Vitamin A is linked to the expression of the AI-CIII-AIV gene cluster in familial combined hyperlipidemia

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Abstract **There is growing evidence of the capacity of vitamin A to regulate the expression of the genetic region that encodes apolipoproteins (apo) A-I, C-III, and A-IV. This region in turn has been proposed to modulate the expression of hyperlipidemia in the commonest genetic form of dyslipidemia, familial combined hyperlipidemia (FCHL). The hypothesis tested here was whether vitamin A (retinol), by controlling the expression of the AI-CIII-AIV gene cluster, plays a role in modulating the hyperlipidemic phenotype in FCHL. We approached the subject by studying three genetic variants of this region: a C_{1100}–T transition in exon 3 of the apoC-III gene, a G_{3206}–T transversion in exon 4 of the apoC-III gene, and a G_{-75}–A substitution in the promoter region of the apoA-I gene. The association between plasma vitamin A concentrations and differences in the plasma concentrations of apolipoproteins A-I and C-III based on the different genotypes was assessed in 48 FCHL patients and 74 of their normolipidemic relatives. The results indicated that the subjects carrying genetic variants associated with increased concentrations of apoA-I and C-III (C_{1100}–T and G_{-75}–A) also presented increased plasma concentrations of vitamin A. This was only observed among the FCHL patients, which suggested that certain characteristics of these patients contributed to this association. The G_{3206}–T was not associated with changes in either apolipoprotein concentrations or in vitamin A. In summary, we report a relationship between genetically determined elevations of proteins of the AI-CIII-AIV gene cluster and vitamin A in FCHL patients. More studies will be needed to confirm that vitamin A plays a role in FCHL which might also be important for its potential application to therapeutical approaches.**—Ribalta, J., J. Girona, J. C. Vallvé, A. E. La Ville, M. Heras, and L. Masana. **Vitamin A is linked to the expression of the AI-CIII-AIV gene cluster in familial combined hyperlipidemia.** ***J. Lipid Res.*** **1999.** 40: **426–431.**

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Familial combined hyperlipidemia (FCHL) is a common genetic lipid disorder which is strongly associated with an increased risk of cardiovascular disease (1, 2). It is characterized by high concentrations of plasma cholesterol and/or triglycerides (3) which are postulated to result from hepatic overproduction of apolipoprotein (apo) B-100 particles (4, 5). However, the exact primary genetic defect in FCHL is not known. Lipoprotein profiles may vary in the patient over time, and different hyperlipidemic phenotypes are also present among the first degree relatives (6), implying that family studies are necessary if a correct diagnosis is to be made. There is considerable phenotypic overlap between FCHL and other lipid and metabolic disorders such as defective lipoprotein lipase (LPL) function (7), glucose intolerance, insulin resistance (8), or hypertension (9). Because this phenotypic heterogeneity has both environmental and genetic bases, a number of studies have attempted to identify these accompanying modifying factors.

The AI-CIII-AIV gene cluster appears to be an important region in modulating the expression of hyperlipidemia in FCHL (10–12). Dallinga-Thie et al. (10) reported a specific modifying effect of the AI-CIII-AIV gene cluster on plasma triglyceride and low density lipoprotein (LDL) cholesterol concentrations in these patients. This observation was confirmed when the same group showed that specific haplotypes of this genetic region made patients more susceptible to developing hyperlipidemia (12). Our group added that this region not only affected the lipid content of the lipoproteins but also the number of these particles which is relevant in view of the existing apoB-100 hepatic overproduction (11).

The mechanism by which the AI-CIII-AIV gene cluster influences the expression of the FCHL phenotype is currently not known. However, biochemical characteristics of FCHL, such as high plasma apoC-III concentrations (13), suggest that the control of the expression of these genes plays a major role.

Several studies indicate that vitamin A is an important regulator of the expression of the AI-CIII-AIV gene cluster

Abbreviations: FCHL, familial combined hyperlipidemia; VLDL, very low density lipoprotein; LDL, low density lipoprotein; PPAR, peroxisome proliferator activated receptor; RXR, retinoid X receptor; HPLC, high pressure liquid chromatography; HDL, high density lipoprotein; RFLP, restriction fragment length polymorphism.

