FAMILIAL HYPERLIPOPROTEINEMIA TYPE III: DEFICIENCY OF A SPECIFIC APOLIPOPROTEIN (APO E-III) IN THE VERY-LOW-DENSITY LIPOPROTEINS

G. UTERMANN, M. JAESCHKE and J. MENZEL
Institut für Humangenetik der Universität 3550 Marburg/Lahn, Bahnhofstrasse 7 A, BRD

Received 13 June 1975

1. Introduction

Familial hyperlipoproteinemia type III is a primary disorder of lipid metabolism characterized by an elevation of cholesterol and triglycerides in plasma and by the occurrence of an atypical lipoprotein in the VLDL fraction of affected individuals. This lipoprotein has β- instead of pre-β-mobility in electrophoresis [1] and is rich in cholesterol [2] and in an arginine-rich apolipoprotein [3]. There is evidence that this lipoprotein is an intermediate in the catabolism of triglyceride-rich lipoproteins which accumulate in hyperlipoproteinemia type III [3].

The disease is associated with premature arteriosclerosis, but is usually not expressed before the age of twenty. The primary biochemical lesion in the familial disorder is not known and there is uncertainty on its mode of inheritance [1,4,5].

In the present study we have shown that one protein component of mol. wt ~ 39 000 is deficient in the VLDL from patients with hyperlipoproteinemia type III. This most likely is the underlying defect in the hereditary dyslipoproteinemia.

2. Materials and methods

Fasting plasma was obtained from eight patients with hyperlipoproteinemia type III, five first degree relatives of type III probands, who had type IV or type II hyperlipoproteinemia, sixteen normolipidemic blood donors, twenty patients with type IV and one with type V hyperlipoproteinemia. The diagnosis of type III hyperlipoproteinemia was established by demonstration of primary hyperlipidemia, β-migrating VLDL and a cholesterol: triglyceride ratio in VLDL considerably higher than 0.2 (between 0.53 and 0.77 in all the patients studied here) [6,7]. The eight type III patients were all from different families. A detailed description of these patients and their relatives will be published elsewhere [8]. Two of the type III patients have already been documented in previous publications (E. M. = III, 19 in [9] and E. R. in [10]) and familial involvement has been verified in both [8,10].

Lipoprotein fractions VLDL, LDL-1, LDL-2 and HDL were isolated by sequential ultracentrifugation in a Spinco model L2-65 B preparative centrifuge according to standard procedures [11] with minor modifications [12]. Electrophoretic analysis of lipoproteins in agarose gels was done as described by van Melsen et al. [13]. Soluble lipoproteins were delipidated by five successive extractions with 50 volumes of acetonethanol 1:1 (v/v) at $-15°C$ and finally dried with nitrogen [14]. The arginine-rich apolipoprotein E and the C apoproteins were isolated from apo VLDL by preparative SDS-PAGE in the discontinous system of acetone-ethanol 1:1 (v/v) at $-15°C$ and finally dried Analytical SDS-PAGE was performed according to Weber and Osborne [16] using a 10% monomer concentration. Analytical isoelectric focusing of the urea soluble apo VLDL polypeptides was done by an adaption [12] of the method of Wrigley [17]. Scanning densitometry of the focusing gels was performed after staining with Coomassie brillant blue in a Vitatron

Abbreviations: VLDL: Very-low-density lipoproteins (d < 1.006 g/ml). LDL-1: Low-density-lipoproteins-1 (d = 1.006–1.019 g/ml). LDL-2: Low-density-lipoproteins-2 (d = 1.019–1.063 g/ml). HDL: High-density-lipoproteins (d = 1.063–1.21 g/ml). PAGE: Polyacrylamide gel electrophoresis. SDS: Sodium dodecyl suphate.

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TLD. Standard curves for quantitative determinations of Apo C and Apo E in the urea soluble fraction of apo VLDL were established by running known amounts of pure apolipoproteins in parallel gels. Protein was determined according to Lowry et al. [18] with 0.1% SDS in the reaction mixture, cholesterol by the procedure of Roeschlau et al. [19] and triglycerides by the method of Eggstein and Kreutz [20].

