Deoxyribonucleic acid polymorphism in the apolipoprotein A-1-C-III gene cluster. Association with hypertriglyceridemia.

A Rees, … , F E Baralle, D J Galton

J Clin Invest. 1985;76(3):1090-1095. https://doi.org/10.1172/JCI112062.

Research Article

A DNA sequence polymorphism, revealed by digestion of human DNA with the restriction endonuclease Sst-1 and hybridization with an apolipoprotein A-I complementary DNA clone, has been shown to be located in or close to the 3' noncoding region of the apolipoprotein C-III gene. This polymorphism is found in significantly increased prevalence (P less than 0.001) in Caucasian hypertriglyceridemic subjects compared with race-matched controls, and its distribution in normal individuals of differing racial origins is reported. Furthermore, no alteration of high density lipoprotein or apolipoprotein A-I and apolipoprotein C-III phenotypes was observed in individuals with or without the polymorphism.

Deoxyribonucleic Acid Polymorphism in the Apolipoprotein A-1–C-III Gene Cluster

Association with Hypertriglyceridemia

A. Rees, J. Stocks, C. R. Sharpe, M. A. Vella, C. C. Shoulders, J. Katz, N. I. Jowett, F. E. Baralle, and D. J. Galton
Medical Professorial Unit and Department of Chemical Pathology, St. Bartholomew's Hospital, London EC1A 7BE, and Sir William Dunn School of Pathology, Oxford, England OX1 3RE

Abstract

A DNA sequence polymorphism, revealed by digestion of human DNA with the restriction endonuclease Sst-1 and hybridization with an apolipoprotein A-I complementary DNA clone, has been shown to be located in or close to the 3′ noncoding region of the apolipoprotein C-III gene. This polymorphism is found in significantly increased prevalence ($P < 0.001$) in Caucasian hypertriglyceridemic subjects compared with race-matched controls, and its distribution in normal individuals of differing racial origins is reported. Furthermore, no alteration of high density lipoprotein or apolipoprotein A-I and apolipoprotein C-III phenotypes was observed in individuals with or without the polymorphism.

Introduction

Hypertriglyceridemia is a common disorder arising from the overproduction or defective clearance of triglyceride-rich lipoproteins. Several studies have indicated that in some cases the disorder has a familial basis (1). The best-established origins are for those rare monogenic forms where there is defective clearance resulting from reduced or absent lipoprotein lipase activity, as in type 1 hyperlipoproteinemia or the absence of apolipoprotein C-II (2), which is an activator of the enzyme. Lipoprotein lipase is the rate-limiting enzyme for the clearance of chylomicrons and very low density lipoprotein from plasma (3). However, in the majority of cases, it is not possible to identify the genes involved, and many familial forms of hypertriglyceridemia do not show a simple Mendelian pattern of inheritance. This probably arises from the strong environmental influence on plasma triglyceride levels, which are markedly affected by intake of carbohydrate, fat, and ethanol. Thus, both genetic and environmental factors may contribute to the expression of the disease.

The analysis of genetic abnormalities can be determined directly in an individual's DNA by using gene-specific DNA probes (4) even when there is no identifiable phenotypic variation. Human DNA shows variation in the nucleotide sequence that can be detected by differences in the size of the discrete DNA fragments produced by digestion with restriction endonucleases when hybridized with the DNA probes. These are not necessarily of any pathologic significance but can serve as markers for particular alleles. The use of these restriction fragment length polymorphisms (RFLP)[1] allows direct analysis of the variations in genotype associated with disorders that may have a familial component. Differences in the hybridization patterns identify different alleles and can be used to determine differences in allele frequency associated with a particular disease compared with the frequency in the general population or to study co-inheritance of a gene and a disease within families. This approach has generally been used in the study of monogenic disorders displaying simple Mendelian inheritance, but it can be equally applicable to metabolic disorders where there is a strong genetic component interacting with environmental factors.

The study of RFLP around the gene coding for apolipoprotein A-1 (apo A-1), the major apoprotein of high density lipoprotein (HDL), has demonstrated a polymorphic restriction site for the endonuclease Sst-1 in the 3′ flanking region of the gene. With the use of a complementary DNA (cDNA) cloned from the messenger RNA (mRNA) of apo A-1 as a hybridization probe, two types of alleles were demonstrated. The common allele, found in >95% of our local population, generates 5.7-kilobase (kb) and 4.2-kb A-1 gene fragments with the restriction endonuclease Sst-1. With the uncommon allele, a 3.2-kb fragment is found instead of the 4.2-kb fragment. This difference arises from the presence of an additional Sst-1 recognition site. Although the uncommon allele is found in <5% of the control group, it was demonstrated in 12 out of 28 patients with types IV and V hyperlipidemia (5).

