UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 4. Lipid, lipoprotein and apolipoprotein parameters in subjects from FCHL families and control families based on genotype.

Variable	FCHL families		Control families	
	CC n=62	CT/TT n=58	CC n=39	CT/TT n=24
Plasma Chol	4.98 (1.14)	5.28(1.28)	4.89(0.96)	5.27(0.81)
Plasma Trig	1.06(1.23)	1.45(1.00)[a]	0.95(0.38)	0.97(0.45)
VLDL				
Chol	0.27(0.49)	0.45(0.50)[c]	0.20(0.15)	0.19(0.17)
Trig	0.55(0.97)	0.79(0.81)[b]	0.42(0.31)	0.41(0.32)
Apo B	6.99(10.03)	14.68(17.08)[b]	8.37(8.33)	8.63(5.01)
IDL				
Chol	0.17(0.12)	0.25(0.25)[a]	0.15(0.08)	0.16(0.16)
Trig	0.14(0.11)	0.17(0.13)[b]	0.11(0.05)	0.11(0.07)
Apo B	4.96(2.36)	6.51(5.36)[a]	4.77(3.31)	5.29(3.38)
LDL				
Chol	3.38(0.98)	3.37(1.03)	3.23(0.78)	3.65(0.67)
Trig	0.25(0.12)	0.27(0.11)	0.23(0.08)	0.26(0.11)
Apo B	76.54(21.09)	71.90(22.35)	70.33(17.85)	77.84(13.77)
HDL2	0.18(0.15)	0.20(0.11)	0.30(0.25)	0.27(0.12)
HDL3	0.93(0.27)	1.02(0.29)	1.05(0.20)	1.09(0.25)
Total HDL	1.13(0.30)	1.23(0.35)	1.35(0.37)	1.36(0.27)
Plasma apo A-I	113.52(18.14)	122.96(23.20)[b]	122.15(18.56)	121.49(18.96)
Plasma apo B	86.72(31.96)	90.12(33.16)	84.29(20.99)	89.67(19.91)
Plasma apo C-II	5.59(1.63)	5.77(1.30)	4.52(0.40)	4.30(0.36)
Plasma apo C-III	11.51(4.71)	12.75(4.64)[a]	10.78(1.26)	11.34(2.07)

Cholesterol and triglycerides in plasma and all fractions are expressed as mmol/L.
Apolipoproteins in plasma and all fractions are expressed in mg/dL.
All values expressed as means (SD).
Statistical differences assessed by ANOVA with data adjusted for age, gender and BMI.
[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$ compared to the CC genotype in FCHL families.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Figure 1

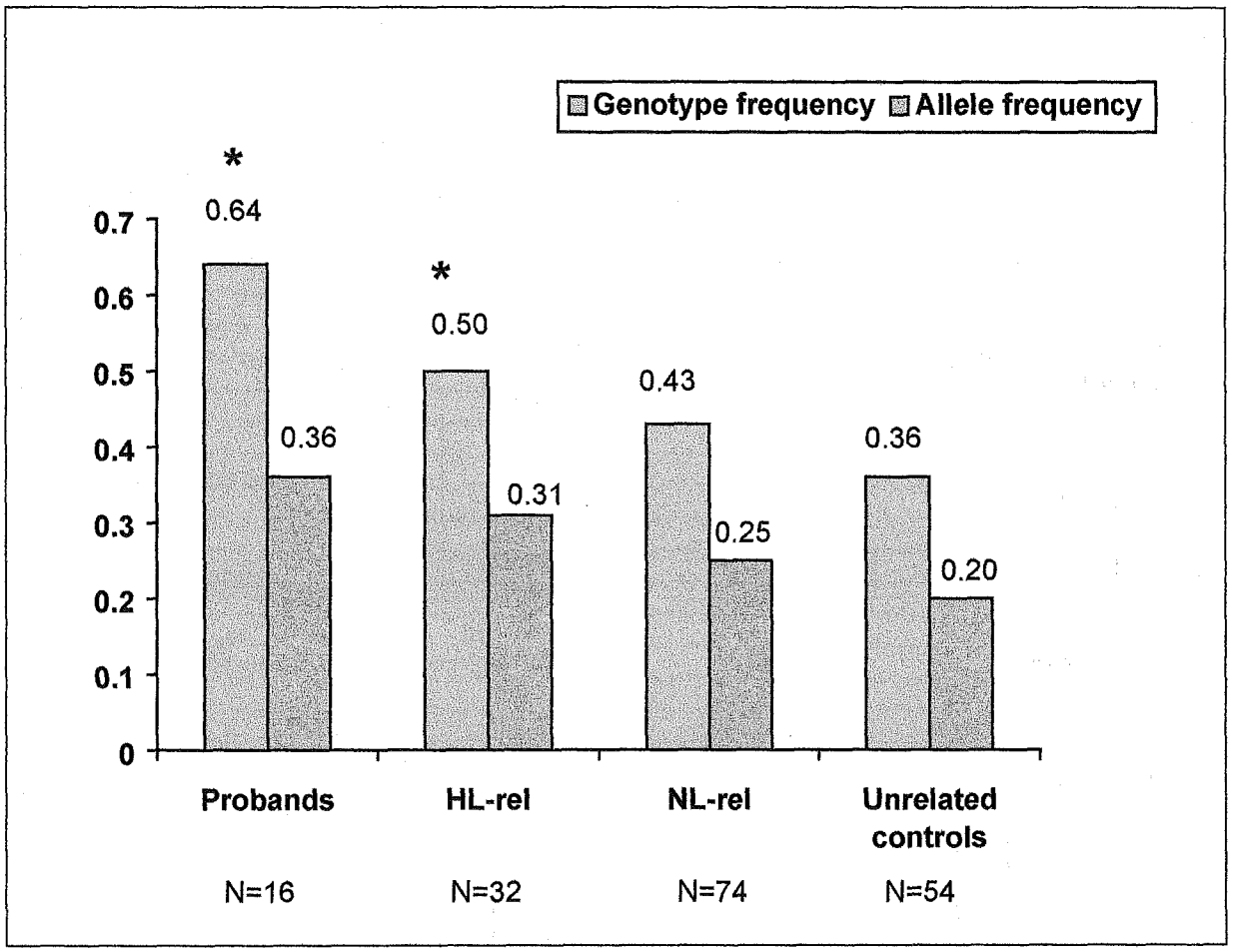

Figure 1.

Genotype (CT/TT) and allelic frequencies of the minor allele (T) in FCHL families: Probands, Hyperlipidemic relatives (HL-rel), Normolipidemic relatives (NL-rel), and unrelated Controls.

* Statistically different from controls. Probands ($p<0.01$), HL-rel ($p<0.001$).

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Figure 2.

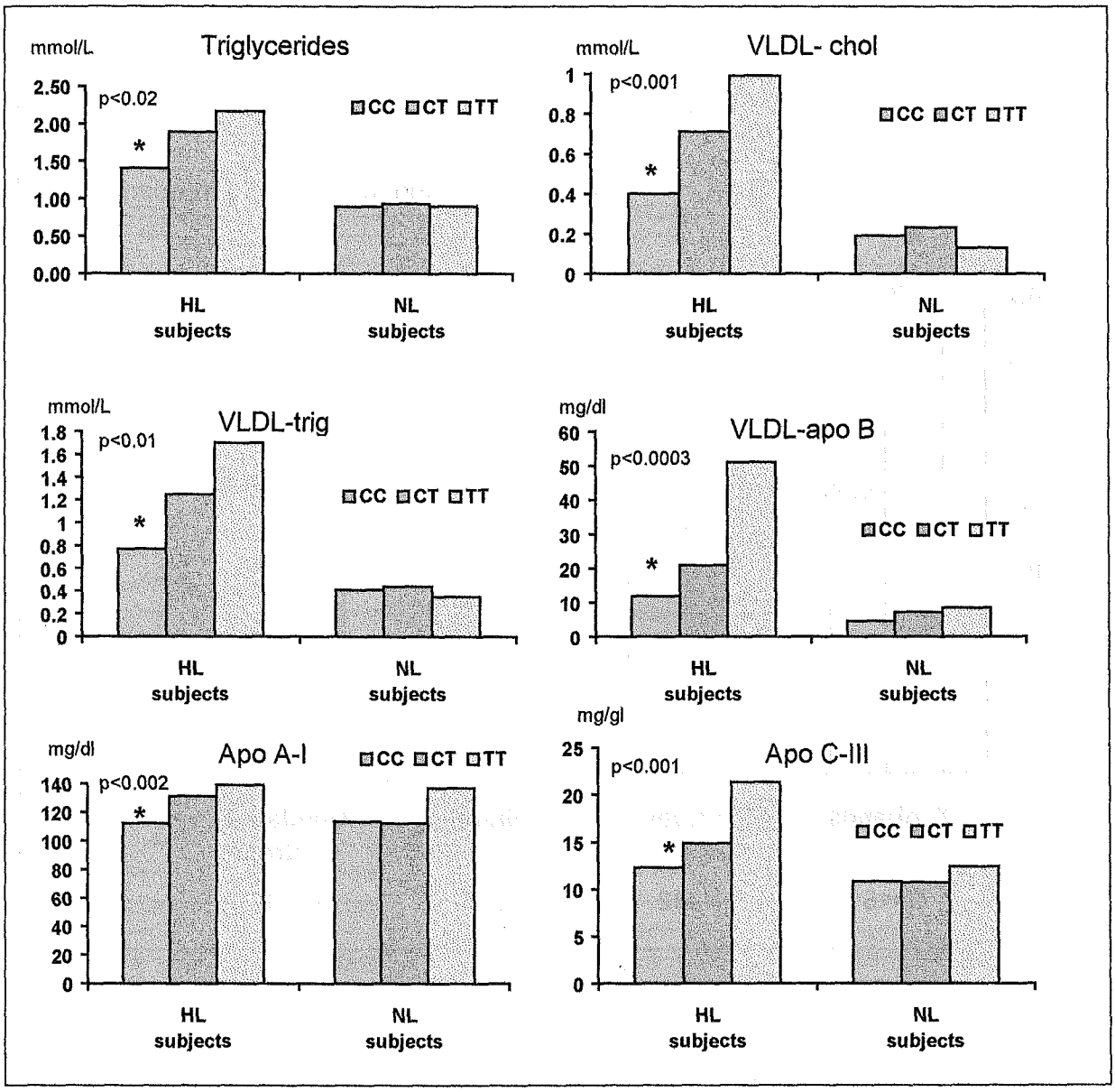

Figure 2.

Genotype-status interaction in relation to concentrations of plasma triglycerides, VLDL-chol, VLDL-trig, VLDL-apoB, apo C-III and apo A-I. Note : Gene dosage observed only among the hyperlipidemic members of FCHL families. * Statistically significant linear correlation.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

4.4. ESTUDI 3

Estudi de les concentracions plasmàtiques de retinol en individus afectes d'hiperlipèmia familiar combinada.

Ribalta J, La Ville AE, Girona J, Vallvé JC, Masana L. *Low plasma vitamin A concentrations in familial combined hyperlipidemia. Arch Intern Med.* (En revisió).

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Low plasma vitamin A concentrations in familial combined hyperlipidemia.

Abstract

Background : Up to 20% of the survivors of acute myocardial infarction present with the heritable form of hyperlipidaemia termed familial combined hyperlipidemia (FCHL). Some of the genes reported to be involved in this disorder, such as that of lipoprotein lipase (LPL) and apolipoprotein (apo) C-III, are controlled by a peroxisome proliferator activated receptor (PPAR) / retinoic acid receptor X (RXR) regulatory system which is retinoic acid dependant. We hypothesised that the availability of retinoic acid or its precursor retinol (vitamin A) could be altered in FCHL and could help explain some aspects of the phenotypic expression of the disease.

Methods : Plasma lipids, lipoproteins, apolipoproteins and retinol concentrations were measured in 30 FCHL subjects (from 16 families) and 56 control subjects from 12 families. Since vitamin A circulates in close association with the plasma lipoproteins, all statistical analyses were performed on data adjusted for plasma cholesterol and triglyceride concentrations.

