of cleavage by Alu 1 restriction endonuclease are indicated. The stippled area represents the 354 base pair Alu 1 fragment used to prepare the nick-translated probe. Its orientation with respect to the preapoC-II amino acid sequence is indicated.

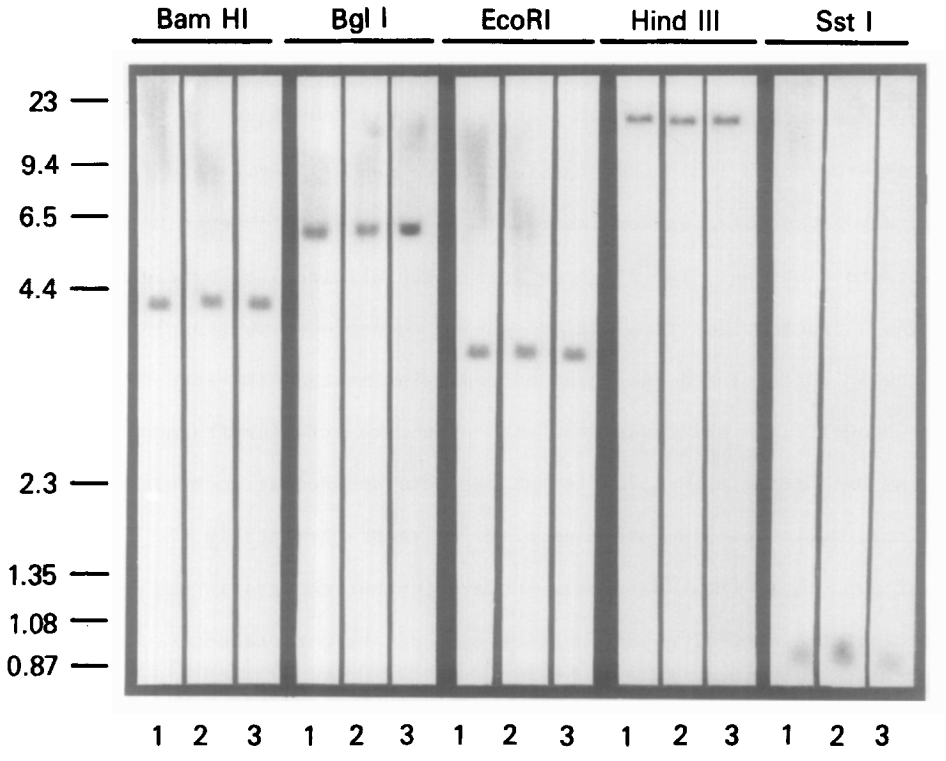

Figure 2. Autoradiogram of Southern blotting analysis of restriction endonuclease-digested chromosomal DNA from 2 patients with apoC-II deficiency (lanes 1 and 2) and a normolipidemic subject (lane 3). The restriction endonuclease enzymes used are indicated at the top.

acids from the prepeptide sequence of apoC-II and included 73 nucleotides from the 3' untranslated end (Fig. 1).

Southern blot analysis of DNA from the 2 apoC-II deficient patients and a representative normal control after digestion with restriction enzymes BamHI, Bgl I, EcoRI, Hind III and SST I is shown in Figure 2. The sizes of the restriction fragments which hybridized to the apoC-II cDNA probe were 4.3 Kb, 6 Kb, 3.6 Kb, 21 Kb, and 0.8 Kb, respectively. In all cases the apoC-II gene of apoC-II deficient patients was identical in size to that of the normal control.

DISCUSSION

In this paper we analyze the apoC-II gene in 2 patients from independent families with apoC-II deficiency. Although deficiency of apoC-II in the plasma of patients with this syndrome has been documented by functional and

immunologic techniques, the basic molecular defect in apoC-II deficiency is not understood (13-18). Alteration of the apoC-II gene or of the genes regulating apoC-II expression or processing could result in the deficiency.

Our study of 2 independent patients with apoC-II deficiency failed to detect any gross deletions or insertions in their apoC-II genes when analyzed by Southern blotting. The 354 base pair probe utilized for hybridization contains 44 nucleotides from the prepeptide sequence as well as 73 nucleotides from the 3' untranslated end, thus encompassing most of the structural gene for preapoC-II. In all cases, the size of the restriction fragments obtained from the DNA of the apoC-II deficient patients and normal controls was identical, ruling out the presence of major rearrangements in the structural gene of apoC-II in these patients. However, minor changes in the nucleotide sequence of the apoC-II gene cannot be excluded by our present study. These findings are similar to those seen in one kindred with apoA-I and C-III deficiency where no restriction enzyme polymorphism was detected (23) but different from a second kindred with apoA-I and C-III deficiency where a 7.5 Kb DNA insertion in one of the exons of the apoA-I gene results in absence of apoA-I and apoC-III (24).