Karathanasis et al. (6) have shown that the gene coding for apolipoprotein C-III (apo C-III) is situated 2.6 kb from the 3′ end of the apo A-1 gene. Thus the polymorphic site should be within or close to the apo C-III gene. The 3′ noncoding region of the gene contains a possible site for this polymorphism (6). Both apoprotein A-1 and C-III have important roles in the metabolism of triglyceride-rich lipoproteins, apoprotein A-1 being a major component of HDL, which serves as an acceptor for surface components of very low density lipoprotein (VLDL) and chylomicrons during their catabolism. Apoprotein C-III is the major low molecular weight apoprotein of VLDL and chylomicrons and may modulate hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase (7) and also influence the hepatic uptake of chylomicron remnants (8). The DNA sequence variation in this gene cluster revealed by the RFLP with Sst-1, which is associated with hypertriglyceridemia, could affect the regulation of the C-III gene or be a marker for an abnormality in the structure or regulation of the genes coding for A-1 or C-III. Alternatively, it could be in linkage disequilibrium with another gene involved in lipoprotein metabolism. To characterize this polymorphism further both at the genetic and phenotypic level, we have determined the lipoprotein and apoprotein levels of

Address requests for reprints to Dr. Galton, Department of Medicine, St. Bartholomew's Hospital, London EC1A 7BE, England.

Received for publication 25 April 1984 and in revised form 16 October 1984.

J. Clin. Invest.

0021-9738/85/09/1090/06 $1.00
Volume 76, September 1985, 1090–1095

1. *Abbreviations used in this paper:* apo, apolipoprotein; kb, kilobase; RFLP, restriction fragment length polymorphism.

individuals with different genotypes to establish whether there is any difference in the levels of apoprotein A-1, HDL, or in C-III isoforms.

The DNA polymorphism has also been investigated to demonstrate whether the polymorphism in hypertriglyceridemic subjects is located within the C-III gene and is thus revealed by hybridization with an apo C-III gene probe which covers the 3′ end of the C-III gene. Finally, because the frequency of DNA polymorphisms can show wide racial differences, we have studied the distribution of the different alleles in various racial groups.

Methods

Subjects. Hypertriglyceridemic subjects were selected from the lipid clinic at St. Bartholomew's Hospital. Hypertriglyceridemia was diagnosed when three fasting serum samples contained triglyceride concentrations > 2 mmol/liter. They were comprised of 13 females and 61 males. 19 of the patients had noninsulin-dependent diabetes. The mean values for cholesterol in the group were 8.2±1.8 mmol/liter and for triglycerides 12.7±19.4 mmol/liter. The Caucasian control subjects were normal healthy males attending a health screening center who had normal fasting triglyceride values (<2 mmol/liter on a single estimation). Control subjects were also chosen from different racial groups from the local population who were screened for hypertriglyceridemia.

Lipoproteins. VLDL triglyceride, HDL cholesterol, and low density lipoprotein (LDL) cholesterol were measured by ultracentrifugation and precipitation with heparin/$MnCl_2$ according to the Lipid Research Clinics standard procedure (9). The analysis of cholesterol and triglyceride in serum and lipoprotein fractions was performed using fully enzymatic methods (Boehringer Corporation, Ltd., United Kingdom).

Apoproteins. Apoprotein A-1 was measured by single radial immunodiffusion using prepared plates and standards according to the manufacturer's instructions (Immuno AG, Vienna, Austria).

VLDL was isolated by ultracentrifugation in 0.195 mmol/liter NaCl, 0.2 mmol/liter EDTA at 40,000 rpm for 16 h in a 8 × 50-ml angle head rotor. HDL was isolated by gradient density centrifugation according to the procedure of Havel (10) and then purified by ultracentrifugation at density 1.20 for 30 h at 50,000 rpm in a 8 × 20-ml angle head rotor.

Lipoproteins were delipidated with ethanol/ether (10) and then the apoproteins were dissolved in freshly prepared urea (6 mol/liter). Apo VLDL was subjected to flatbed isoelectric focusing as described previously (11). Apo HDL was focused in vertical rods at pH 4–7 using the procedure of Swaney and Gidez (12).