Findings : Plasma retinol concentrations in FCHL subjects were significantly lower than that of control subjects (418.9 ± 229.5 vs. 888.9 ± 352.2 µg/L, respectively; $p < 0.0001$). Interestingly, plasma retinol concentration correlated significantly with plasma cholesterol ($R = 0.38$; $p < 0.003$), triglycerides ($R = 0.23$; $p < 0.05$), LDL cholesterol ($R = 0.32$; $p < 0.01$) and apolipoprotein B ($R = 0.42$; $p < 0.001$) in controls whereas, in the FCHL subjects, it correlated significantly only with parameters of reverse cholesterol transport; HDL cholesterol ($R = 0.44$; $p < 0.01$) and apolipoprotein A-I ($R = 0.53$; $p < 0.002$).

Interpretation : This novel finding of highly significant decreased concentrations of plasma retinol in FCHL relative to control subjects gives support to the hypothesis that vitamin A might be involved in the expression of this disorder.

Introduction

Familial combined hyperlipidemia (FCHL)[1] is the commonest genetic form of hyperlipidaemia and is present in about 20% of myocardial infarction survivors.[2] FCHL patients may present with hypercholesterolaemia, hypertriglyceridaemia or both. Moreover, the FCHL phenotype may vary among family members and even in the individual patient over time.[3] Although the aetiology of this highly heterogeneous disorder is not well understood,[4] several metabolic features such as very low density lipoprotein (VLDL) apolipoprotein (apo) B overproduction,[5] small dense low density lipoprotein (LDL) particles,[6] decreased lipoprotein lipase (LPL) activity[7,8] and elevated plasma apo C-III concentrations[9] have been described. Mutations in the LPL[10,11] and apo C-III[12,13] genes associated with decreased LPL activity or elevated apo C-III concentrations have been reported to be more frequent among FCHL subjects. The interaction between these genetic variants and environmental factors such as diet and obesity contribute to the individual expression of the FCHL phenotype. Hence, it is of considerable importance to identify the mechanisms by which these factors regulate gene expression. For example, the LPL and apo C-III genes are stimulated and repressed, respectively, by fibrate therapy and dietary fatty acids.[14-16] More precisely, the metabolic perturbation induced by environmental stimuli leads to the activation of a class of proteins belonging to the nuclear receptor superfamily termed Peroxisome Proliferator Activated Receptors (PPARs)[17] which, by heterodimerising with the 9-*cis*-retinoic acid receptor X (RXR), recognise response elements (RE) of the promoter region of the above-mentioned target genes and, hence, control their expression. Both elements of this signalling pathway, PPAR and RXR, need to be physiologically activated to promote a regulatory effect on the target genes. PPARs are activated by multiple stimuli directly resulting from the action of hypolipidaemic agents (such as the fibrates), diet and lipid or glucose metabolism whereas RXR is activated by retinoic acid,[16,17] which is an intracellular active form of dietary retinol (vitamin A). This is, therefore, a regulatory system in which the physiological response to drug or diet-induced changes would be directly dependent on the availability of vitamin A. In view of the role that this signalling pathway has on the regulation of genes known to be involved in FCHL, we hypothesised that the availability (i.e. concentrations in plasma) of vitamin A could be playing a role in the

expression of FCHL phenotype.

Subjects and methods

FCHL subjects

As part of a larger investigation into the inheritance of FCHL, sixteen families diagnosed as having the disease were identified from the Lipid Clinic of the Hospital Universitari de Sant Joan in Reus (Spain). Diagnosis was based on the index patient having plasma concentrations of cholesterol (chol) and triglycerides (tg) $\geq$ 6.4 mmol/L and/or $\geq$ 2.8 mmol/L, respectively, detected at any time in the clinical history and at least one first-degree relative with a hyperlipidaemic phenotype different from that of the proband. Among these families all those with plasma cholesterol or triglycerides $\geq$ 6.4 mmol/L and $\geq$ 2.8 mmol/L were assigned the FCHL phenotype (n = 30).

Biochemical analyses were conducted to rule out secondary causes of hyperlipidaemia and apo E genotyping was performed to exclude type III hyperlipidemia. Sixteen were on lipid-lowering diets and had been taken off lipid-lowering medication for a period of at least two months when recalled for the study. The rest (n = 14) were identified and recruited before any therapeutic intervention was initiated.

Normolipidaemic control subjects

Fifty six clinically-healthy individuals belonging to 12 normolipidaemic families volunteered to participate and were included as controls in this study. These individuals were recruited from among the clinical and laboratory staff. Subjects undergoing lipid lowering therapy or with secondary causes of hyperlipidaemia were excluded. None of the families met the criteria to be classified as FCHL.

All patients and control subjects recruited into 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.

Analytical methods

A 10 ml venous blood sample was withdrawn after an overnight fast of 12 hours. One aliquot of the plasma samples was immediately frozen at -70ºC in opaque containers for

plasma retinol and HDL cholesterol or apo A-I concentrations was detected in this group. Conversely, in the FCHL group, positive significant correlation between plasma retinol and HDL cholesterol (R = 0.44; p=0.01) and apo A-I (R = 0.53; p = 0.002) was observed.

Discussion

The present report forms part of a wider investigation into the genotypic and phenotypic expression of FCHL. The hypothesis that vitamin A could be involved in the expression of familial combined hyperlipidemia was based on observations which indicated that the increased synthesis and decreased catabolism of VLDL, which is characteristic of FCHL patients, is reverted by the action of fibrate therapy[20,21] the mode of action being the stimulation of the expression of the triglyceride lipase enzyme and repression of the synthesis of its inhibitor, apo C-III.[14-16] Further, this regulatory effect occurs via the PPAR / RXR system which depends on retinoic acid, the intracellular form of plasma vitamin A, being physiologically active.[16,17] Since vitamin A is largely of dietary origin, this aspect could have some significance in the observed variation in the patterns of FCHL expression.

Our results indicating that plasma retinol concentrations were significantly lower in FCHL relative to control subjects are intriguing in that several phenomena associated with FCHL would, now, need to be explored.

Firstly, dietary intake of vitamin A could account for the 50% reduction in the circulating levels of retinol observed in FCHL subjects compared with controls[22,23]. This possibility, however, cannot be the case in the present study since plasma retinol concentrations were no different between those FCHL subjects who were on lipid lowering diet (regularly monitored in the Lipid Clinic) and those who were not. Moreover, preliminary results indicated that vitamin A concentrations were also decreased in normolipidaemic members of FCHL families compared to control subjects (data not shown). These two aspects suggest that low plasma vitamin A concentrations could be more a feature of FCHL rather than a consequence of dietary restriction. Alterations of vitamin A absorption and its binding to RBP[24] could affect the assembly, transport and subsequent hepatic clearance of chylomicrons, the lipoprotein which delivers dietary vitamin A to the liver and which is reported to have a delayed post-prandial clearance in FCHL.[9] *In vivo* lipoprotein kinetics using stable isotopes may help elucidate these aspects.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Secondly, the proposed hypothesis that vitamin A modulates the effect of the PPAR/RXR system on lipoprotein metabolism in response to fibrate or dietary therapy relies on the assumption that intracellular retinoic acid availability is dependent on the plasma concentration of its precursor, vitamin A. In the present study, vitamin A values in the FCHL subjects were not in the range that could be considered as a state of vitamin A deficiency (none of the subjects had plasma vitamin A concentrations below the mean minus two standard deviations of the control values) and, therefore, the extent to which the observed reductions could affect the intracellular availability of retinoic acid needs to be investigated since there is evidence indicating that vitamin A regulates the expression of apo A-I and C-III genes in a tissue specific manner in rats.[25]

Thirdly, controversy exists regarding the closeness of the relationship between retinoids and lipids since, on one hand, hypertriglyceridaemia has been reported to develop as a result of the therapeutic use of retinoids[26] and, on the other hand, epidemiological studies have demonstrated that long-term vitamin A intake does not produce clinically-significant hypertriglyceridemia.[27] Again, *in vivo* lipoprotein kinetics using the recently-developed non-radioactive tracer methodologies in conjunction with *in vitro* testing of this signalling pathway by means of gene expression assays would resolve these questions; studies that are currently under way in our metabolic ward.

In conclusion, the novel observation of low vitamin A concentrations in subjects with FCHL is consistent with our hypothesis that vitamin A could be involved in the pathogenesis of this disorder. The cause of these low vitamin A concentrations requires prompt investigation also because of its reported anti-oxidant and, hence, anti-atherogenic characteristics.

lipoprotein lipase gene (Asp9-Asn) contributes to the expression of familial combined hyperlipidemia. Hum Mol Genet 1995; 4: 1543-1549.

12 Xu CF, Talmud P, Schuster H, Houlston R, Miller G, Humphries S. Association between genetic variation in the AI-CIII-AIV gene cluster and familial combined hyperlipidemia. *Clin Genet* 1994; **46**: 385-397.

13 Dallinga-Thie G, Bu X-D, van Linde-Sibenius Trip M, Rotter JI, Lusis AJ, de Bruin TWA. Apolipoprotein A-I/C-III/A-IV gene cluster in familial combined hyperlipidemia : effects on LDL-cholesterol and apolipoproteins B and C-III. *J Lipid Res* 1996; **37**: 136-147.

14 Staels B, Vu-Dac N, Kosykh VA, et al. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. *J Clin Invest* 1995; **95**: 705-712.

15 Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, et al. PPARa and PPARg activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 1996; **15**: 5336-5348.

16 Auwerx J, Schoonjans K, Fruchart JC, Staels B. Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis* 1996; **124**: S29-S37.

17 Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1996; **1302**: 93-109.

18 Schumaker VR, Puppione DL. Sequential flotation ultracentrifugation. *Method Enzymol* 1986; **128**: 155-170.

19 Bieri JC, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am J Clin Nutr* 1979; **10**: 2143-2149.

20 Kosykh VA, Podrez EA, Novikov DK, et al. Effect of bezafibrate on lipoprotein secretion by cultured human hepatocites. *Atherosclerosis* 1987; **68**: 67-76.

21 Goldberg AP, Applebaum-Bowden DM, Bierman EL, et al. Increase in lipoprotein lipase during clofibrate treatment of hypertriglyceridemia in patients on hemodialysis. *N Eng J Med* 1979; **301**: 1073-1076.

22 Goodman DW. Vitamin A and retinoids in health and disease. *N Eng J Med* 1984; **310**: 1023-1030.

References

1 Rose HG, Kranz P , Weinstock M, Juliano J, Haft JI. Inheritance of combined hyperlipidemia: Evidence for a new lipoprotein phenotype. *J Lipid Res* 1973; **54**: 148-160.

2 Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in coronary heart disease II: Genetic analyses of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest* 1973; **52**: 1544-1568.

3 Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E, Mc Donald GB. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. *J Lipid Res* 1983; **24**: 147-155.

4 Kwiterovich PO. Genetics and molecular biology of familial combined hyperlipidemia. *Curr Opin Lipidol* 1993; **4**: 133-143.

5 Janus ED, Nicoll AM, Turner PR, Magill P, Lewis B. Kinetic bases of the primary hyperlipidemias: Studies of apolipoprotein B turnover in genetically defined subjects. *Eur J Clin Invest* 1980; **10**: 161-172.

6 Austin MA, Horowitz H, Wijsman E, Krauss RM, Brunzell J. Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. *Atherosclerosis* 1992; **92**: 67-77.

7 Babirak SP, Iverius P-H, Fujimoto WY, Brunzell JD. Detection and characterisation of the heterozigote state of lipoprotein lipase deficiency. *Arteriosclerosis* 1989; **9:** 326-334.

8 Babirak SP, Brown BG, Brunzell JD. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscl Thromb* 1992; **12**: 1176-1183.

9 Castro-Cabezas M, de Bruin TWA, Jansen H, Kock AW, Kortland W, Erkelens DW. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler Thromb* 1993; **13**: 804-814.