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(14–17). Animal studies have shown that vitamin A modulates lipid transport in normal rats by altering apolipoprotein levels (14) and this effect has been confirmed in vitamin A-deficient rats (15). On the other hand, studies exploring the mode of action of certain drugs such as fibrates have shown that the expression of the apoA-I and C-III genes is controlled by a class of proteins that belong to the nuclear receptor superfamily, called peroxisome proliferator activated receptors (PPARs), and that heterodimerize with the retinoid X receptor (RXR). PPARs are activated by multiple stimuli directly resulting from the action of hypolipidemic agents (such as fibrates), diet, or lipid and glucose metabolism while RXR is activated by retinoic acid, an intracellular active form of dietary vitamin A (16, 17). The reported differences in the plasma concentrations of vitamin A between FCHL patients and normolipidemic controls also suggest that vitamin A has a role in FCHL (18).

Putting all these data together we hypothesized that vitamin A, by controlling the expression of the AI-CIII-AIV gene cluster, might play a role in FCHL. The aim of the present study was to explore whether variations in the AI-CIII-AIV gene cluster, which are associated with an increased expression of apolipoproteins A-I and C-III in patients with familial combined hyperlipidemia, are also associated with differences in plasma vitamin A concentrations in these patients. Three genetic variants of this region were selected: the C_{1100}–T polymorphism in exon three of the apoC-III gene was selected because it has a frequency which is nearly double in FCHL patients than in controls (11, 19) and it is associated with higher concentrations of apoC-III and also apoA-I in such patients (11). The G_{-75}–A substitution in the promoter of the apoA-I gene was chosen because it is in very strong linkage disequilibrium with the XmnI RFLP (19) which has shown linkage with the FCHL phenotype in a number of families (20) and has been reported to be consistently associated with elevated concentrations of apoA-I (21). Finally, the G_{3206}–T transversion in the non-translated region of exon 4 of the apoC-III gene was selected because it has been reported to present with increased frequency in combined hyperlipidemia (19) but is not associated with increased concentrations of either apoC-III or apoA-I.

SUBJECTS AND METHODS

Subjects

All subjects recruited for the study gave fully informed written consent and the protocol was approved by the Scientific and Ethical Committee of the Hospital Universitari de Sant Joan.

Sixteen FCHL families were identified by the following criteria: an index patient with plasma concentrations of cholesterol (chol) ⩾6.4 mmol/L and triglycerides (trig) ⩾2.8 mmol/L detected at any time in the clinical history and at least one first degree relative with a hyperlipoproteinemic phenotype different from that of the index patient. All available family members of index patients were recruited, a total of 106 individuals. Of the 122 subjects, those with plasma cholesterol and/or triglyceride concentrations ⩾6.4 mmol/L and 2.8 mmol/L, respectively, or above the 95th percentile for age and gender were included in the FCHL group (n = 48). Relatives who did not meet this criteria (n = 74) were assigned to the normolipidemic relatives (NL) group.

Biochemical analyses were conducted to rule out secondary causes of hyperlipidemia and apoE genotyping was performed to exclude type III hyperlipoproteinemia.

Analytical methods

A 10-ml venous blood sample was withdrawn after an overnight fast of 12 h. Triglyceride and cholesterol concentrations were measured using enzymatic kits (Boehringer Mannheim, Germany) adapted for a Cobas Mira centrifugal analyzer (Roche Pharmaceuticals, Switzerland) with Precinorm® (Boehringer Mannheim, Germany) as quality control. Immunoturbidometry was used for the apolipoproteins using specific antiserum for apoA-I and apoB-100 (Boehringer Mannheim, Germany) and apoC-III (Daichii Chemicals, Japan). Low density lipoproteins were separated by sequential preparative ultracentrifugation (22) using a Kontron 45.6 fixed-angle rotor in a Centrikon 75 (Kontron Instruments, Italy). Total high density lipoprotein (HDL) cholesterol was measured after precipitation with polyethylene glycol (Immuno AG, Austria).