A-1 genotyping. DNA was prepared from the nuclear fraction of blood lysed with Triton X-100 using the procedure of Kunkel et al. (13). 10 ml of frozen whole blood in EDTA was thawed, diluted with 90 ml of cold buffer (0.32 mmol/liter sucrose; 10 mmol/liter Tris, pH 7.5; 5 mmol/liter $MgCl_2$; and 1% vol/vol Triton X-100), and centrifuged at 10°C and 5,000 *g* for 10 min. The pellet was resuspended in 5 ml of 0.075 mmol/liter NaCl-EDTA, and 250 μl of 10 g/dl sodium dodecyl sulphate and 100 μl of 10 mg/ml proteinase K was added and the suspension was incubated for 16 h at 37°C. It was then mixed with 5 ml of phenol and then the phenol was extracted by the addition of 5 ml of chloroform/octanol (24:1 vol/vol). The mixture was centrifuged to separate the phases, and then the aqueous phase was extracted with an equal volume of chloroform/octanol. DNA was precipitated from the aqueous phase by addition of 2 vol of ethanol and then redissolved in 10 mmol/liter Tris–1 mmol/liter EDTA, pH 7.4, over a period of 48 h.

15–20 μg of DNA was digested with 2 U Sst 1/μg DNA (Bethesda Research Laboratories) for 16 h. The restricted DNA was electrophoresed in 0.85% agarose at 2 V/cm for 16 h and then transferred to nitrocellulose membranes by Southern blotting (14). The filters were preincubated in 50% formamide, 3× standard saline citrate, 20 μg/ml herring sperm DNA, 2 mg/ml polyvinylpyrrolidine, 2 mg/ml Ficoll, and 2 mg/ml bovine serum albumin for 16–24 h at 42°C. Hybridization was carried out in the same medium with the addition of 0.1 g/ml dextran sulphate and 5 × 10^6 cpm of ^{32}P-labeled genomic A-1 or C-III probe for 24–36 h at 42°C. The filters were washed successively with 3×, 1×, and 0.1× standard saline citrate for 30 min at 62°C and then autoradiographed for 2–7 d at −70°C. The M-13 clones used in the hybridization were prepared from subclones of the human genomic library as described (15), or from a human liver cDNA library. They were labeled using the primer extension procedure as described to an activity of 1 × 10^8 cpm/μg.

Results

Sst-1 polymorphism in the A-1–C-III gene cluster and hypertriglyceridemia. Digestion of DNA from normolipidemic Caucasian individuals with Sst-1 commonly yields two fragments, 5.7 and 4.2 kb long, which hybridize to the apo A-1 probe. They arise from intragenic cleavage in the third exon of the A-1 gene and in the 5′ and 3′ flanking regions. A different allele is found in some individuals who yield a 3.2-kb fragment instead of the 4.2-kb fragment. Heterozygotes for the uncommon allele show both 3.2- and 4.2-kb fragments (Fig. 1). The 3.2-kb allele was not found in any of 52 male Caucasians who had fasting plasma triglycerides below 2 mmol/liter. However, the 3.2-kb allele was found in 30% of Caucasian subjects with type IV and V hypertriglyceridemia (Table I). In our sample, three subjects were homozygous for the 3.2-kb allele, 23 were heterozygous, and 48 showed the normal pattern. There was no difference in the frequency of the 3.2-kb allele in hypertriglyceridemics with or without type 2 diabetes.

Distribution of the polymorphism in racial and ethnic groups. Whereas the 3.2-kb allele is rare in normolipidemic Caucasians, it was found in much greater frequency among normolipidemic individuals from different racial groups. It was found in ~65% of Chinese and in 30% of Africans and Indian Asians. Gene frequencies in the different racial groups are given in Table I. There was no significant difference in triglyceride levels between

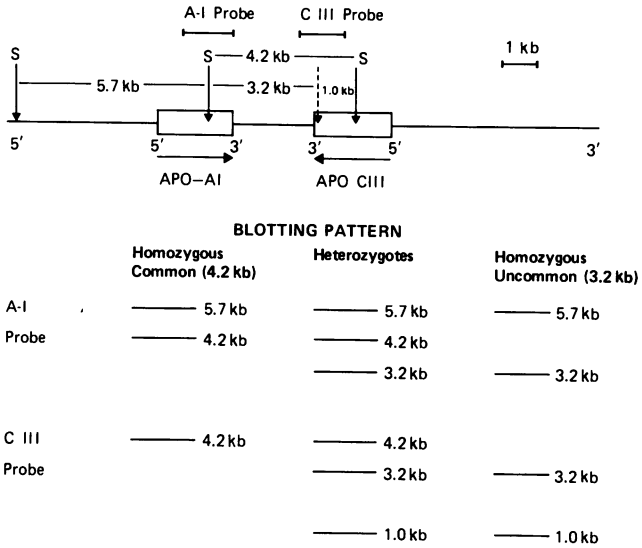

Figure 1. Map of the apo A-1 and C-III genes with the Sst-1 sites and the polymorphic locus. The polymorphic Sst-1 site is indicated (↓). Note that the polymorphic locus is due to the creation of a new Sst-1 site at the 3′ end of the C-III gene so that 3.2- and 1-kb fragments are produced instead of a 4.2-kb fragment. The blotting patterns illustrated are those obtained with the different genotypes, i.e., homozygous for the 4.2- or 3.2-kb alleles and heterozygotes, when each is hybridized with an A-1 genomic probe or a C-III genomic probe.