10 Mailly F, Tugrul Y, Reymer PW, et al. A common variant in the gene for lipoprotein lipase (Asp9-Asn). Functional implications and prevalence in normal and hyperlipidemic subjects. *Arterioscl Thromb Vasc Biol* 1995; **15** : 468-478.

11 Reymer PW, Groenemeyer BE, Gagne E, et al. A frequently occurring mutation in the

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

23 Blomhoff R, Green M, Morum K. Vitamin A: Physiological and biochemical processing. *Ann Rev Nutr* 1990; **49**: 1-12.

24 Kanai M, Raz A, Goodman DS. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J Clin Invest* 1968; **47**: 2025-2044.

25 Nagasaki A, Kikuchi T, Kurata K, Masushige S, Hasegawa T, Kato S. Vitamin A regulates the expression of apolipoprotein AI and CIII genes in the rat. *Biochem Bioph Res Co* 1994; **205**: 1510-1517.

26 Cohen PR. The use of gemfibrozil in a patient with chronic myelogenous leukemia to successfully manage retinoid-induced hypertriglyceridemia. *Clin Investig* 1993; **71**: 74-77.

27 Omenn GS, Goodman GE, Thornquist M, Brunzell JD. Long-term vitamin A does not produce clinically significant hypertriglyceridemia: results from CARET, the beta-carotene and retinol efficacy trial. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 711-713.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 1. Biometric characteristics of the FCHL and control group of subjects.

	FCHL (n=30)	CONTROL (n=56)
Age	44.1± 19.5	40.0 ± 17.2
BMI (Kg/m^2)	26.9 ± 5.6	24.4 ± 3.8
Gender (M/F)	16/14	26/30

Body mass index (BMI). Gender (Male/Female).

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 2. Lipid, lipoprotein and apolipoprotein values in the FCHL and Control group of subjects.

	FCHL (n=30)	CONTROL (n=56)
Chol	6.47 ± 0.93#	4.92 ± 0.91
Tg	1.82 ± 0.83#	0.99 ± 0.53
VLDL		
Chol	0.65 ± 0.47#	0.20 ± 0.17
Tg	1.11 ± 0.67#	0.46 ± 0.42
IDL		
Chol	0.38 ± 0.27#	0.15 ± 0.09
Tg	0.24 ± 0.12#	0.12 ± 0.05
LDL		
Chol	4.34 ± 0.87#	3.27 ± 0.76
Tg	0.36 ± 0.13#	0.25 ± 0.08
Total HDL	1.09 ± 0.33	1.32 ± 0.35
HDL_2	0.17 ± 0.09	0.27 ± 0.15
HDL_3	0.92 ± 0.28	1.05 ± 0.26
Lp(a)	20.8 ± 17.9	23.0 ± 14.0
Apo A-I	122.3 ± 26.3	121.9 ± 20.4
Apo B	122.1 ± 22.6#	83.7 ± 21.3
Apo C-II	6.6 ± 1.3#	4.7 ± 0.5
Apo C-III	14.5 ± 3.0#	10.9 ± 2.0

Cholesterol and triglycerides in plasma and lipoprotein fractions are expressed as mmol/L.
Lp (a) and apolipoproteins are expressed as mg/dL.
Significantly different from control.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 3. Plasma retinol correlations with lipid and apolipoprotein concentrations.

Group	Chol	Tg	LDLc	HDLc	Apo B	Apo A-I
FCHL						
	0.15	0.12	0.05	0.44	0.20	0.53
	(NS)	(NS)	(NS)	(p<0.01)	(NS)	(p<0.002)
CONTROL						
	0.38	0.23	0.32	0.004	0.42	0.22
	(P<0.003)	(P<0.05)	(P<0.01)	(NS)	(p<0.001)	(NS)

Correlation (R) values adjusted for age, gender and BMI. Statistical significance in brackets.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Figure 1.

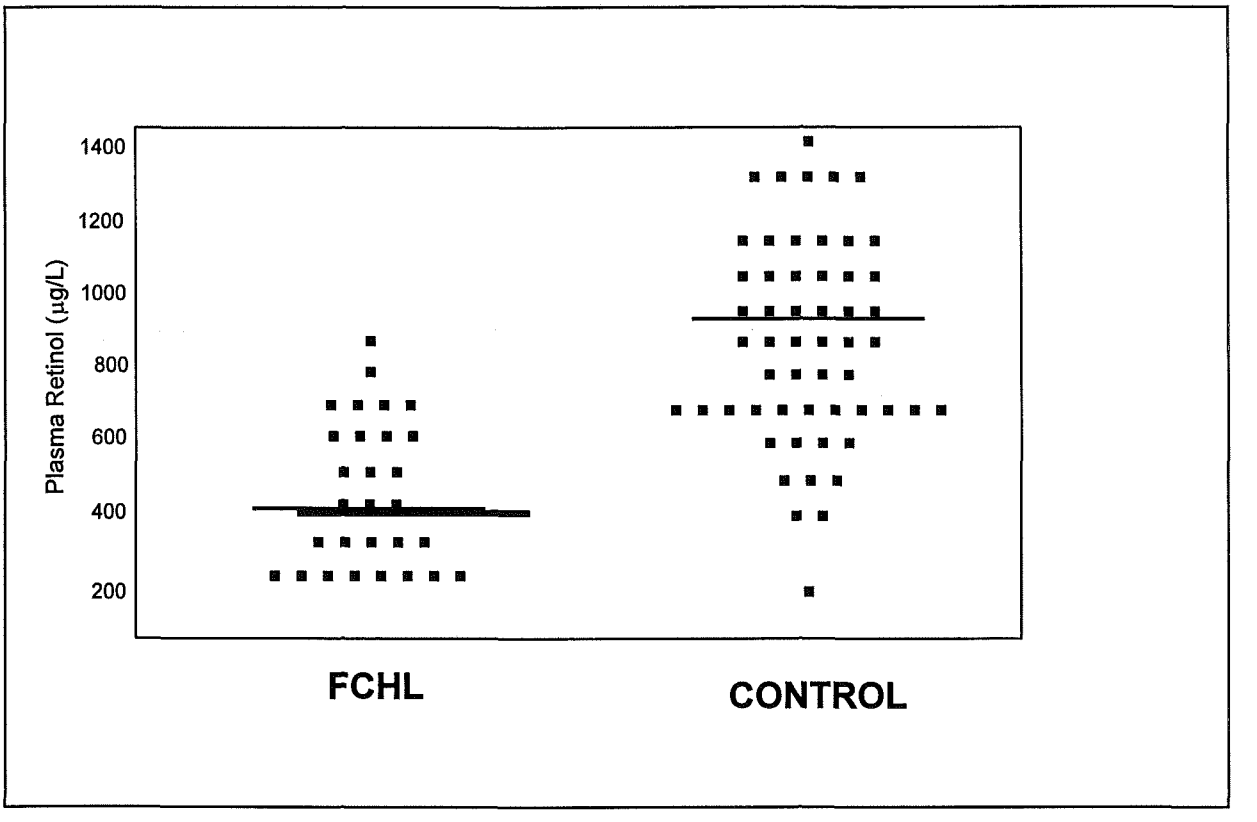

Figure 1

Plasma retinol concentrations in the individual FCHL (n=30) and control subjects (n=56). Horizontal line indicates mean plasma retinol concentrations of each group.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

4.5. ESTUDI 4

Estudi de les lipoproteïnes d'alta densitat i la seva relació amb l'activitat de l'enzim LCAT en pacients amb hiperlipèmia familiar combinada.

Ribalta J, La Ville AE, Vallvé JC, Girona J, Masana L. *Lecithin :cholesterol acyltransferase activity is not altered in familial combined hyperlipidemia. Atherosclerosis.* (En revisió).

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Lecithin:cholesterol acyltransferase (LCAT) activity is not altered in Familial combined hyperlipidemia.

Abstract

Elevated concentrations of plasma cholesterol and triglycerides are characteristic of Familial Combined Hyperlipidemia (FCHL) which may also present reduced high density lipoprotein (HDL) cholesterol concentrations. Lecithin:cholesterol acyltransferase (LCAT) plays a key role in the reverse cholesterol transport by converting unesterified cholesterol to cholesterol ester in the process of maturation of HDL and, therefore, could influence HDL metabolism in these patients.

We aimed to study high density lipoproteins and LCAT activity in 25 FCHL subjects and 48 controls. Total HDL (p=0.018) and HDL2 (p=0.008) were significantly decreased in the FCHL group compared with controls. After analyses with adjusted data only HDL2 remained significantly decreased in the FCHL group (p=0.050). The LDLc/HDLc and A-I/HDLc ratios were significantly elevated in the FCHL group, the latter suggesting the existence of compositional differences in the HDL particles of the FCHL individuals.

LCAT activity assessed in the FCHL (19.94 ± 3.95 nmol/ml/h) and control (20.13 ± 6.86 nmol/ml/h) groups showed no statistically significant differences. A significant positive correlation of LCAT activity with total HDL (r=0.42), HDL3 cholesterol (r=0.46) and apolipoprotein A-I (r=0.47) was observed in affected subjects but not in controls.

An association between a G_{-75}-A variation in the promoter region of the apo A-I gene and elevated concentrations of total HDL cholesterol (p=0.052) and apo A-I (p=0.009) was observed. This association was strongly influenced by the status of the subject (FCHL or control) providing further evidence for a regulatory role of this genetic region in the expression of FCHL.

Our data suggests that LCAT activity is normal in FCHL and, therefore, does not account for the abnormalities observed in these patients essentially with regard to the HDL2 subfraction.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Introduction

Familial combined hyperlipidemia (FCHL) is the commonest genetic form of hyperlipidemia among survivors of premature myocardial infarction (10-20%) with an estimated prevalence of 0.5-2% in the general population [1,2]. Family studies are necessary to diagnose FCHL in an individual patient since the FCHL phenotype may vary among family members and, over a temporal sequence, even in the individual patient, with hypercholesterolemia, hypertriglyceridemia, or both, being expressed [3,4]. Increased flux of apolipoprotein (apo) B of very low density lipoprotein (VLDL) and decreased catabolism of VLDL and intermediate density lipoproteins (IDL) have been reported in these patients [5-8]. Increase of small dense low density lipoproteins (LDL) [9,10] and impaired lipoprotein lipase (LPL) activity [11,12] are also features associated with the FCHL phenotype in a subset of patients.

Low concentrations of high density lipoprotein (HDL) have been reported in relation to FCHL [4,13,14] and could account for the increased risk of coronary artery disease (CAD) associated with this disorder [15,16]. If these reductions can be considered a specific characteristic of these patients remains to be elucidated since factors of a either metabolic (hypertriglyceridemia, insulin resistance, menopause) [17-19], environmental (smoking, physical exercise) [20,21] or genetic (apo E genotype) [22] nature may partially explain these reductions. Conversely, several reports suggest that key proteins involved in reverse cholesterol transport, such as, Lecithin :cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) might be altered in these patients [23,24]. LCAT converts unesterified cholesterol to cholesterol ester in the process of maturation of HDL [25,26] whereas CETP exchanges neutral lipids between triglyceride-rich lipoproteins and HDL [27]. While CETP has been reported to have increased activity in patients with combined hyperlipidemia [24], the potential role of LCAT in FCHL has been pointed out in animal studies [23] but remains to be clarified in humans. More precisely, familial hypercholesterolemic swine, which resemble FCHL, present elevations in HDL cholesterol in response to drug treatment which are accompanied of increased LCAT activity.

The fact that the gene encoding for apo A-I, the activator of LCAT, is located in the AI-CIII-AIV gene cluster to which extensive data links to the aetiology of FCHL [28-30], further supports a potential role of LCAT in the expression of FCHL.

We aimed to study high density lipoproteins and LCAT activity in subjects with familial combined hyperlipidemia and also its relation with a polymorphism in the promoter region of the apo A-I gene in order to investigate a potential genetic component underlying the reverse

transport of cholesterol in FCHL.

Subjects and methods

All subjects recruited into 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.