3. Results and discussion

In a search for the primary metabolic defect in familial hyperlipoproteinemia type III, we have studied the polypeptide composition of VLDL in patients with hyperlipoproteinemia type III, first degree relatives of type III probands with and without other phenotypic expressions of dyslipoproteinemia and for comparison a control group comprising of normolipidaemic volunteers and of patients with type IV and V hyperlipoproteinemia.

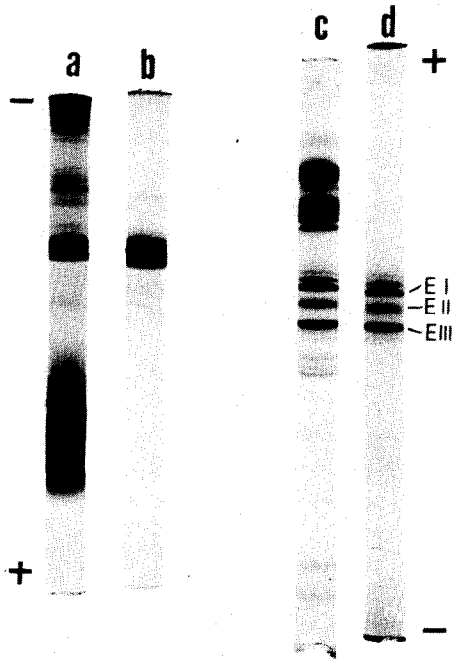

Fig.1. (Left) SDS-PAGE of apo VLDL (a) and purified Apo E (b). (Right) Analytical isoelectric focusing of urea soluble apo VLDL in polyacrylamide gels at pH 3.5–10 (c) and the same apo E preparation as in gel b (d).

The characteristic polypeptide composition of VLDL as demonstrated by SDS-PAGE is shown in fig.1. There are three main components in this fraction: Apo B, Apo C and an arginine-rich protein we have designated Apo E [14]. The latter protein, which has an apparent mol. wt of ~39 000 in SDS-PAGE, is heterogenous and shows three major components in analytical isoelectric focusing in the presence of 6 M urea (fig.1, [14]).

The three Apo E polypeptides were demonstrated in a ratio of roughly 2:1:2 in the apo VLDL of all the thirty-seven controls. Quantitatively Apo E represented 18% of the urea soluble fraction of normolipidaemic subjects (table 1) and about 10% of the total apo VLDL [14]. On the other hand, none of type III patients demonstrated this normal apo VLDL focusing pattern. Two deviations from normal were evaluated by inspection and standardizing scanning densitometry of the stained gels (fig.2, table 1). First the percentage of apo E in the urea soluble apo VLDL was on the average 57% which is considerably higher than in the controls. This finding is in general agreement with previous results of Havel and Kane [3]. Despite the predominance of the arginine-rich Apo E in this fraction, none of the eight type III probands showed significant amounts of a protein preliminary designated Apo E-III ([14] fig.1,2) which was present in all the controls.

Apo E-III could not be detected in any of the other lipoprotein fractions of the patients and seems thus to be deficient in their lipoprotein system. Only traces of Apo E were detected in the HDL and LDL-2 of the type III patients by SDS-PAGE or isoelectric focusing. Their LDL-1, however, contained particles of β-mobility which were extremely rich in total Apo E, but the specific Apo E-III was also deficient in this fraction (fig.3).

The catabolism of triglyceride-rich lipoproteins proceeds through different steps [21]. One of these is apparently impaired in familial type III hyperlipoproteinemia, resulting in the accumulation of the intermediate β-VLDL [3]. Recent evidence along with that presented here suggests that Apo E is intimately associated with the conversion of triglyceride-rich to cholesterol ester-rich lipoproteins and is primarily involved in the transport and metabolism of cholesterol [3,8,22–24]. The specific function of the arginine-rich Apo E in this process is not yet

Table 1
Characteristics of VLDL fractions from patients with primary hyperlipoproteinemia type III