Table I. Frequency of the Polymorphic Sst-1 Site (3.2-kb Allele) at the 3' End of the apo C-III Gene in Various Racial Groups and in Normolipidemic and Hypertriglyceridemic Caucasians

Group	Number of individuals	Number with 3.2-kb allele: Homozygotes	Number with 3.2-kb allele: Heterozygotes	Frequency of 3.2-kb allele
	n			
Normolipidemic				
Chinese	20	6	7	0.475
Japanese	21	0	8	0.19
Africans	20	0	6	0.15
Indian Asians	28	0	10	0.18
Caucasians	52	0	0	0
Hypertriglyceridemic*				
Caucasian	74	3	23	0.19

* Analysis of difference between number of Caucasian hypertriglyceridemic subjects with the 3.2-kb allele compared with the Caucasian control subjects $\chi^2 = 23.0$ ($P < 0.001$).

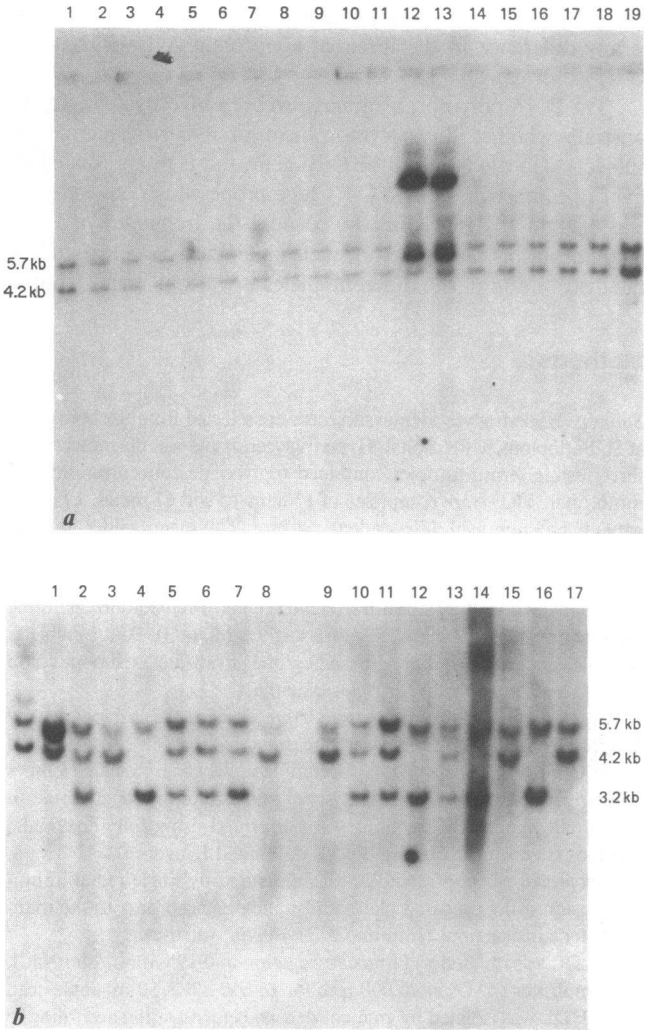

Figure 2. Autoradiograms of Sst-1 digests of DNA with the A-1 probe. (*a*) Normolipidemic Caucasians; (*b*) normolipidemic Chinese. 15 μg of DNA was digested with Sst-1, electrophoresed in 0.85% agarose, and Southern blotted onto nitrocellulose. The filters were hybridized with a ^{32}P-labeled M-13 genomic A-1 probe and autoradiographed. Tracks 4, 12, and 16 are homozygous for the 3.2-kb allele. Tracks 2, 5, 6, 7, 10, 11, and 13 are heterozygous.

the different genotypes among the non-Caucasian racial groups. Autoradiograms showing the polymorphism in Chinese subjects and the lack of the polymorphism in normolipidemic Caucasians are shown in Figure 2.