FCHL families

Nine index patients diagnosed as having FCHL were recruited from the Lipid Clinic of the Hospital Universitari de Sant Joan in Reus (Spain). Diagnosis was based on the subject having plasma concentrations of cholesterol (chol) and triglycerides (trig) ≥ 6.4 mmol/L and ≥ 2.8 mmol/L, respectively, detected at any time in the clinical history and at least one first degree relative with a hyperlipidemic phenotype different from that of the proband. Mean plasma cholesterol and triglyceride values of index subjects during selection were 7.5 ± 0.9 and 4.5 ± 1.5 mmol/l, respectively.

Biochemical analyses were conducted to rule out secondary causes of hyperlipidemia and apo E genotyping was performed to exclude type III hyperlipidemia. All index subjects were on a lipid lowering diet and had been taken off lipid lowering medication for a period of at least two months when recalled for the study. All available family members of index patients with plasma cholesterol concentrations ≥ 6.4 mmol/L and/or plasma triglycerides ≥ 2.8 mmol/L or elevated above the 95th percentile for age and gender were assigned the FCHL phenotype (n=16). The FCHL group totalled with the index subjects 25 individuals .

Normolipidemic control families

Forty eight individuals belonging to 12 normolipidemic families volunteered to participate and were included as controls in this study. The families were from among the clinical and laboratory staff. Subjects undergoing lipid lowering therapy or with secondary causes of hyperlipidemia were excluded. None of the families met the criteria to be classified as FCHL.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Analytical methods

A 10 ml venous blood sample was withdrawn after an overnight fast of 12 hours. Triglycerides and cholesterol in plasma and lipoprotein fractions were measured using enzymatic kits (Boehringer Mannheim, Germany) adapted for a Cobas Mira analyzer (Roche Pharmaceuticals, Switzerland) with Precilip EL® and Precinorm® (Boehringer Mannheim, Germany) as quality controls. Immuno-turbidometry was employed for the measurement of the apolipoproteins using specific antiserum purchased from Boehringer Mannheim, Germany (for apo A-I and apo B), Daiichi Chemicals, Japan (for apo C-II and apo C-III) and Incstar Corporation, U.S.A (for Lp(a)).

Sequential preparative ultracentrifugation

Lipoproteins were separated by sequential preparative ultracentrifugation [31], using a Kontron 45.6 fixed-angle rotor in a Centrikon 1075 (Kontron Instruments, Italy). The lipoprotein fractions isolated were VLDL (d<1.006 g/ml), IDL (d=1.006-1.019 g/ml) and LDL (d=1.019-1.063 g/ml). Total HDL and HDL3 cholesterol were measured subsequent to the precipitation of the apo B-containing lipoproteins with polyethylene glycol (Immuno AG, Austria). HDL2 cholesterol was calculated from the difference between total HDL and HDL3 cholesterol.

LCAT activity assay

LCAT activity was assessed using the liposome substrate prepared according to Albers et al [32]. The reaction was initiated by mixing 15 μL of plasma and 100 μL of the substrate. Samples were incubated for 30 minutes at 37ºC and the reaction stopped by addition of 2 ml of ethanol. LCAT was extracted into hexane and the hexane phase was evaporated under nitrogen, and the free and esterified cholesterol fractions were separated by thin layer chromatrography. The cholesterol ester bands were visualized with rhodamine scraped from the plates and put into liquid scintillation fluid for beta counting. LCAT activity is expressed as nmol cholesterol esterified/ml/hour.

DNA analyses

DNA was extracted from an aliquot of frozen white cells by the salting out method [33]. PCR [34] was carried out to amplify the region containing the G_{-75}-A substitution. The

reaction mixture (50 μL) for amplification contained 250 ng of each of the two primers (Boehringer Mannheim, Germany), 5' primer, 5'-AGGGACAGAGCTGATCCTTGAACTCTTAAG-3' and 3' primer, 5'-TTAGGGGACACCTAGCCCTCAGGAAGAGCA-3', 200 ng of genomic DNA and 1 U of Taq polymerase (Boehringer Mannheim, Germany). Magnesium concentration in the reaction buffer was 1.5 mM. The reactions were performed on a Hybaid Omnigene thermocycler at 95ºC for 5 min, 55ºC for 1 min and 72ºC for 2 min followed by 35 cycles of 95ºC for 1 min, 55 ºC for 1 min and 72ºC for 2 min. Twenty microliters of PCR product were digested overnight with 9 U of the restriction enzyme *MspI* (Boehringer Mannheim) 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* results 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 are produced [35].

Statistical Analyses

The χ^2 test was used to compare the frequency of the A_{-75} genotypes (GA/AA) among groups. The Z test for comparison of proportions was used to compare allele frequencies. ANOVA was performed to compare the means of the lipid and LCAT activity adjusted data and $\log_{10}$ transformed when the variables were not normally distributed. Deviation from Hardy-Weinberg equilibrium was tested with the χ^2 goodness-of-fit test. Results are expressed as means ± standard deviations. Statistical significance was accepted at the 0.05 level.

Results

According to the criteria mentioned above, the population of study was divided in two groups. Those subjects belonging to FCHL families expressing the FCHL phenotype (*FCHL* ; n=25) and a group of healthy controls (*controls* ; n=48). Biometric data of the two groups is summarised in table 1. The two groups were comparable with relation to age and gender distribution. The percentage of cigarette smokers (26% and 33%, respectively) and pre-menopausal women (71% and 83%, respectively) in FCHL subjects and controls was not

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

significantly different. The BMI was elevated in the FCHL group relative to controls (p=0.035)

Lipoproteins and apolipoproteins

In the affected group 13 subjects with hypercholesterolemia alone, 4 subjects with hypertriglyceridemia alone and 8 subjects presenting both, were identified. Plasma concentrations of lipoproteins related to peripheral lipid transport (VLDL, IDL and LDL) (Table 2) and to reverse cholesterol transport (total HDL, HDL2 and HDL3) (table 3) were studied in the two groups. Cholesterol and triglycerides in plasma, VLDL, IDL and LDL together with apolipoproteins B, C-II and C-III were significantly elevated among FCHL subjects compared with control individuals.

In relation to HDL metabolism, total HDL (p=0.018) and HDL2 (p=0.008) cholesterol concentrations were decreased among FCHL subjects. After ANOVA analyses on data adjusted for age, gender, BMI and fasting plasma triglycerides only differences regarding the HDL2 fraction remained significant (p=0.050). Significantly elevated values for the apo A-I/HDLc (p<0.0001) and LDLc/HDLc (p<0.0001) ratios were observed in the FCHL group compared with controls.

LCAT activity

LCAT activity was determined in FCHL and control subjects. ANOVA analyses on adjusted data did not show any difference between groups regarding LCAT activity (table 4). Significant differences between groups were observed with the correlation of LCAT activity with apo A-I and HDL cholesterol concentrations. Statistically significant correlation (r) values of 0.47, 0.42 and 0.46 were obtained with LCAT activity and apo A-I, total HDL and HDL3, respectively, in the FCHL group. LCAT activity significantly correlated only with HDL2 (r=0.27) in controls.

G_{-75}-A polymorphism in the promoter of the apo A-I gene

The observed frequencies of the GG, GA and AA genotypes in the two groups were not different from those predicted by the Hardy-Weinberg distribution. The genotype (GA+AA) (0.37 vs 0.27) and allele frequencies (0.21 vs 0.13) in the FCHL and control groups were not significantly different.

The effect of the A_{-75} allele on lipids, lipoproteins and apolipoproteins was studied.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

IDL cholesterol (p=0.032), apo C-III (p=0.041) and apo A-I (p=0.009) were significantly elevated in subjects carrying the A_{-75} allele. Total HDL showed elevations in the A_{-75} which almost reached statistical significance (p=0.052). Genotype-status interaction was investigated by means of an ANOVA model showing that the relationship between this polymorphism and triglyceride-rich lipoproteins was significantly modified by the status of the subject (FCHL or controls). This interaction was also found to affect total HDL and apo A-I.

LCAT activity was also studied in relation with the G-A substitution of the apo A-I gene. LCAT activity in subjects with the GG genotype (19.83 ± 6.97 nmol/ml/hour) was almost identical to that of the A allele carriers (19.51 ± 3.90 nmol/ml/hour). No significant genotype-status interaction was detected with regard to the activity of the enzyme.

Discussion

The population studied had unusually normal mean plasma triglyceride concentrations for a FCHL group when recruited for the study and this is for two reasons. First, the presence of several FCHL subjects with plasma triglyceride concentrations above the 95th percentile for age and gender although not necessarily meaning above 2.8 mmol/l, the cut-off point value for an adult FCHL patient. Second, a clear over-representation of isolated hypercholesterolemia (n=13) versus isolated hypertriglyceridemia (n=4). Given the well known effect that hypertriglyceridemia has on HDL metabolism, this group of subjects seemed to be an appropriate sample to explore any possible alteration of HDL related to FCHL, minimising the effect of hypertriglyceridemia.

Concentrations of HDL cholesterol might be reduced in FCHL [4,13,14]. However, before this can be considered a specific characteristic of FCHL, several aspects should be taken into account. The diversity of lipoprotein phenotypes present among FCHL subjects, as well as, a number of factors such as high fasting plasma triglycerides, cigarette smoking, alcohol intake or physical exercise [17-22] can account for the low levels of HDL observed in these subjects. Our results confirm this point by showing that the reductions in total HDL and HDL3 among FCHL subjects are essentially explained by their age, gender, BMI and plasma triglycerides. However, HDL2 cholesterol concentrations are significantly reduced in this group and this is consistent with the data of Brunzell and co-workers [4] indicating that reductions in HDL mostly affect the HDL2 fraction in FCHL.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

The increased LDLc/HDLc and A-I/HDLc ratios observed in the FCHL group compared with controls remained significant in all the cases. While the former is most likely a consequence of the reported hepatic overproduction of VLDL and, to a lesser extent, of LDL [7], the understanding of the latter might be of importance. Assuming that apo A-I is almost completely contained in the HDL fraction, an elevated A-I/HDLc ratio suggests the existence of some alterations in the composition of the HDL particles. Whether these alterations might result in less efficient reverse cholesterol transport which could, subsequently, contribute to the increased risk of CAD associated with FCHL, remains to be elucidated. Should it be so, those proteins involved in the modelling of HDL particles, LCAT and CETP, could play a role in this process. While CETP has recently been reported to have an increased activity in patients with combined hyperlipidemia [24], LCAT has not been studied in much detail in relation to FCHL.

To clarify whether LCAT could play a role in the regulation of HDL cholesterol in these patients, its activity was assessed *in vitro* in the both groups of study. Our results showed that LCAT activity was the same in FCHL or control subjects, indicating that a defective form of the enzyme associated with FCHL ought to be discarded. However, since the ability of LCAT to esterifiy cholesterol is assessed, *in vitro*, using an artificial substrate [32], a defective function of the enzyme, *in vivo,* due to the specific composition of the HDL particles of these patients should not be ruled out. This possibility is strengthened by several studies which indicate that the phospholipid or apolipoprotein composition of HDL might have an important effect on LCAT activity [37,38]. Further studies of LCAT activity *in vivo* in these patients should be made in order to clarify this point.

Of interest is also the markedly different correlation between LCAT activity and HDL parameters in FCHL and control subjects. A positive significant correlation of LCAT with apo A-I, total HDL and HDL3 was observed in FCHL subjects but not in controls. This could suggest that HDL cholesterol concentrations are more importantly influenced by LCAT in those subjects with a hyperlipidemic profile.

A potential genetic component underlying the observed differences was assessed by studying a $G_{-75}A$ substitution in the promoter region of the apo A-I gene [35]. Apo A-I, the major protein component of HDL and the activator of LCAT, is encoded in the AI-CIII-AIV gene cluster to which extensive data link to FCHL [28-30]. This variation showed to be associated with elevations in IDL cholesterol and apolipoproteins A-I and C-III. Interestingly, the associations between this polymorphism and parameters related to triglyceride-rich

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

metabolism were found to be significantly different in FCHL and control subjects. This genotype-status interaction confirms previous observations by our group [39] and supports the reported regulatory role of the AI-CIII-AIV gene cluster in FCHL [30].