Vitamin A analyses

Vitamin A in plasma was measured using the method of Bieri, Tolliver, and Catignani (23). Briefly, vitamin A was extracted from 100 μl of plasma into n-hexane and 100 μl of a solution of all-*trans*-retinyl acetate (Sigma Chemical Co., St. Louis, MO) in ethanol was added as internal standard. The amount of retinyl acetate lost during the extraction was used to estimate how much retinol was lost during the procedure. The samples were centrifuged and 150 μL of the hexane layer was removed and evaporated under a stream of nitrogen. The reconstituted lipid residue was analyzed by high pressure liquid chromatography (HPLC) using the Hewlett-Packard 1050 series system (Hewlett-Packard GmbH, Germany) in which the separation column was Spherisorb ODS 2 and the mobile phase was methanol–water 98:2 (v/v). A standard curve of all-*trans*-retinol (Sigma Chemical Co.) was used to determine vitamin A concentrations. Absorbancies were recorded on a UV variable wavelength detector set at 325 nm.

DNA analyses

DNA was extracted from an aliquot of frozen white cells by the salting out method (24). The genetic variants in the AI-CIII-AIV gene cluster studied were: a C_{1100}–T transition in exon 3 of the apoC-III gene, a G_{3206}–T transversion in exon 4 of the apoC-III gene, and a G_{-75}–A substitution in the promoter region of the apoA-I gene. The region containing each of the variants was amplified separately by PCR in a reaction mixture that contained 250 ng of each of the two primers, 200 ng of genomic DNA, 1.5 mm $MgCl_2$, and 1 U of Taq polymerase (Boehringer Mannheim, Germany). The reactions were performed on a Hybaid Omnigene thermocycler at 95°C for 5 min, 55°C for 1 min, 72°C for 2 min followed by 35 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. Specific primers were: 5′ primer, 5′-CA ATGGGTGGTCAAGCAGAAGC-3′ and 3′ primer, 5′-GAGCACCT CCATTCCATTGTTGG-3′ (for C_{1100}–T); 5′ primer, 5′-CATGGT TGCCTACAGAGGATG-3′ and 3′ primer, 5′-TGACCTTCCGC ACAAAGCTGT-3′ (for G_{3206}–T); and 5′ primer, 5′-AGGGACAGA GCTGATCCTTGAACTCTTAAG-3′ and 3′ primer, 5′-TTAGGGG ACACCTAGCCC TCAGGAAGAGCA-3′ (for G_{-75}–A). For the C_{1100}–T and G_{3206}–T genotypes, PCR products were run on a 2% agarose gel and double-blotted on Hybond-N^+ membrane (Amersham International, U.K.) and subsequently hybridized with

the following oligonucleotides for C_{1100}–T: C allele 5′-ATG CAGGGCTACATGAA-3′ and T allele 5′-TTCATGTAACCCTG CAT-3′; for G_{3206}–T: G allele: 5′-ACAGGGGCAGCCCTG-3′ and T allele 5′- CAGGGCTTCCCCTGT-3′ according to the conditions described elsewhere (25). For the G_{-75}–A polymorphism, 20 μL of PCR product was digested overnight with 9 U of the restriction enzyme *MspI* (Boehringer Mannheim, Germany) in a total volume of 30 μL using the buffer recommended by the manufacturer. The DNA fragments were separated by electrophoresis on 2% agarose gels. Digestion with *MspI* resulted in four fragments of 48, 67, 110, and 207 bp. In the absence of the *MspI* cutting site, three fragments of 48, 177, and 207 bp were produced (26).

Statistical analyses

The chi-square (χ^2) test was used to compare the frequency of the genotypes among groups and to test for the Hardy-Weinberg equilibrium. The Z-test for comparison of proportions was used to compare allele frequencies that were determined by gene counting. ANOVA was performed to compare the means of lipid, lipoprotein, apolipoprotein, and vitamin A data adjusted for age and gender and BMI and log transformed when the variables were not normally distributed. Comparisons using unadjusted data were made with the Student's *t*-test. Results are expressed as mean (standard deviation). Statistical significance was accepted at the 0.05 level. Analyses were carried out with the SPSS 7.5. 2S version statistical package.