Localization of the polymorphic Sst-1 restriction site. This polymorphism is located in the 3' flanking region of the A-1 gene and previous mapping data has indicated that it arises from the creation of an additional Sst-1 recognition site (Fig. 1 and Reference 6). Because the apo C-III gene is located 2.6 kb from the 3' end of the A-1 gene, the polymorphism should also be detected with a C-III probe. In order to establish this, Sst-1 digests of DNA from individuals homozygous for the 4.2-kb allele and hypertriglyceridemic subjects homozygous or heterozygous for the 3.2-kb allele were hybridized with a genomic C-III probe. An autoradiogram of a filter showing the resulting hybridization pattern is shown in Fig. 3. Homozygotes for the common allele produce 4.2-kb fragments hybridizing with the C-III probe, the same as with the A-1 probe, whereas homozygotes for the uncommon allele produce 3.2- and 1-kb fragments. All three bands are found with heterozygotes. The same hybridization patterns with the A-1 and C-III probes were found with DNA from normolipidemic Chinese individuals. It follows that the polymorphic restriction site is within the common 4.2-kb fragment and is detected by hybridization with a C-III probe which spans the restriction site and reveals a 3.2- and 1-kb fragment in individuals showing a 3.2-kb fragment with the A-I probe. This confirms the observation (6) that the polymorphic site is located in or close to the 3' end of the C-III gene and furthermore that it arises from a new Sst-1 site, 3' of the nonpolymorphic Sst-1 within the C-III gene. The restriction sites within the A-1–C-III gene cluster, together with the blotting patterns produced with the two probes, are illustrated in Fig. 1. If the polymorphic restriction site is different between Caucasian hypertriglyceridemics and other racial groups, one can calculate from sequence data of the 3'-end of the C-III gene that the minimum size difference of the 1-kb fragment should be 45 base-pairs (bp) and therefore detectable by electrophoresis. We have determined the size of this fragment by hybridization with the C-III probe and can find no difference between the size of the band in Oriental or Caucasian subjects (Fig. 4). Thus the site appears to be identical.

The Mendelian inheritance of the uncommon allele was shown in a two-generation family in which one parent and two children were heterozygous for the allele (Fig. 5). The parent with the 3.2-kb allele was hypertriglyceridemic, and both children had triglyceride levels above normal for their age.

Apoprotein and lipoprotein levels in hypertriglyceridemic individuals with the different genotypes. To establish whether there was any phenotypic difference between hypertriglyceridemic individuals and the different genotypes expressed as an alteration of HDL or apoprotein levels, we compared the levels of apoprotein A-1 and HDL in hypertriglyceridemic subjects with the different A-1 genotypes and our Caucasian control group. Plasma HDL levels were reduced compared with those of controls, but this was irrespective of genotype (Table II). This reduction of HDL is typical of hypertriglyceridemia whatever the cause. There

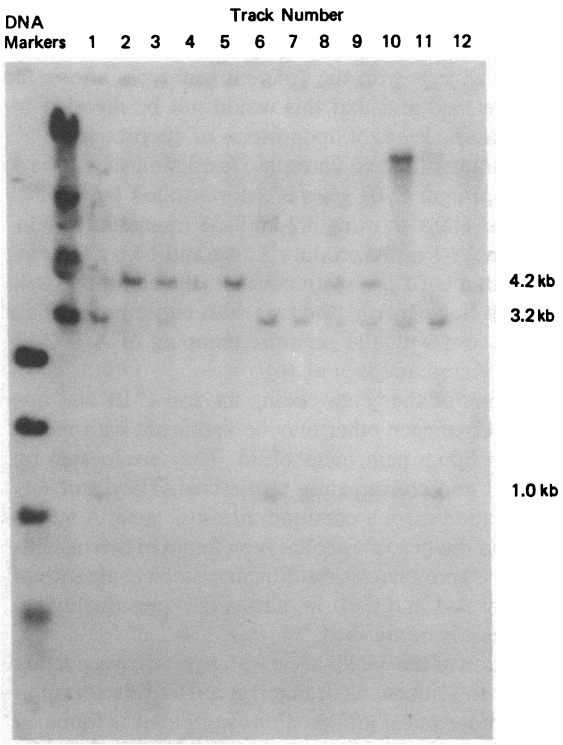

Figure 3. Autoradiogram of an Sst-1 digest of hypertriglyceridemic individuals with different A-1–C-III genotypes hybridized with the C-III probe. Tracks 1, 6, 7, 10, and 11 are individuals homozygous for the 3.2-kb allele; tracks 2, 4, 5, and 12 are individuals homozygous for the 4.2-kb allele; tracks 3, 8, and 9 are heterozygotes. Note: individuals with the 3.2-kb allele all show a 1-kb fragment hybridizing with the C-III probe.