We conclude that LCAT enzyme is normal in FCHL and, therefore, does not account for the abnormalities observed in these patients essentially with regard to the HDL2 subfraction.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Acknowledgments

The authors wish to thank Prof. Steve Humphries and Dr. Chung-Fang Xu for invaluable help and advice in the setting-up of the molecular biology techniques; Mercedes Heras and Silvia Olivé, for their excellent technical support; Drs. Núria Plana, Pilar Sardà, Rosa Solà and Carlos Alonso-Villaverde for their clinical assistance. This study has been supported, in part, by the Fundació Joan Abelló Pascual and the Ministerio de la Salud (DGICYT # PM 92-0209).

References

[1] Rose HG, Kranz P, Weinstock M, Juliano J, Haft JI. Inheritance of combined hyperlipidemia: Evidence for a new lipoprotein phenotype. J Lipid Res 1973 ; 54: 148.

[2] Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. Hyperlipidaemia in coronary heart disease II: Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidaemia. J Clin Invest 1973 ; 52: 1544.

[3] Nikkilä EA, Aro A. Family study of serum lipids and lipoproteins in coronary heart disease. Lancet 1973 ; 1: 954.

[4] Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszek E, Mc Donald GB. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. J Lipid Res 1983 ; 24: 147.

[5] Chait A, Albers JJ, Brunzell JD. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridemia. Eur J Clin Invest 1980 ; 10: 17.

[6] Cortner JA, Coates PM, Bennett MJ, Cryer DR, Le NA. Familial combined hyperlipidemia: use of stable isotopes to demonstrate overproduction of very-low-density lipoprotein apolipoprotein B by the liver. J Inherited Dis 1991 ; 14: 915.

[7] Janus ED, Nicoll AM, Turner PR, Magill P, Lewis B. Kinetic bases of the primary hyperlipidemias: studies of apolipoprotein B turnover in genetically defined subjects. Eur J Clin Invest 1980 ; 10: 161.

[8] Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. Stable isotopes show a direct relation between VLDL apo B overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. Arterioscl Thromb 1993 ; 13: 1110.

[9] Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. Arteriosclerosis 1990 ; 10: 520.

[10] Austin MA, Horowitz H, Wijsman E, Krauss RM, Brunzell J. Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. Atherosclerosis 1992 ; 92: 67.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

[11] Babirak SP, Iverius P-H, Fujimoto WY, Brunzell JD. Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. Arteriosclerosis 1989 ; 9: 326.

[12] Babirak SP, Brown BG, Brunzell JD. Familial combined hyperlipidemia and abnormal lipoprotein lipase. Arterioscl Thromb 1992 ; 12: 1176.

[13] Castro Cabezas M, de Bruin TWA, de Valk HA, Shoulders CC, Jansen H, Erkelens DW. Impaired fatty acid metabolism in familial combined hyperlipidemia. J Clin Invest 1993 ; 92: 160.

[14] Brunzell J, Mazzone T, Motulsky A, Albers JJ. Abnormalities in high density lipoprotein composition in familial combined hyperlipidemia. Arterioscler Thromb 1982 ; 2: 416a.

[15] Castro Cabezas M, de Bruin TWA, Erkelens DW. Familial combined hyperlipidemia: 1973-1991. Neth J Med 1992 ; 40: 83.

[16] Kwiterovich PO. Genetics and molecular bilogy of familial combined hyperlipidemia. Curr Opin Lipidol 1993 ; 4: 133.

[17] Frayn KN. Insulin resistance and lipid metabolism. Curr Opin Lipidol 1993 ; 4: 197.

[18] Kauma H, Savolainen MJ, Heikkila R, Rantala AO, Lilja M, Reunanen A, Kesaniemi YA. Sex difference in the regulation of plasma high density lipoprotein cholesterol by genetic and environmental factors. Hum Genet 1996 ; 97: 156.

[19] Murakami T, Michelagnoli S, Longhi R, Gianfrancheschi G, Pazzuconi F, Calabresi L, Sirtori CR, Franceschini G. Triglycerides are major determinants of cholesterol esterification/transfer and HDL remodelling in human plasma. Arterioscler Thromb Vasc Biol 1995 ; 15: 1819.

[20] Wilson PW, Evans JC. Coronary artery disease prediction. Am J Hypertens 1993 ; 6: 309S.

[21] Angotti CM, Levine MS. Review of 5 years of a combined dietary and physical fitness intervention for control of serum cholesterol. J Am Diet Assoc 1994 ; 94: 634.

[22]. Sijbrands EJ, Westendorp RG, Hoffer MJ, Havekes LM, Frants RR, Meinders AE, Frolich M, Smelt AH. Effect of insulin resistance, apo E2 allele, and smoking on combined hyperlipidemia. Arterioscl Thromb 1994 ; 14: 1576.

[23] Hasler-Rapacz J, Kempen HJ, Princen HM, Kudchodkar BJ, Lacko A, Rapacz J. Effects of simvastatin on plasma lipids and apolipoproteins in familial

hypercholesterolemic swine. Arterioscl Thromb Vasc Biol 1996 ; 16: 137.

[24] Tato F, Vega GL, Tall AR, Grundy SM. Relation between cholesterol ester transfer protein and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. Arterioscl Thromb Vasc Biol 1995 ; 15: 112.

[25] Applebaum-Bowden D. 1995. Lipases and lecithin :cholesterol acyltransferase in the control of lipoprotein metabolism. Curr Opin Lipidol 1995 ; 6: 130.

[26] Leroy A, Dallongeville J, Fruchart JC. Apolipoprotein A-I-containing lipoproteins and atherosclerosis. Curr Opin Lipidol 1995 ; 6: 281.

[27] Lagrost L. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vivo and in vitro studies. Biochim Biophys Acta 1994 ; 1215: 209.

[28] Wojciechowski AP, Farrall M, Cullen P, Wilson TME, Bayliss JD, Griffin BA, Caslake MD, Packard CJ, Shepherd J, Thakker R, Scott J. Familial combined hyperlipidemia linked to the apolipoprotein A-I/C-III/A-IV gene cluster on chromosome 11q23-q24. Nature 1991 ; 349: 161.

[29] Xu CF, Talmud P, Schuster H, Houlston R, Miller G, Humphries S. Association between genetic variation at the apo AI-CIII-AIV gene cluster and familial combined hyperlipidemia. Clin Genet 1994 ; 46: 385.

[30] Dallinga-Thie GM, Bu X-D, v L-S. Trip M, Rotter JI, de Bruin TWA. Apolipoprotein A-I/C-III/A-IV gene cluster in familial combined hyperlipidemia: effects on LDL-cholesterol and apolipoproteins B and C-III. J Lipid Res 1996 ; 37: 136.

[31] Schumaker VR, Puppione DL. Sequential flotation ultracentrifugation. Method Enzymol 1986 ; 128: 155.

[32] Albers JJ, Chen C-H, Lacko AG. Isolation, characterization and assay of lecithin-cholesterol acyltransferase. Method Enzymol 1986 ; 129: 776.

[33] Miller SA, Dykes DD, Polesky HF. A simple salting out method procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988 ; 16: 1215.

[34] Saiki RK, Ferrell RE, Stoffel S, Scharff SJ, Higuchi R, Horn GT, Mullis KB, Erlich H. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988 ; 239: 487.

[35] Xu CF, Angelico F, Del Ben M, Humphries S. Role of genetic variation at the apo AI-CIII-AIV gege cluster in determining plasma apo AI levels in boys and girls. Genet Epidemiol 1993 ; 10: 113.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

[36] Castro cabezas M, de Bruin TWA, Jansen H, Kock AW, Kortland W, Erkelens DW. Impaired chylomicron remnant clearance in Familial Combined Hyperlipidemia. Arterioscler Thromb 1993 ; 13: 804.

[37] Bolin DJ, Jonas A. Binding of lecithin :cholesterol acyltransferase to reconstituted high density lipoproteins is affected by their lipid but not apolipoprotein composition. J Biol Chem 1994 ; 269: 20156.

[38] Duverger N, Ghalim N, Theret N, Fruchart JC, Castro G. Lipoproteins containing apo A-IV : composition and relation to cholesterol esterification. Biochim Biophys Acta 1994 ; 1211: 23.

[39] Ribalta J, La Ville AE, Vallvé JC, Humphries SE, Turner PR, Masana L. A variation in the apolipoprotein C-III gene is associated with an increased number of circulating VLDL and IDL particles in familial combined hyperlipidemia. J Lipid Res 1997 ; (In press).

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 1. Biometric characteristics

	FCHL	CONTROL
Number	25	48
Age	41.3 ± 21.1	40.9 ± 18.1
Gender (M/F)	14/11	23/25
BMI (Kg/m^2)	26.1 ± 5.4[a]	24.4 ± 3.7
Diastolic BP	77 ± 16	78 ± 14
Systolic BP	128 ± 24	125 ± 20

[a] Significantly different from controls ; p=0.035. Gender Male/Female.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 2. Lipids and apolipoproteins related to VLDL, IDL and LDL.

Variable	FCHL (n=25)	CONTROLS (n=48)
Plasma Chol	6.30 ± 1.01[a]	5.04 ± 1.04
Plasma Trig	1.79 ± 0.65[a]	1.07 ± 0.65
VLDL		
Chol	0.63 ± 0.47[a]	0.23 ± 0.21
Trig	1.11 ± 0.70[a]	0.50 ± 0.50
IDL		
Chol	0.36 ± 0.27[a]	0.17 ± 0.13
Trig	0.23 ± 0.12[a]	0.12 ± 0.06
LDL		
Chol	4.24 ± 0.89[a]	3.39 ± 0.84
Trig	0.35 ± 0.13[a]	0.26 ± 0.10
Lp(a)	20.6 ± 17.4	20.9 ± 13.4
Plasma apo B	118.0 ± 25.7[a]	88.3 ± 25.2
Plasma apo C-II	6.54 ± 1.28[a]	4.65 ± 0.48
Plasma apo C-III	14.7 ± 4.52[a]	10.8 ± 1.90

[a] Significantly different from controls $p<0.0001$

Statistical differences assessed by ANOVA with data adjusted for BMI, gender and age. Cholesterol and triglycerides are expressed in mmol/L. Apolipoproteins and Lp(a) are expressed in mg/dl.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 3. Lipids and apolipoproteins related to HDL metabolism.

Variable	FCHL (n=25)	CONTROL (n=48)	p^a=	p^b=
Total HDL	1.12 ± 0.35	1.28 ± 0.30	0.018	NS
HDL_2	0.17 ± 0.10	0.24 ± 0.15	0.008	0.050
HDL_3	0.95 ± 0.30	1.03 ± 0.23	NS	NS
Plasma apo A-I	123.6 ± 25.8	120.6 ± 19.2	NS	NS
A-I / HDLc	115.5 ± 20.0	97.2 ± 13.6	0.0001	0.014
LDLc / HDLc	4.15 ± 1.47	2.78 ± 0.88	0.0001	0.001

p^a= statistical significance with unadjusted data.

P^b= statistical significance for ANOVA with data adjusted for age, BMI, gender and fasting plasma triglycerides.

HDLc = HDL cholesterol ; LDLc = LDL cholesterol

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 4. LCAT activity and correlation with apo A-I and HDL parameters in FCHL and control subjects.

		FCHL (n=25)	CONTROL (n=48)
LCAT activity		19.94 ± 3.95	20.13 ± 6.86
Correlation			
	Apo A-I	r=0.47 (p=0.027)	r=0.01 (NS)
	Total HDL	r=0.42 (p=0.047)	r=-0.01 (NS)
	HDL2	r=0.12 (NS)	r=0.27 (p=0.040)
	HDL3	r=0.46 (p=0.030)	r=-0.19 (NS)
	A-I/HDLc	r=-0.16 (NS)	r=-0.02 (NS)
	LDLc/HDLc	r=-0.21 (NS)	r=-0.23 (NS)

LCAT activity is expressed in nmol cholesterol esterified/ml/hour.
Values of LCAT activity and correlation with HDL parameters obtained from data adjusted for age, gender and BMI.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 5. Lipids and apolipoproteins related to VLDL, IDL and LDL based on genotype.