The analysis of the underlying molecular defect in apoC-II deficiency may provide new information on factors which normally modulate apoC-II biosynthesis and processing.

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REFERENCES

1. LaRosa, J.C., Levy, R.I., Herbert, P., Lux, S.E. and Fredrickson, D.S. (1970) Biochem. Biophys. Res. Comm. 41, 45-62.
2. Havel, R.J., Shore, J.G., Shore, B. and Bier, D.M. (1970) Circ. Res. 27, 597-600.
3. Smith, L.C., Pownall, H. and Gotto, A.M. (1978) Ann. Rev. Biochem. 47, 751-777.
4. Fojo, S.S., Law, S.W. and Brewer, H.B. Jr. (1984) Proc. Natl. Acad. Sci. USA (in press).
5. Hospattankar, A.V., Fairwell, T., Ronan, R. and Brewer, H.B. Jr. (1983) J. Biol. Chem. 259, 318-322.

6. Fojo, S.S., Law, S.W., Brewer, H.B., Jr., Sakaquchi, A.Y. and Naylor, S.L. (1984) Biochem. Biophys. Res. Comm. 122, 687-693.
7. Jackson, C.L., Bruns, G.A. and Breslow, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 2945-2949.
8. Olaisen, B., Teisburg, P. and Gedde-Dahl, T. Jr. (1982) Human Genet. 62, 233-236.
9. Francke, U., Brown, M.S. and Goldstein, J.L. (1984) Proc. Natl. Acad. Sci. USA 81, 2826-2830.
10. Wu, A.L. and Windmueller, H.G. (1979) J. Biol. Chem. 254, 7316-7322.
11. Osborne, J.C. Jr. and Brewer, H.B. Jr. (1977) Adv. Protein Chem. 31, 253-337.
12. Eisenberg, S., Bilheimer, D.W., Levy, R.I. and Lindgren, F.T. (1973) Biochem. Biophys. Acta. 326, 361-377.
13. Breckenridge, W.C., Little, J.A., Steiner, G., Chow, A. and Poapst, M. (1978) N. Engl. J. Med. 298, 1265-1273.
14. Yamamura, T., Sudo, H., Ishikawa, K. and Yamamoto, A. (1979) Atherosclerosis 34, 53-65.
15. Crepaldi, G., Fellin, R., Baggio, G., Augustin, J. and Greten, H. (1980) In: Atherosclerosis V. (A.M. Gotto, Jr., L.C. Smith and B. Allen, eds.) Springer-Verlag, New York, pp. 250-254.
16. Miller, N.E., Rao, S.N., Alaupovic, P., Nobel, N., Slack, J., Brunzell, J.D. and Lewis, B. (1981) Europ. J. Clin. Invest. 11, 69-76.
17. Stalenhalf, A.F.H., Caspaire, A.F., Demacher, P.N.M., Stouten, J.T.J., Lutterman, J.A. and Van't Laar, A. (1981) Metabolism 30, 919-926.
18. Catapano, A.I., Mills, G.L., Roma, P., LaRosa, M. and Capurso, A. (1983) Clin. Chem. Acta 130, 317-327.
19. Rigby, R.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
20. Sprecher, D.L., Taam, L. and Brewer, H.B. Jr. (1984) Clin Chem. (in press).
21. Bell, G.I., Karam, J.H. and Rutter, W.J. (1979) Proc. Natl. Acad. Sci. USA 78, 5759-5763.
22. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
23. Schaefer, E.J., Ordovas, J.M., Law, S.L., Ghiselli, G., Kashyap, M.L., Srivastava, L.S., Heaton, W.H., Albers, J.J., Connor, W.E., Lindgren, F.T., Lemeshev, Y, Segrest, J.P. and Brewer, H.B., Jr. (1984) Manuscript in preparation for publication.
24. Karathanasis, S.K., Norum, R.A., Zannis, V.I. and Breslow, J.L. (1983) Nature 301, 718-720.