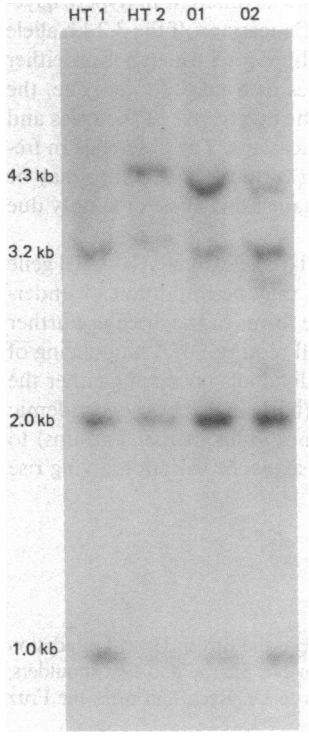

Figure 4. An autoradiogram of Sst-1 digests of DNA from hypertriglyceridemic and Oriental normolipidemic subjects hybridized with a full-length cDNA apo C-III probe. Note that there are no differences in size of the 1.0-kb fragment between Oriental and Caucasian subjects. Abbreviations: HT1, hypertriglyceridemic homozygous-rare individual; HT2, hypertriglyceridemic heterozygous subject; 01, normolipidemic Oriental subject; 02, normolipidemic Oriental subject.

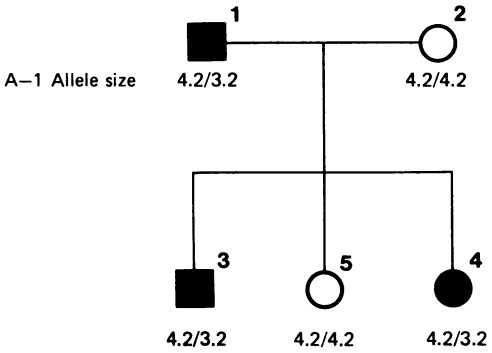

Figure 5. Inheritance of the 3.2-kb A-1 allele and hypertriglyceridemia. The proband (*1*) was hypertriglyceridemic and the mother (*2*) is normolipidemic. The children, (*3*) aged 12 yr and (*4*) aged 11 yr, had triglyceride values above normal for their age. The child (*5*), age 18 yr, was normolipidemic.

was no significant difference in A-1 levels between subjects with the different genotypes (Table II).

Isoelectric focusing of apoprotein A-1 and apoprotein C-III. Isoelectric focusing of apo HDL in the range pH 4–7 showed that the isoelectric point of the main apo A-1 isoform (5.5–5.6) was identical in hypertriglyceridemic individuals with either the 3.2 or 4.2-kb allele. The proportions of the isoforms of apo C-III, apo C-III-0, -1, and -2 were determined by isoelectric focusing and scanning densitometry of apo VLDL from individuals with the different genotypes. There was no significant difference between the proportions of the different sialylated forms of C-III or in the ratio of C-II/III (Table III).

Discussion

This report demonstrates that the DNA polymorphism originally described in the 3′ flanking region of the A-1 gene is located within the gene for apo C-III, and furthermore that it may provide a genetic marker for some forms of hypertriglyceridemia because one particular allele, producing 3.2-kb DNA fragments hybridizing with the apo A-1 or C-III gene probe is found in a much higher proportion of Caucasian individuals with hypertriglycer-

Table II. Apoprotein A-1 and HDL Levels in Hypertriglyceridemic Subjects with Different A-1–C-III Alleles

Group	*n*	Apoprotein A-1	HDL cholesterol
		g/liter	*mmol/liter*
Hypertriglyceridemic subjects with 3.2-kb allele	12*	1.29±0.33	0.86±0.17
Hypertriglyceridemic subjects without 3.2-kb allele	9	1.18±0.29	0.84±0.31
Normolipidemic control subjects	21	1.24±0.34	1.22±0.31

* The 12 hypertriglyceridemic subjects with the 3.2-kb allele include two homozygotes and 10 heterozygotes. Results are means±standard deviations.

Table III. Apoprotein C Composition of Triglyceride-rich Lipoproteins of Hypertriglyceridemic Subjects Possessing the 3.2-kb C-III Allele Compared to Hypertriglyceridemic Subjects with the Common Genotype

		% total apoprotein C			
Hypertriglyceridemia	*n*	C-II	C-III-2	C-III-1	C-III-0
with 3.2-kb allele*	8	17.5±3.6	26.2±6.5	49.1±7.0	8.9±3.1
with 4.2-kb allele	6	21.0±4.4	25.6±6.6	46.9±7.6	5.8±4.1

* Includes two homozygotes for 3.2-kb allele. All the subjects were Caucasian. The % apoprotein composition was determined by scanning densitometry of isoelectric focusing gels of apo VLDL. The values are not corrected for differences in chromogenicity between C-II and C-III. Results are means±standard deviations.

idemia when compared with a racially matched control group. In fact, the 3.2-kb allele was not found in any of 52 normolipidemic control subjects but was found in 30% of individuals with primary hypertriglyceridemia or diabetic hypertriglyceridemia.