	GG	GA/AA	p=	Status interaction
Number	49	24		
Plasma Chol	5.40 ± 1.10	6.02 ± 1.28	NS	NS
Plasma Trig	1.29 ± 0.78	1.59 ± 0.90	NS	0.007
VLDL				
Chol	0.34 ± 0.30	0.54 ± 0.53	NS	0.0001
Trig	0.69 ± 0.80	0.93 ± 0.76	NS	0.002
IDL				
Chol	0.20 ± 0.14	0.35 ± 0.31	0.032	0.007
Trig	0.15 ± 0.08	0.21 ± 0.13	NS	0.009
LDL				
Chol	3.69 ± 0.99	3.95 ± 0.89	NS	NS
Trig	0.28 ± 0.12	0.33 ± 0.11	NS	NS
Lp(a)	20.21 ± 15.24	21.32 ± 15.56	NS	NS
Plasma apo B	97.4 ± 28.0	110.5 ± 29.7	NS	0.033
Plasma apo C-II	5.28 ± 1.11	5.87 ± 1.60	NS	0.006
Plasma apo C-III	11.9 ± 2.4	14.1 ± 5.5	0.041	0.0001

Statistical differences assessed by ANOVA with data adjusted for BMI, gender and age. Cholesterol and triglycerides are expressed in mmol/L. Apolipoproteins and Lp(a) are expressed in mg/dl. Status interaction indicates a significant effect of being a FCHL subject on the asociation between the polymorphism and lipid parameters.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Table 6. Lipids and apolipoproteins related to HDL based on genotype.

	GG	GA/AA	p=	Status interaction
Number	49	24		
Total HDL	1.19 ± 0.35	1.24 ± 0.29	0.052	0.029
HDL_2	0.21 ± 0.15	0.23 ± 0.09	NS	NS
HDL_3	0.99 ± 0.27	1.01 ± 0.25	0.083	NS
Plasma apo A-I	118.1 ± 18.3	129 ± 27.2	0.009	0.001
A-I / HDLc	104.0 ± 19.4	107.4 ± 18.4	NS	NS
LDLc / HDLc	3.41 ± 1.45	3.35 ± 1.10	NS	NS

HDL cholesterol expressed as mmol/l. Apo A-I expressed as mg/dl. Statistical differences ssessed by ANOVA with data adjusted for age, BMI, gender and fasting plasma triglycerides.. Status interaction indicates a significant effect of being a FCHL subject on the asociation between the polymorphism and lipid parameters.

HDLc = HDL cholesterol ; LDLc = LDL cholesterol

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

4.6. Resum dels resultats

- La freqüència d'individus portadors del polimorfisme C_{1100}-T del gen de l'apolipoproteïna C-III és més elevada en pacients HLFC (64%) que en controls (36%).

- Els portadors del polimorfisme C_{1100}-T tenen concentracions més elevades de triglicèrids plasmàtics, de colesterol, triglicèrids i apo B en VLDL i IDL, i d'apo C-III.

- L' associació entre el polimorfisme C_{1100}-T i els esmentats paràmetres lipídics només es detecta en els individus HLFC.

- Les concentracions plasmàtiques de retinol son més baixes en individus HLFC que en controls (418.9 vs 888.9 μg/L). Aquestes diferències no són degudes a factors dietètics.

- L'activitat *in vitro* de l'enzim LCAT no és diferent entre pacients HLFC i controls.

- La concentració de colesterol en la fracció HDL2 és més baixa en els pacients HLFC tot i que aquesta diferència està al límit de la significació estadística.

- El polimorfisme G_{-75}-A del gen de l'apolipoproteïna A-I està associat a concentracions més elevades d'apo A-I, apo C-III i colesterol de la fracció IDL.

- La prevalença d'hiperlipèmia entre descendents menors de 19 anys de pacients HLFC és del 43%. Aquests, a diferència dels individus adults, no presenten alteracions en la fracció IDL.

- La determinació de la isoforma de l'apolipoproteïna E a partir del fenotip suposa un 6% d'error respecte del genotip.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

5. CONCLUSIONS GLOBALS

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

5. Conclusions globals

- Les associacions dels polimorfismes C_{1100}-T (apo C-III) i G_{-75}-A (apo A-I) amb els triglicèrids plasmàtics, les fraccions VLDL i IDL i l'apolipoproteïna C-III, suggereixen la presència en la regió de l'agregat AI-CIII-AIV d'una **mutació funcional** amb un efecte clar sobre el metabolisme dels triglicèrids. L'elevada prevalença del polimorfisme C_{1100}-T entre els pacients HLFC també suggereix que aquesta **mutació funcional** pot estar implicada en l'etiopatogènia d'aquesta alteració.

- Les associacions entre aquests polimorfismes i els paràmetres lipídics són diferents entre pacients i controls. Això fa pensar que aquesta hipotètica **mutació funcional** estaria altament modulada per factors metabòlics o ambientals i que actuaria com un **factor de predisposició** en aquests pacients.

- L'associació del polimorfisme C_{1100}-T amb un major número de partícules VLDL i IDL, però no amb un augment dels nivells plasmàtics totals d'apo B, fa pensar que aquests pacients podrien tenir un catabolisme deficient d'aquestes partícules amb una base genètica, possiblement lligada a un augment en la síntesi d'apo C-III.

- La hipòtesi que els mecanismes de regulació gènica depenents d'àcid retinoic poden estar involucrats en la HLFC es veu recolzada per les baixes concentracions plasmàtiques de vitamina A que aquests pacients presenten respecte dels controls. Aquests baixos nivells de vitamina A no són deguts a factors dietètics i podrien tractar-se d'una característica específica d'aquests pacients.

- En pacients HLFC no es pot parlar d'una clara disminució de les concentracions de colesterol en les lipoproteïnes d'alta densitat tot i que aquestes tendeixen a estar disminuïdes.

- Els pacients HLFC tenen una activitat LCAT *in vitro* normal. Això descarta la presència d'una forma defectuosa de l'enzim associada a aquesta patologia, però no descarta un

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

funcionament anormal *in vivo* degut a les característiques específiques de les lipoproteïnes d'aquests individus.

- En les famílies HLFC, la prevalença d'hiperlipèmia entre descendents menors de 19 anys és de prop del 50%, tal com caldria esperar en una alteració de transmissió autosòmica dominant. A més, aquests resultats confirmen la possibilitat de detectar de manera precoç aquests individus per a fer-ne un seguiment preventiu. L'absència d'alteracions en la fracció IDL suggereix que aquestes poden estan involucrades en l'evolució d'aquesta malaltia.

- D'entre els diferents mètodes de determinar la isoforma de l'apolipoproteïna E és més fiable i senzill determinar el genotip que el fenotip.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

6. REFERÈNCIES BIBLIOGRÀFIQUES

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. 1996. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.* 271 : 518-520.

Aguilar Salinas CA, Barrett PHR, Pulai J, Zhu XL, Schonfeld G. 1997. A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism : kinetics of apolipoprotein B during placebo and pravastatin therapy. *Arterioscler Thromb Vasc Biol.* 17 : 72-82.

Albers JJ, Chen CH, Adolphson JL. 1981. Lecithin :cholesterol acyltransferase (LCAT) mass : Its relationship to LCAT activity and cholesterol esterification rate. *J Lipid Res.* 22 : 1206-1213.

Austin MA, Breslow JL, Hennekens CH, Burning JE, Willett WC, Krauss RM. 1987. Low density lipoprotein subclass pattern and risk of myocardial infarction. *JAMA.* 260 : 1917-1921.

Austin MA, Horowitz H, Wijsman E, Krauss RM, Brunzell J. 1992. Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. *Atherosclerosis.* 92 : 67-77.

Austin MA, King M-A, Vranizan KM, Krauss RM. 1990. Atherogenic lipoprotein phenoype. A proposed genetic marker for coronary heart disease risk. *Circulation.* 82 : 495-506.

Austin MA, King M-C, Vranizan KM, Newman B, Krauss RM. 1988. Inheritance of low density lipoprotein subclass patterns : Results of complex segregation analysis. *Am J Hum Genet.* 43 : 838-846.

Austin MA, Krauss RM. 1986. Genetic control of low density lipoprotein subclasses. *Lancet.* ii : 592-595.

Austin MA, Wijsman E, Guo S, Krauss RM, Brunzell JD, Deeb S. 1991. Lack of evidence for linkage between low-density lipoprotein subclass phenotypes and the apolipoprotein B locus in familial combined hyperlipidemia. *Genet Epidemiol.* 8 : 287-297.

Auwerx J, Leroy P, Schoonjans K. 1992. Lipoprotein lipase : recent contributions from molecular biology. *Crit Rev Cl Lab Sci.* 29 : 243-268.

Auwerx J, Schoonjans K, Fruchart JC, Staels B. 1996. The transcriptional control of triglyceride metabolism : fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis.* 124 : S29-S37.

Babirak SP, Iverius P-H, Fujimoto WY, Brunzell JD. 1989. Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. *Arteriosclerosis.* 9 : 326-334.

Bansal BC, Sood AK, Bansal CB. 1986. Familial hyperlipidemia in stroke in the young. *Stroke.* 17 : 1142-1145.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Beaumont JL, Carlson LA, Cooper GR, Fejfar Z, Fredrickson DS, Strasser T. 1970. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. WHO.* 43 :891-908.

Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* 1989. 341 : 162-164.

Berg K. 1963. A new serum type system in man - the Lp system -. *Acta Pathol Microbiol Scand.* 59 (suppl) : 166-167, 369-382.

Bielicki JK, Johnson WJ, Glick JM, Rothblat GH. 1991. Efflux of phospholipids from fibroblasts with normal and elevated levels of cholesterol. *Biochem Biophys Acta.* 1084 : 7-14.

Brown MS, Goldstein JL. 1983. Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. *J Clin Invest.* 72 : 743-747.

Brown MS, Goldstein JL. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* 232 : 34-47.

Brunzell JD, Albers JJ, Chait A, Grundy SM, Groszeck E, McDonald GB. 1983. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. *J Lipid Res.* 24 : 147-155.

Brunzell JD, Austin MA. 1989. Plasma triglyceride levels and coronary disease. *New Engl J Med.* 320 : 1273-1275.

Brunzell JD, Hazzard WR, Motulsky AG, Bierman EL. 1974. Role of obesity in phenotypic expression of familial combined hyperlipidemia. *Clin Res.* 22 : 462 A.

Brunzell JD, Sniderman AD, Albers JJ, Kwiterovich PO Jr. 1984. Apoproteins B and A-I and coronary artery disease in humans. *Arteriosclerosis.* 4 : 79-83.

Bu G, Maksymovitch EA, Nerbonne JM, Schwartz AL. 1994. Expression and function of the low density lipoprotein receptor-related protein (LRP) in mammalian central neurons. *J Biol Chem.* 269 : 18521-18528.

Caladaras C, Hadzopoulou-Caladaras M, Nolte RT, Atkinson D, Zannis VI. 1986. The complete sequence ans structural analysis of human apolipoprotein B-100 : relationship between apo B-100 and apo B-48. *EMBO J.* 5 : 3495-3507.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Cardin AD, Jackson RL, Johnson JD. 1982. 5-Dimethylaminonaphtalene-1-sulfonyl 3-amino tyrosil apolipoprotein C-III. Preparation, characterization and interaction with phospholipid vesicles. *J Biol Chem.* 257 : 4987-4992.

Castro cabezas M, de Bruin TWA, de Valk HW, Shoulders CC, Jansen H, Erkelens DW. 1993. Impaired fatty acid metabolism in familial combined hyperlipidemia. *J Clin Invest.* 92 : 160-168.

Castro cabezas M, de Bruin TWA, Erkelens DW. 1992. Familial combined hyperlipidemia : 1973-1991. *Neth J Med.* 40 : 83-95.

Castro cabezas M, de Bruin TWA, Jansen H, Kock LAW, Kortlandt W, Erkelens DW. 1993a. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. *Arterioscler Thromb.* 13 : 804-814.