RESULTS

The FCHL and NL groups had a similar male/female distribution. The FCHL group presented significantly higher age, BMI, and blood pressure values (**Table 1**). Plasma lipids, lipoproteins and apolipoproteins, including apoA-I, were significantly elevated in the FCHL group with the exception of HDL chol which was not different between groups (**Table 2**). Plasma cholesterol/triglyceride ranges in the FCHL and NL groups were (4.28–8.35 mmol/L)/(0.50–3.49 mmol/L) and (2.45–6.12 mmol/L)/(0.36–2.52 mmol/L), respectively.

Plasma vitamin A concentrations

Plasma vitamin A concentrations were not different between the FCHL (527.5 (240.3) μg/L) and the normolipidemic (489.6 (335.9) μg/L) groups (**Fig. 1**). **Table 3** shows the correlation between plasma vitamin A levels and lipids, lipoproteins and apolipoproteins which were very low in the two groups although in some cases they reached statistical significance. The correlation between vitamin A and apolipoproteins A-I and C-III gave a correlation coefficient (*r*) of 0.23 and 0.30 for the FCHL group and 0.07 and 0.26 for the NL group, respectively. Correlations were just as low, when all subjects were analyzed together, between vitamin A and plasma cholesterol ($r = 0.18$), plasma triglycerides ($r = 0.19$), LDL chol ($r = 0.16$) and apoB-100 ($r = 0.24$). When the two groups were studied separately, statistical significance was only maintained in the normolipidemic group, most probably due to the size of the sample. Linear regression analysis indicated that neither cholesterol nor triglyceride accounted for a significant percentage of the vitamin A variability in this sample. All this indicated a lack of association between the variations in vitamin A and lipid, lipoprotein and apolipoprotein concentrations.

TABLE 1. Biometric characteristics of the studied groups

	FCHL	NL	*P*
n	48	74	
Age (years)	40.0 (21.0)	31.2 (17.3)	0.01
Gender (M/F)	26/22	31/43	NS
BMI (kg/m^2)	26.3 (5.7)	24.1 (6.0)	0.02
Diastolic BP	78 (16)	69 (14)	0.005
Systolic BP	128 (22)	115 (20)	0.002

Values expressed as mean (SD). Gender Male/Female. Normolipidemic relatives (NL).

TABLE 2. Plasma lipid, lipoprotein, and apolipoprotein concentrations

	FCHL	NL	*P*
n	48	74	
Plasma Chol	6.22 (1.03)	4.42 (0.82)	<0.0001
Plasma Trig	1.71 (0.89)	0.88 (0.41)	<0.0001
LDL chol	4.22 (0.84)	2.89 (0.72)	<0.0001
Total HDL	1.13 (0.34)	1.20 (0.30)	NS
ApoA-I	122.9 (23.8)	114.8 (18.6)	0.025
ApoB-100	115.9 (26.7)	71.7 (18.4)	<0.0001
ApoC-III	14.4 (4.4)	10.7 (2.4)	<0.0001

Cholesterol and triglycerides are expressed as mmol/L and apolipoproteins as mg/dL. Statistical differences were assessed by ANOVA with data adjusted for age, gender, and BMI. Normolipidemic relatives (NL).

G_{-75}–A polymorphism in the apoA-I gene

The frequencies of the GG, GA, and AA genotypes were not different from those predicted by the Hardy-Weinberg distribution. The A_{-75} allele and the GA/AA genotype frequencies were not different between the FCHL (0.18 and 0.31, respectively) and the normolipidemic group (0.17 and 0.32, respectively). Due to the low frequency of the