The involvement of apolipoproteins in lipoprotein metabolism, as activators of enzymes or as ligands for receptors involved in the cellular uptake of lipoproteins, suggest that abnormalities of apolipoproteins, either structural or regulatory, may represent some of the genetic factors predisposing to hyperlipidemia. This is demonstrated by the association of the apoprotein E-2 allele with type III hyperlipidemia (16) and by the hypertriglyceridemia found in apo C-II deficiency (3). Abnormalities characterized by low levels of apo A-1, such as fish-eye disease (17) and Tangier disease (18), or structural abnormalities of A-1 such as A-1 Milano and A-1 Marburg (19) are often accompanied by a disturbance of lipoprotein metabolism resulting in a varying degree of hypertriglyceridemia.

Primary and secondary abnormalities of apo C-III are also associated with hypertriglyceridemia. Apo C-III is the major low molecular weight apoprotein of VLDL and chylomicrons and may influence the clearance of triglyceride-rich lipoproteins by inhibiting the activity of lipoprotein lipase and also by reducing the hepatic uptake of triglyceride-rich chylomicron remnants. An increase in the proportion of the more sialylated isoforms of apoprotein C-III, apo C-III-2, is found in some patients with type V hyperlipoproteinemia (20). Their triglyceride-rich lipoproteins display impaired interaction with lipoprotein lipase due to this excess sialylation of apo C-III.

It follows that the association of hypertriglyceridemia with the uncommon (3.2 kb) allele may be due to the fact that the polymorphic site may be in a sequence of the C-III gene which regulates its expression, or alternatively, that it is not etiological but may represent a linkage marker for a structural or functional abnormality of either apo A-1 or C-III or another neighboring gene involved in lipoprotein metabolism. Although there is a highly significant association in our Caucasian population between the 3.2-kb allele and hypertriglyceridemia, we can detect no phenotypic differences between hypertriglyceridemic individuals with the 3.2-kb allele and hypertriglyceridemic individuals without it. Furthermore, there is no discernible difference in the isoelectric points of apo A-1 and apo C-III between the different genotypes nor is there any difference in the proportions of C-III isoforms. Nevertheless, it is possible that there is a difference in the rate of induction of apoprotein A-1 or C-III synthesis between subjects with the different genotypes after a fat or carbohydrate load and that this would not be revealed by differences in fasting levels of lipoproteins or apoproteins.

The localization of the polymorphic restriction site to the 3′ end of the apoprotein C-III gene is demonstrated by the fact that individuals' DNA showing 3.2-kb Sst-1 fragments hybridizing with an apo A-1 probe produce 3.2-kb and 1-kb fragments hybridizing with a C-III probe. In contrast, the common allele produces 4.2-kb fragments hybridizing with either probe. This finding is in accord with the genomic mapping of A-1–C-III gene cluster by Karathanasis et al. (6).

The clustering of the genes coding for apo C-III and apo A-1 within 2.6 kb of each other may be significant with regard to their roles in lipoprotein metabolism. They are located on chromosome 11 and convergently transcribed. They probably arose by duplication from a common ancestral gene. A 6.5-kb DNA insertion in this gene cluster has been found in two patients with premature atherosclerosis resulting in complete absence of both apoproteins A-1 and C-III in plasma (21) presumably because neither gene is transcribed.

The association of the 3.2-kb allele with hypertriglyceridemia may only apply to Caucasians in that it is a relatively common allele in some other racial groups. For example, it is found in over 65% of Chinese individuals. It is possible that the polymorphic site may be heterogeneous, i.e., the Sst-1 site found in hypertriglyceridemic Caucasians and other racial groups may be different but situated close enough to produce very similarly sized restriction fragments. Karathanasis et al. (6) have found a cDNA with a single C-G transmutation in the 3′ noncoding region of the C-III mRNA. However, there are four other hexanucleotide sequences close to this side in the 3′ end of the gene where a single nucleotide change would create a new Sst-1 restriction site (15). Our hybridization data suggests it is unlikely that the polymorphic restriction site is different in hypertriglyceridemic Caucasians and in non-Caucasians. If the 3.2-kb allele is linked to a mutation affecting lipoprotein metabolism either in the apo A-1 or apo C-III genes or a neighboring gene, the mutation may have arisen after the divergence of the races and thus not be linked in the non-Caucasians. The difference in frequency of the 3.2-kb allele among various racial groups may be due to selective pressure for or against this allele or simply due to neutral drift.