Chait A, Albers JJ, Brunzell JD. 1980. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridemia. *Eur J Clin Invest.* 10 : 17-20.

Chen GC, Zhu S, Hardman DA, Schilling JW, Lau K, Kane JP. 1989. Structural domains of human apolipoprotein B-100. Differential accessibility to limited proteolysis of B-100 in low density and very low density lipoproteins. *J Biol Chem.* 264 : 14369-14375.

Chen S-H, Habib G, Yang C, Gu Z, Lee BR, Weng S-A, Silberman SR, Cai S-J, Deslypere JP, Rosseneu M, Gotto AM Jr, Li W-H, Chan L. 1987. Apolipoprotein B-48 is a product of a messenger RNA with an organ-specific in-frame stop codon. *Science.* 238 : 363-366.

Cheung MC, Albers JJ. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography ; particles containing A-I but not A-II. *J Biol Chem.* 259 : 12201-12209.

Coresh J, Beaty TH, Kwiterovich PO, Antronarakis S. 1992. Pedigree and sib-pair linkage analysis suggest the apolipoproteinB gene is not the major gene influencing plasma apolipoproteinB levels. *Am J um Genet.* 50 : 1038-1045.

Cortner JA, Coates PM, Bennett MJ, Cryer DR, Le N-A. 1991. Familial combined hyperlipidemia : use of stable to demonstrate overproduction of very low-density lipoprotein apolipoprotein B by the liver. *J Inher Metab Dis.* 14 : 915-922.

Cortner JA, Coates PM, Gallagher PR. 1990. Prevalence and expression of familial combined hyperlipidemia in childhood. *J Pediatr.* 116 : 514-519.

Cullen P, Farren B, Scott J, Farral M. 1994. Complex segragtion analysis provides evidence for a major gene acting on serum triglyceride levels in 55 british families with familial combined hyperlipidemia. *Arterioscler Thromb.* 14 : 1233-1249.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Dallinga-Thie G, Bu X-D, van Linde-Sibenius Trip M, Rotter JI, Lusis AJ, de Bruin TWA. Apolipoprotein A-I/C-III/A-IV gene cluster in familial combined hyperlipidemia : effects on LDL-cholesterol and apolipoproteins B and C-III. *J Lipid Res.* 37 : 136-147.

Davis CG, Driel IR, Russell DW, Brown MS, Goldstein JL. 1987. The low density lipoprotein receptor. Identification of amino acids in cytoplasmatic domain required for rapid endocytosis. *J Biol Chem.* 262 : 4075-4082.

Diard P, Malewiak M-I, Lagrange D, Griglio S. 1994. Hepatic lipase may act as a ligand in the uptake of artificial chylomicron remnant-like particles by isolated rat hepatocytes. *Biochem J.* 299 : 889-894.

Dolphin PJ. 1985. Lipoprotein metabolism and the role of apolipoproteins as metabolic programmers. *Can J Biochem.* 63 : 850-869.

Drayna D, Jarnagin AS, McLean J, Henzel W, Kohr W, Fielding C, Lawn R. 1987. Cloning and sequencing of human cholesteryl ester transfer protein cDNA. *Nature.* 327 : 632-634.

Drayna DT, McLean J, Wion KL, Trent JM, Drabkin HA, Lawn RM . 1987. Human apolipoprotein D gene : gene sequence, chromosome localization and homology to the α_{2u}–globulin superfamily. *DNA.* 6 : 199-204.

Ehnholm C, Garoff H, Renkonen O, Simons K. 1972. Protein and carbohydrate composition of Lp(a) lipoprotein from human plasma. *Biochemistry.* 11 : 3229-3232.

Eisenberg S, Sehayek E, Olivecrona T, Vlodavsky I. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J Clin Invest.* 90 : 2013-2021.

Fan J, Wang J, Bensadoun A, Lauer SJ, Dang Q, Mahley RW, Taylor JM. 1994. Overexpression of hepatic lipase in transgenic rabbits leads to a marked reduction of plasma high density lipoproteins and intermediate density lipoproteins. *Proc Natl Acad Sci USA.* 91 : 8724-8728.

Fless GM, Rolih CA, Scanu AM. 1984. Heterogeneity of human plasma lipoprotein (a). Isolation and characterization of the lipoprotein subspecies and their apoproteins. *J Biol Chem.* 259 : 11470-11478.

Francone OL, Gong EL, Ng EL, Fielding CJ, Rubin EM. 1995. Expression of human lecithin-cholesterol acyltransferase in transgenic mice. Effect of human apolipoprotein A-I and human apolipoprotein A-II of plasma lipoprotein cholesterol metabolism. *J Clin Invest.* 96 : 1440-1448.

Gagné E, Genest J Jr, Zhang H, Clarke LA, Hayden MR. 1994. Analysis of DNA changes in the LPL gene in patients with familial combined hyperlipidemia. *Arterioscler Thromb.* 14 :1250-1257.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Genest J, Sniderman AD, Cianflone K, Teng B, Wacholder S, Marcel Y, Kwiterovich PO Jr. 1986. Hyperapobeta-lipoproteinemia. Plasma lipoprotein responses to oral fat load. *Arteriosclerosis.* 6 : 297-304.

Glueck CJ, Fallat R, Buncher CR, Tsang R, Steiner P. 1973. Familial combined hyperlipoproteinaemia : studies in 91 adults and 95 children from 33 kindreds. *Metabolism.* 22 : 1403-1428.

Goldstein JL, Ho YK, Basu SK, Brown MS. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci USA.* 76 : 333-337.

Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG. 1973. Hyperlipidemia in coronary heart disease II. Genetic analyses of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest.* 52 : 1544-1568.

Gómez-Coronado D, Lasunción MA, Herrera E. 1989. Lipoproteínas transportadoras de triglicéridos (II). *Clin Invest Arterios.* 1 : 116-129.

Gordon JI, Sims HF, Eldstein C, Scanu AM, Strauss AW. 1986. Extracellular processing of proapolipoprotein AII in HepG2 cell cultures is mediated by a 54-kDa protease immunologically related to cathepsin B. *J Biol Chem.* 260 : 14824-14831.

Gotto AM Jr, Pownall HJ, Havel RJ. 1986. Introduction to the plasma lipoproteins. *Methods Enzymol.* 128 : 3-41.

Granot E, Tabas I, Tall AR. 1987. Human plasma cholesteryl ester transfer protein enhances the transfer of cholestery ester from high density lipoproteins into cultured HepG2 cells. *J Biol Chem.* 262 : 3182-3187.

Gregg RE, Brewer HB Jr. 1988. The role of apolipoprotein E and lipoprotein receptors in modulating the in vivo metabolism of apolipoprotein B-containing lipoproteins in human. *Clin Chem.* 34 : B28-B32.

Grundy SM, Chait A, Brunzell JD. 1987. Familial combined hyperlipidemia workshop. *Arteriosclerosis.* 7 : 203-207.

Havel RJ, Eder HA, Bragdon JH. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest.* 34 : 1345-1350.

Havel RJ, Shore VG, Shore B, Bier DM. 1970. Role of specific glycopeptides of human serum lipoproteins in the activation of lipoprotein lipase. *Circ Res.* 27 : 595-600.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Havel RJ. 1988. Regulation of lipoprotein metabolism by lipoprotein receptors. *Atherosclerosis Rev.* 17 : 1-18.

Havel RJ. 1994. Postprandial hyperlipidemia and remnant lipoproteins. *Curr Opin Lipidol.* 5 : 102-109.

Hesler C, Swenson T, Tall AR. 1987. Purification and characterization of the human plasma cholesteryl ester transfer protein. *J Biol Chem.* 262 : 2275-2282.

Hoffmann SL, Russell DW, Brown MS, Goldstein JL, Hammer RE. 1988. Overexpression of low density lipoprotein (LDL) receptor eliminates LDL from plasma in transgenic mice. *Science.* 239 : 1277-1281.

Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Laouel J-M, Williams RR. 1989. Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia (Study of Utah patients with familial dyslipemic hypertension). *Arteriosclerosis.* 9 : 335-344.

Imaizumi K, Havel RJ, Fainaru M, Vigne JL. 1978. Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats. *J Lipid Res.* 19 : 1038-1046.

Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR. 1990. Increased high density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med.* 323 : 1234-1238.

Innerarity TL, Mahley RW. 1978. Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoprotein. *Biochemistry.* 17 : 1440-1447.

Ishida BY, Albee D, Paigen B. 1991. Interconversion of prebeta-migrating lipoproteins containing apolipoprotein A-I and HDL. *J Lipid Res.* 31 : 227-236.

Jackson RJ, Morrisett JD, Gotto AM Jr. 1976. Lipoprotein srtucture and metabolism. *Physiol Rev.* 56 : 256-270.

Janus ED, Nicoll AM, Turner PR, Magill P, Lewis B. 1980. Kinetic bases of the primary hyperlipidaemias : studies of apolipoprotein B turnover in genetically defined subjects. *Eur J Clin Invest.* 10 : 161-172.

Jarvik GP, Beaty TH, Gallagher PR, Coates PM, Cortner JA. 1993. Genotype at a major locus with large effects on apolipoprotein B levels predicts familial combined hyperlipidemia. *Genet Epidemiol.* 10 : 257-270.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Jarvik GP, Brunzell JD, Austin MA, Krauss RM, Motulsky AG, Wijsman E. 1994. Genetic predictors of FCHL in four large pedigrees. *Arterioscler Thromb.* 14 : 1687-1694.

Jiang X, Moulin P, Quinet E, Goldberg IJ, Yacoub LK, Agellon LB, Compton D, Polokoff R, Tall AR. 1991. Mammalian adipose tissue ans muscle are major sources of lipid transfer protein mRNA. *J Biol Chem.* 266 : 4631-4639.

Kane JP, Hardman DA, Paulus HE. 1980. Heterogeneity of apolipoprotein B : Isolation of new species from human chylomicrons. *Proc Natl Acad Sci USA.* 77 : 2465-2469.

Kane JP, Havel RJ. 1994. Disorders of the biogenesis and secretion of lipoproteins containing the B-apolipoproteins. In : Scriver CR, Beaudet AL, Sly WS *et al*, eds. *The Metabolic Basis of Inherited Disease.* New York. : McGraw-Hill.

Kayden HJ, Traber MG. 1993. Absorption, lipoprotein transport and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res.* 34 : 343-358.

Kishimoto A, Goldstein JL, Brown MS. 1987. Purification of a catalytic subunit of low density lipoprotein receptor kinase and identification of heat-stable activator protein. *J Biol Chem.* 262 : 9367-9373.

Kowal RC, Herz J, Goldstein JL, Esser V, Brown MS. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc Natl Acad Sci USA.* 86 : 5810-5814.

Krauss RM, Lindgren FT, Williams PT, Kelsey SF, Brensike J, Vranizan K, Detre KM, Levy RI. 1987. Intermediate-density lipoproteins and progression of coronary artery disease in hypercholesterolemic men. *Lancet.* 2 : 62-66.

Krieger M. 1992. Molecular flypaper and atherosclerosis : structure of the macrophage scavenger receptor. *Trends Biochem Sci.* 17 : 141-146.

Kristensen T, Moestrup SK, Gliemann J, Bendtsen L, Sand O, Sottrup-Jensen L. 1990. Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the alpha-2-macroglobulin receptor. *FEBS Lett.* 276 : 151-155.

Kunitake ST, Mendel CM, Hennessy LK. 1992. Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities. *J Lipid Res.* 33 : 1807-1816.

Kwiterovich PO Jr, Coresh J, Bachorik PS. 1993. Prevalence of hyperapobetalipoproteinemia and other lipoprotein phenotypes in men (< 50 years) and women (< 60 years) with coronary artery disease. *Am J Cardiol.* 71 : 631-639.

La Ville AE, Turner PR, Pittilo MR, Martini S, Marenah CB, Rowles PM, Morris G, Thompson GA, Woolf N, Lewis B. 1987. Hereditary hyperlipidemia in the rabbit due to overproduction of lipoproteins. I. Biochemical studies. *Arterioscleorsis.* 7 : 105-112.