Patient	Phenotype	C/TG	Mobility prae-β	β	Urea soluble apoproteins (weight %) Apo C	Apo E	Apo E (% of densitometric area) E-I	E-II	E-III
H. Sch.	III	0.53	+	+	60	40	42	58	–
E. R.	III	0.53	+	+	27	73	41	59	–
E. M.	III	0.66	+	+	44	56	42	58	–
Ch. J.	III	0.55	+	+	41	59	51	49	–
S. J.	III	0.77	+	+	45	55	42	58	–
Mean ± SD		0.63 ± 0.09			43 ± 12	57 ± 12	43.6 ± 4.4	56.4 ± 4.4	–
Control[a]	N	0.24	+	–	82	18	38	23	39

C/TG = Cholesterol/Triglyceride ratio
[a]VLDL isolated from a pool of 12 normolipidemic blood donors.

understood. The data known on the metabolic role of Apo E, however, is compatible with the hypothesis that a defect in the regulation or structure of Apo E-III is responsible for the hereditary human disease hyperlipoproteinemia type III.

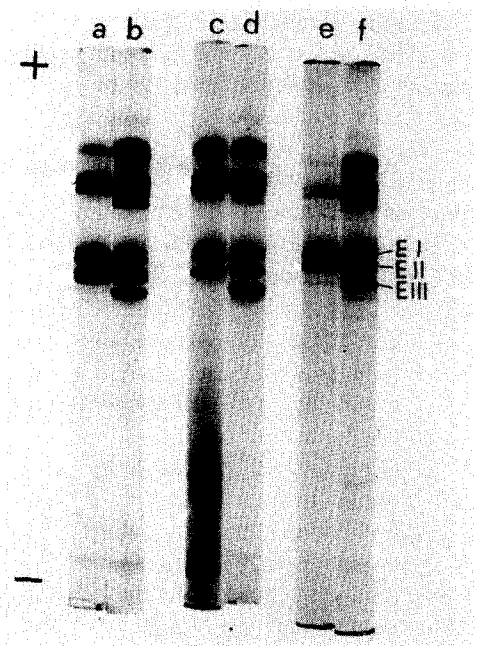

Fig.2. Analytical isoelectric focusing in polyacrylamide gels at pH 3.5–10 of apo VLDL from patients E. M. (a), H.Sch. (c) and E. R. (e) with familial hyperlipoproteinemia type III, the son O. M. of proband E. M. who had a type IV pattern (b) and controls (d,f).

The data presented here suggest that Apo E-III deficiency is a genuine marker for familial broad-β-disease. This marker may help to establish the diagnosis in uncertain cases and allows to differentiate secondary forms [8].

Apo E-III was not deficient in the apo VLDL from first degree relatives of type III probands, who had

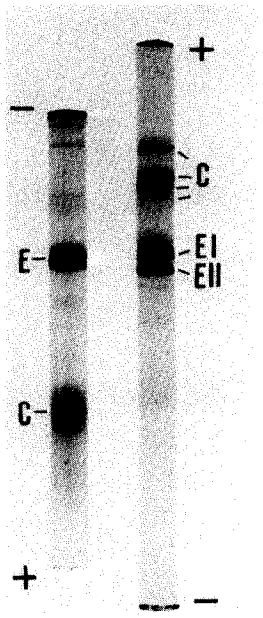

Fig.3. SDS-PAGE (left) and analytical isoelectric focusing at pH 3.5–10 (right) of urea-soluble apo LDL-1 proteins from patient E. M. with hyperlipoproteinemia type III.

other phenotypic forms of hyperlipidemia (fig.2), and in both parents of one type III proband (E. R.). The genetical aspects of these observations and a family study which is in progress in our laboratory will be discussed elsewhere in detail [8]. The preliminary results from the family study indicate that the deficiency of Apo E-III is transmitted by an autosomal recessive mode of inheritance and that patients with type III are homozygous for this defect.

Acknowledgments

We thank Miss Annegret Knauf and Miss Lucie Theus for skillful technical assistance. We are indepted to Professor Canzler (Hannover), Professor Kaffarnik, Drs G. and O. Mühlfellner, Dr J. Schneider (Marburg/Lahn), Dr W. Schoenborn (Starnberg) and Dr K. H. Vogelberg (Düsseldorf) for providing plasma from their hyperlipidaemic patients.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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