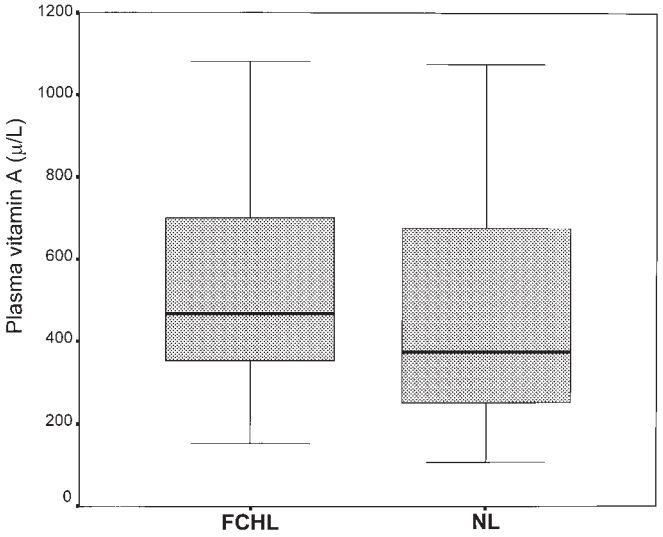

Fig. 1. Box-plot representation of plasma vitamin A concentrations (μg/L) in FCHL patients (FCHL) and their normolipidemic relatives (NL).

TABLE 3. Plasma vitamin A concentrations and their correlation with apolipoproteins A-I and C-III and lipid and lipoprotein parameters

	All	FCHL	NL
n	122	48	74
Vitamin A (μg/L)	503.1 (303.0)	527.5 (240.3)	489.6 (335.9)
ApoA-I	$r = 0.13$ (NS)	$r = 0.23$ (NS)	$r = 0.07$ (NS)
ApoC-III	$r = 0.18$ ($P = 0.006$)	$r = 0.30$ ($P = 0.029$)	$r = 0.26$ ($P = 0.015$)
Plasma Chol	$r = 0.18$ ($P = 0.028$)	$r = 0.20$ (NS)	$r = 0.20$ ($P = 0.051$)
Plasma Trig	$r = 0.19$ ($P = 0.024$)	$r = 0.19$ (NS)	$r = 0.32$ ($P = 0.004$)
LDL Chol	$r = 0.16$ ($P = 0.049$)	$r = 0.07$ (NS)	0.020 ($P = 0.055$)
Total HDL	$r = -0.11$ (NS)	$r = 0.01$ (NS)	$r = -0.15$ (NS)
Apo B-100	$r = 0.24$ ($P = 0.006$)	$r = 0.32$ ($P = 0.02$)	$r = 0.27$ ($P = 0.011$)

Differences in vitamin A plasma concentrations (μg/L) were assessed by ANOVA with data adjusted for age, gender and BMI. Normolipidemic relatives (NL). Vitamin A presented as mean (SD).

minor allele, all carriers of the A_{-75} (heterozygotes and homozygotes) were pooled for statistical analysis. The association between the G_{-75}–A polymorphism and vitamin A, apoA-I, and apoC-III, investigated in all the subjects, showed that the GA/AA genotype was associated with increased concentrations of apoA-I ($P = 0.010$), apoC-III ($P = 0.031$), and with elevations of vitamin A which almost reached statistical significance ($P = 0.054$) (**Table 4**). When the two groups were studied separately it was observed that the GA/AA genotype was associated with elevated concentrations of apoA-I ($P = 0.002$), apoC-III ($P = 0.017$), and vitamin A ($P = 0.015$) in the FCHL group while none of these associations was detected among the normolipidemic subjects.

The A_{-75} allele was associated with significantly higher plasma cholesterol ($P = 0.040$) and elevated plasma triglyceride ($P = 0.054$) in the FCHL group.

C_{1100}–T and G_{3206}–T polymorphisms in the apoC-III gene

The frequencies of the genotypes for both polymorphisms were not different from those predicted by the Hardy-Weinberg distribution. The T_{1100} allele and CT/TT genotype frequencies of the C_{1100}–T polymorphism were not different between the FCHL (0.33 and 0.54, respectively) and the normolipidemic (0.26 and 0.45, respectively) groups. Carriers of the T_{1100} alllele were pooled for statistical analyses. When all subjects were considered together, the CT/TT carriers had significantly higher concentrations of apoA-I ($P = 0.024$) and apoC-III ($P = 0.047$). This was only observed among the affected subjects when the groups were analyzed separately. More importantly, only the FCHL subjects also presented significantly higher concentrations of vitamin A ($P = 0.043$) (**Table 5**). The T_{1100} allele was associated with significantly higher plasma triglyceride ($P = 0.028$) in the FCHL group.