The association of one particular allele of the A-1–C-III gene cluster with hypertriglyceridemia may be important in understanding the genetic basis of some forms of the disease. Further elucidation of the relationship will require DNA sequencing of the 3′ end of the C-III gene in individuals possessing either the common or uncommon alleles (both in hypertriglyceridemic Caucasians and in individuals from different racial groups) to determine the precise nucleotide sequence variation giving rise to the different restriction site.

Acknowledgments

This work was supported by grants from the British Heart Foundation and the Medical Research Council to Dr. Sharpe and Dr. Shoulders, from the British Diabetic Association to Dr. Rees, and from the Fritz Thyssen Foundation to Dr. Katz.

References

1. Francois, J., F. Lentini, P. Hoste, and R. Rottiers. 1977. Genetic study of hyperlipoproteinaemia type IV and V. *Clin. Genet.* 12:202–207.

2. Breckenridge, W. C., A. Little, G. I. Steiner, A. Chow, and M. Poapst. 1978. Hypertriglyceridaemia associated with a deficiency of apolipoprotein C-II. *N. Engl. J. Med.* 298:1265–1273.

3. Robinson, D. S., and D. R. Wing. 1971. *In* Plasma Lipoproteins. Smellie, R. M. S., editor. Academic Press, Ltd., London. 123–135.

4. Weatherall, D. J. 1982. The New Genetics and Clinical Practice. Nuffield Provincial Hospital Trust, London.

5. Rees, A., C. C. Shoulders, J. Stocks, D. J. Galton, and F. E. Baralle. 1983. DNA polymorphism adjacent to the human apoprotein A-1 gene: relation to hypertriglyceridaemia. *Lancet.* I:444–446.

6. Karathanasis, S. K., V. I. Zannis, and J. L. Breslow. 1983. Linkage of human apolipoprotein A-1 and C-III genes. *Nature (Lond.).* 304:371.

7. Baginsky, M. L., and W. V. Brown. 1977. Differential characteristics of purified hepatic triglyceride lipase and lipoprotein lipase from human post heparin plasma. *J. Lipid Res.* 18:423–437.

8. Windler, E., Y. S. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effect of apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* 255: 8303–8307.

9. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations. Bethesda, MD. DHEW Publication No. 75-628.

10. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins. *Anal. Biochem.* 44:576–588.

11. Stocks, J., G. Holdsworth, P. M. Dodson, and D. J. Galton. 1981. An abnormal triglyceride rich lipoprotein carrying excess apolipoprotein C-II. *Atherosclerosis.* 38:1–9.

12. Swaney, J. B., and L. I. Gidez. 1977. Analysis of rat serum apolipoproteins by isoelectric focussing. II. Studies on the low molecular weight subunits. *J. Lipid Res.* 18:69–76.

13. Kunkel, H. M., K. D. Smith, S. H. Boyer, S. Digamber, S. S. Borgaonkar, V. Miller, W. R. Breg, H. W. Jones, and J. M. Rary. 1977. Analysis of human Y chromosome-specific reiterated DNA in chromosome variants. *Proc. Natl. Acad. Sci. USA.* 74:1245–1249.

14. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–17.

15. Sharpe, C. R., A. Sidoli, C. S. Shelley, M. A. Lucero, C. C. Shoulders, and F. E. Baralle. 1984. Human apolipoproteins AI, AII, CII and CIII. cDNA sequences and mRNA abundance. *Nucleic Acid Res.* 12: 3917–3932.

16. Zannis, V. I., and J. L. Breslow. 1981. Human very low density lipoprotein polymorphism is explained by genetic variation and post translational modification. *Biochemistry.* 20:1033–1041.

17. Carlson, L. A. 1982. Fish-eye disease: a new familial condition with massive corneal opacities and dyslipoproteinaemia. *Eur. J. Clin. Invest.* 12:41.

18. Fredrickson, D. S., P. H. Aetrocchi, L. V. Avioli, D. S. Goodman, and H. C. Goodman. 1961. Tangier disease. *Ann. Intern. Med.* 55:1061–1031.

19. Franceschini, G., C. R. Sirtori, A. Capurso, K. H. Weisgraber, and R. W. Mahley. 1980. A-I Milano apoprotein: decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J. Clin. Invest.* 66:892–900.

20. Holdsworth, G., J. Stocks, P. M. Dodson, and D. J. Galton. 1982. An abnormal triglyceride-rich lipoprotein containing excess sialylated apolipoprotein C-III. *J. Clin. Invest.* 69:932–939.

21. Karathanasis, S. K., V. I. Zannis, and J. L. Breslow. 1983. A DNA insertion in the human apolipoprotein A-1 gene locus related to the development of atherosclerosis. *Nature (Lond.).* 301:718.