TABLE 4. Association between the G_{75}–A polymorphism and plasma vitamin A, apoA-I, and apoC-III concentrations in FCHL families

	GG	GA/AA	P
All			
n	83	39	
Frequency	0.68	0.32	
Vitamin A	466.4 (320.1)	574.5 (292.4)	0.054
ApoA-I	113.9 (18.5)	126.4 (23.6)	0.010
ApoC-III	11.6 (2.8)	13.3 (5.1)	0.031
FCHL			
n	33	15	
Frequency	0.69	0.31	
Vitamin A	474.2 (217.0)	650.1 (244.7)	0.015
ApoA-I	116.3 (18.5)	135.2 (28.2)	0.002
ApoC-III	13.1 (2.8)	16.9 (5.6)	0.017
NL			
n	50	24	
Frequency	0.68	0.32	
Vitamin A	462.1 (366.6)	522.7 (316.8)	NS
ApoA-I	112.4 (18.5)	120.2 (18.0)	NS
ApoC-III	10.7 (2.4)	10.8 (2.6)	NS

Apolipoproteins are expressed as mg/dL and vitamin A as (μg/L). Statistical differences were assessed by ANOVA with data adjusted for age, gender, and BMI. Normolipidemic relatives (NL). GG, homozygote subjects for the G allele; GA/AA, homozygote plus heterozygote subjects for the A allele.

TABLE 5. Association between the C_{1100}–T polymorphism and plasma vitamin A, apoA-I, and apoC-III concentrations in FCHL families

	CC	CT/TT	P
All			
n	62	58	
Frequency	0.52	0.48	
Vitamin A	482.0 (334.7)	513.9 (276.3)	NS
ApoA-I	113.3 (17.5)	123.2 (23.5)	0.024
ApoC-III	11.4 (2.3)	12.9 (4.7)	0.047
FCHL			
n	21	25	
Frequency	0.46	0.54	
Vitamin A	457.8 (210.1)	595.7 (251.4)	0.043
ApoA-I	112.3 (14.6)	132.4 (26.6)	<0.0001
ApoC-III	12.3 (2.5)	16.1 (5.0)	0.005
NL			
n	41	33	
Frequency	0.55	0.45	
Vitamin A	494.0 (397.0)	462.1 (283.5)	NS
ApoA-I	113.8 (18.9)	116.2 (18.3)	NS
ApoC-III	10.9 (2.12)	10.6 (2.8)	NS

Apolipoproteins are expressed as mg/dL and vitamin A as (μg/L). Statistical differences were assessed by ANOVA with data adjusted for age, gender, and BMI. Normolipidemic relatives (NL); CC, homozygote subjects for the C allelle; CT/TT, homozygote plus heterozygote subjects for the T allelle.

TABLE 6. Association between the G_{3206}–T polymorphism and plasma vitamin A, apoA-I, and apoC-III concentrations in FCHL families

	GG	GT	TT	*P*
All				
n	45	37	23	
Frequency	0.43	0.36	0.21	
Vitamin A	461.1 (235.3)	446.9 (292.8)	614.3 (344.4)	0.060
ApoA-I	116.2 (19.3)	116.0 (22.0)	121.5 (24.9)	NS
ApoC-III	12.4 (4.7)	11.7 (3.1)	12.2 (3.1)	NS
FCHL				
n	18	16	11	
Frequency	0.40	0.36	0.24	
Vitamin A	564.0 (234.1)	530.7 (286.2)	461.2 (197.0)	NS
ApoA-I	117.7 (22.3)	121.8 (20.8)	132.2 (31.3)	NS
ApoC-III	15.6 (5.8)	13.0 (2.9)	14.1 (2.8)	NS
NL				
n	27	22	11	
Frequency	0.45	0.37	0.18	
Vitamin A	395.6 (216.3)	386.3 (293.8)	725.6 (392.3)	0.009
ApoA-I	115.2 (17.5)	111.9 (22.5)	111.7 (11.5)	NS
ApoC-III	10.3 (2.1)	10.9 (3.0)	10.4 (2.3)	NS

Apolipoproteins are expressed as mg/dL and vitamin A as (μg/L). Statistical differences were assessed by ANOVA with data adjusted for age, gender, and BMI. Normolipidemic relatives (NL); GG, homozygote subjects for the G allelle; TT, homozygote subjects for the T allelle; GT, heterozygotes.

The allelic frequency of the minor T_{3206} allele in the FCHL group (0.42) was no different from that of the normolipidemic group (0.37). As this was a fairly common variation, genotypes were not grouped for association analysis and, therefore, GG, TG, and TT genotypes were considered separately. This polymorphism showed no association with either apoA-I or apoC-III plasma concentrations in any of the groups studied. However, a significant association with higher plasma vitamin A concentrations in the subjects carrying the TT genotype ($P = 0.009$) was detected (**Table 6**). Separate analysis of the FCHL and NL groups indicated that this association was confined to the group of normolipidemic relatives. There were no significant differences between the genotypes with regard to plasma cholesterol or triglyceride.

DISCUSSION

Our hypothesis was that vitamin A influences the hyperlipidemic phenotype in FCHL by contributing to the control of the expression of the AI-CIII-AIV gene cluster. We approached the subject by studying genetically determined elevations of apolipoproteins A-I and C-III and their relation to plasma vitamin A concentrations in FCHL patients.

Our results indicate that, among FCHL subjects, the genetic variants in the AI-CIII-AIV gene cluster associated with higher plasma concentrations of apoA-I and C-III are also associated with higher vitamin A levels. This is physiologically important because it can be observed in the group of patients but not in their unaffected relatives which suggests that characteristics of these patients contribute to the relationship between the AI-CIII-AIV gene cluster and vitamin A.

These results, although indicative of the possibility that vitamin A plays a role in FCHL expression, do not provide evidence that vitamin A directly controls the expression of this genetic region. Other factors such as lipemia are important determinants of the variability of these two proteins. In the present study the A_{-75} and the T_{1100} alleles were associated with increased cholesterol and triglyceride in plasma, respectively. Moreover, linear regression analysis indicated that 65% of the variability of plasma apoC-III was explained by total triglyceride whereas 59% of the variation of apoA-I was explained by cholesterol in the HDL fraction (data not shown). However, plasma lipids or lipoproteins did not explain a significant percentage of the variability observed in plasma vitamin A. This is not surprising as the vitamin A measured in this study is not associated with lipoproteins (as would be the case for carotenoids or retinyl esters), but is mainly bound to retinol binding protein and is very tightly regulated by the liver. Therefore the nature of these variations associated with genetic variants is somewhat intriguing and suggests that vitamin A has an indirect relationship with the AI-CIII-AIV gene cluster which, based on the results of linear regression analysis, is probably non-mediated by lipoproteins. This lack of a direct association between vitamin A and apolipoproteins A-I and C-III and vitamin A strongly suggests that other factors also contribute to the observed effect.

One crucial aspect is to elucidate by which mechanism vitamin A is capable of regulating the expression of certain proteins at a genetic level. Pharmacological studies that have investigated the mode of action of fibrates have partly responded to this (16, 17). Fibrates were found to activate the transcription factor PPARα which forms a heterodimer with another transcription factor RXRα. The active heterodimer recognizes response elements of certain genes (such as apoA-I and C-III) and controls their expression. In the context of vitamin A it is important to note that RXRα must be activated by retinoic acid, the intracellular active form of vitamin A. Most of the intracellular retinoic acid derives from plasma vitamin A which is 100 times more abundant, which may mean that the availability of vitamin A is important for the efficiency of this system. This point has actually been confirmed by Mukherjee et al. (27) who showed that substances with vitamin A activity that are also agonists of RXRα can activate PPARα-inducible genes and promote a more efficient effect of fibrates. In the light of this evidence, the intracellular concentrations of retinoic acid, PPARα or RXRα appear as potential candidates for the observed association between plasma vitamin A concentrations and apolipoproteins A-I and C-III.

In summary we report a relationship between increased plasma concentrations of apolipoproteins A-I and C-III and vitamin A in FCHL patients that is not mediated by the accompanying hyperlipidemia. More studies will be needed to confirm that vitamin A has a role in FCHL, which might be relevant also for its potential application to therapeutical approaches.

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