LaBelle M, Austin MA, Rubin E, Krauss RM. 1991. Linkage analysis of low density lipoprotein subclass phenotypes and the apolipoprotein B gene. *Genet Epidemiol.* 8 : 269-275.

LaBelle M, Krauss RM. 1990. Differences in carbohydrate content of low density lipoproteins associated with low density lipoprotein subclass patterns. *J Lipid Res.* 31: 1577-1588.

LaRosa JC, Levy RI, Herbert P, Lux SE, Fredrickson DS. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochim Biophys Res Commun.* 41 : 57-68.

Levy RI, Frederickson DS. 1965. Heterogeneity of plasma high density lipoproteins. *J Clin Invest.* 44 : 426-441.

Liu M-S, Jirik F-R, Le Boeuf RC, Henderson H, Castellani LW, Lusis AJ, Ma Y, Forsythe IJ, Zhang H, Kirk E, Brunzell JD, Hayden MR. 1994. Alteration of lipid profiles in plasma of transgenic mice expressing human lipoprotein lipase. *J Biol Chem.* 269 : 11417-11424.

Luo C-C, Li W-H, Moore MN, Chan L. 1986. Structure and evolution of the apolipoprotein multigene family. *J Mol Biol.* 187 : 325-340.

Lutton C. 1991. Dietary cholesterol, membrane cholesterol and cholesterol synthesis. *Biochemie.* 73 : 1327-1334.

Mahley RW, Hui DY, Innerarity TL. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine and man. *J Clin Invest.* 68 : 1197-1206.

Mahley RW, Innerarity TL, Rall SC, Weisgraber KH. 1984. Plasma lipoproteins : apolipoprotein structure abs function. *J Lipid Res.* 25 : 1277-1294.

Marcel YL, Hogue M, Theolis R Jr, Milne RW. 1982. Mapping of antigenic determinants of human apo B using monoclonal antibodies against low density lipoproteins. *J Biol Chem.* 257 : 13165-13168.

Marotti KR, Castle CK, Murray RW, Rehberg EF, Polites HG, Melchior GW. 1992. The role of cholestery ester transfer protein in primate apolipoprotein A-I metabolism. Insights from studies with transgenic mice. *Arterioscler Thromb.* 12 : 736-744.

McLean JW, Tomlinson JE, Kuang W-J, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. 1987. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. *Nature.* 330 : 132-137.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Merrill AH Jr. 1993. Apo A-IV : a new satiety signal. *Nutr Rev.* 51 : 273-275.

Nevin DN, Brunzell JD, Deeb SS. 1994. The LPL gene in individuals with familial combined hyperlipidemia and decreased LPL activity. *Arterioscler Thromb.* 14 : 869-873.

Nichols AV, Blanche PJ, Gong EL, Shore VG, Forte TM. 1985. Molecular pathways in the transformation of model discoidal lipoprotein complexes induced by lecithin :cholesterol acyl transferase. *Biochim Biophys Acta.* 834 : 285-300.

Nikkilä EA, Aro A. 1973. Family study of serum lipids and lipoproteins in coronary heart disease. *Lancet.* 1 : 954-959.

Nozaki S, Kubo M, Sudo H, Matsuzawa Y, Tarui S. 1986. The role of hepatic triglyceride lipase in the metabolism of intermediate-density lipoprotein-postheparin lipolytic activities determined by sensitive, nonradioisotopic method in hyperlipidemic patients and normals. *Metabolism.* 35 : 53-58.

Nykjaer A, Bengtsson-Olivecrona G, Lookene A, Moestrupt SK, Petersen CM, Weber W, Beisigel U, Glieman J. 1993. The alpha$_2$-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and beta-migrating very low density lipoprotein associated with the lipase. *J Biol Chem.* 268 : 15048-15055.

Nykjaer A, Nielsen M, Lookene A, Meyer N, Roigaard H, Etzerodt M, Beisiegel U, Olivecrona G, Gliemann J. 1994. A carboxyl-terminal fragment of lipoprotein lipase binds to the low density lipoprotein receptor-related protein and inhibits lipase-mediated uptake of lipoprotein to cells. *J Biol Chem.* 269 : 31747-31755.

O'Brien R. 1994. Biological importance of low-density-lipoprotein subfractions. *J Cardiovasc Risk.* 1 : 207-211.

Ohta T, Fidge NH, Nestel PJ. 1984. Characterization of apolipoprotein AIV complexes and IV isoforms in human lymph and plasma lipoproteins. *J Biol Chem.* 259 : 14888-14893.

Olivecrona T, Bengtsson-Olivecrona G. 1993. Lipoprotein lipase and hepatic lipase. *Curr Opin Lipidol.* 4 : 187-196.

Olofsson S-O, Bjursell G, Bostrom K, Carlsson P, Elovson J, Protter AA, Reuben MA, Bondjers G. 1987. Apolipoprotein B : structure, biosynthesis and role in lipoprotein assembly process. *Atherosclerosis.* 68 : 1-17.

Oram JF, Brinton EA, Bierman EL. 1983. Regulation of high density lipoprotein receptor activity in cultured human arterial smooth muscle cells. *J Clin Invest.* 1611-1621.

Ordovas JM, Civeira F, Genest J Jr, Craig S, Robbins AH, Meade T, Pocovi M, Frossard PM, Masharani U, Wilson PWF, Salem DN, Ward RH, Schaefer EJ. 1991. Restriction fragment lenght polymorphisms of the apolipoprotein A-I, C-III, A-IV gene locus. *Atherosclerosis.* 87 : 75-86.

Osborne JC, Bengtsson-Olivecrona G, Lee N, Olivecrona T. 1985. Studies on inactivation of lipoprotein lipase : role of the dimer to monomer dissociation. *Biochemistry.* 24 : 5606-5611.

Patsch W, Franz S, Schonfeld G. 1983. Role of insulin in lipoprotein secretion in cultured hepatocytes. *J Clin Invest.* 71 : 1161-1174.

Phillips NR, Havel RJ, Kane JP. Levels and interrelationship of serum and lipoprotein cholesterol and triglycerides. Association with adiposity and the consumption of ethanol, tobacco, and beverages containing caffeine. *Arteriosclerosis.* 1 : 13-24.

Pittman RC, Knecht TP, Rosenbaum MS, Taylor CA. 1987. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J Biol Chem.* 262 : 2443-2450.

Radding CHM, Steinberg D. 1960. Studies on the synthesis and secretion of serum lipoproteins by rat liver slices. *J Clin Invest.* 39 : 1560-1569.

Rader DJ, Cain W, Ikewaki K, Talley G, Zech LA, Usher D, Brewer HB Jr. 1994. The inverse association of plasma lipoprotein (a) concentrations with apolipoprotein (a) isoform size is not due to differences in Lp(a) catabolism but to differences in production rate. *J Clin Invest.* 93 : 2758-2763.

Rader DJ, Ikewaki K, Duverger N, Schmidt H, Pritchard H, Frohlich J, Clerc M, Dumon M-F, Fairwell T, Zech L, Santamarina-Fojo S, Brewer HB Jr. 1994. Markedly accelerated catabolism of apolipoprotein A-II (apo A-II) and high density lipoproteins containing apo A-II in classic lecithin :cholesterol acyltransferase deficiency and fish-eye disease. *J Clin Invest.* 93 : 321-330.

Raugh G, Schuster H, Müller B, Schewe S, Keller C, Wolfram G, Zöllner. 1990. Genetic evidence from 7 families that the apo B gene is not involved in familial combined hyperlipidemia. *Genet Epidemiol.* 8 : 287-297.

Reichl D, Miller NE. 1989. Pathophysiology of reverse cholesterol transport. Insights from inherited disorders of lipoprotein metabolism. *Arteriosclerosis.* 9 : 785-797.

Rose HG, Kranz P, Weinstock M, Juliano J, Haft JI. 1973. Inheritance of combined hyperlipoproteinaemia : evidence for a new lipoprotein phenotype. *Am J Med.* 54 : 148-160.

Runquist EA, Havel RJ. 1992. Acid hydrolases in early and late endosome fractions from rat liver. *J Biol Chem.* 266 : 22557-22563.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Sakai J, Hishino A, Takahashi S, Miura Y, Ishii H, Suzuki H, Kawarabayasi Y, Yamamoto T. 1994. Structure, chromosome location, and expression of the human very low density lipoprotein gene. *J Biol Chem.* 269 : 2173-2181.

Schoonjans K, Peinado-Onsurbe J, Lefebvre A-M, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. 1996. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* 15 : 5336-5348.

Schoonjans K, Staels B, Auwerx J. 1996. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta.* 1302 : 93-109.

Schultz JR, Gong EL, McCall MR, Nichols AV, Clift SM, Rubin EM. 1992. *J Biol Chem.* 267 : 21630-21636.

Schumaker VN, Phillips ML, Chatterton JE. 1994. Apolipoprotein B and low density lipoprotein structure : Implications for biosynthesis of triglyceride-rich lipoproteins. *Ad Protein Chem.* 45 : 205-248.

Seed M, Mailly F, Vallance D, Doherty E, Winder A, Talmud P, Humphries SE. 1994. Lipoprotein lipase activity in patients with familial combined hiperperlipidemia. *Clin Investig.* 72 : 100-106.

Shachter NS, Ebara T, Ramakrishnan R, Steiner G, Breslow JL, Ginsberg HN, Smith JD. 1996. Combined hyperlipidemia in transgenic mice overexpressing human apolipoprotein C-I. *J Clin Invest.* 98 : 846-855.

Shafi S, Brady SE, Bensadoun A, Havel RJ. 1994. Role of hepatic lipase in the uptake and processing of chylomicron remnants in the liver. *J Lipid Res.* 35 : 709-720.

Shelburne F, Hanks J, Meyers W, Quarfordt S. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J Clin Invest.* 65 : 652-658.

Shimano H, Namba Y, Ohsuga J, Kawamura M, Yamamoto K, Shimada M, Gotoda T, Harada K, Yazaki Y, Yamada N. 1994. Secretion-recapture process of apolipoprotein E in hepatic uptake of chylomicron remnants in transgenic mice. *J Clin Invest.* 93 : 2215-2223.

Shoulders CC, Harry PJ, Lagrost L, White SE, Shah NF, North JD, Gilligan M, Gambert P, Ball MJ. 1991. Variation at the apo AI/CIII/AIV gene complex is associated with elevated plasma levels of apo C-III. *Atherosclerosis.* 87 : 239-247.

Sigurdsson G Jr, Gudnason V, Sigurdsson G, Humphries SE. 1992. Interaction between a polymorphism of the apo A-I promoter region and smoking determines plasma levels of HDL and apo A-I. *Arterioscler Thromb.* 12 : 1017-1022.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Sniderman A, Kwiterovich PO Jr. 1987. Hyperapobetalipoproteinemia and LDL and HDL_2 heterogeneity. *Proceedings from a workshop on lipoprotein heterogeneity. NIH publication Nº 87-2646. US department of health and human services : Washington DC.* 293-304.

Sniderman A, Shapiro S, Marpole D, Malcolm I, Skinner B, Kwiterovich PO Jr. 1980. The association of coronary atherosclerosis and hyperapobetalipoproteinemia (Increased protein content but normal cholesterol content in human plasma low density lipoprotein). *Proc Natl Acad Sci USA.* 97 : 604-608.

Sniderman A, Teng B, Genest J, Cianflone K, Wacholder S, Kwiterovich P. 1985. Familial agregation and early expression of hyperapobetalipoproteinemia. *Am J Cardiol.* 55 : 291-295.

Staels B, Vu-Dac N, Kosykh VA, Saladin R, Fruchart JC, Dallongeville J, Auwerx J. 1995. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. *J Clin Invest.* 95 : 705-712.

Stalenhoef AFH, Malloy MJ, Kane JP, Havel RJ. 1984. Metabolism of apolipoprotein B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc Natl Acad Sci USA.* 81 : 1839-1843.

Sultan F, Lagrange D, Jansen H, Griglio S. 1990. Inhibition of hepatic lipase activity impairs chylomicron remnant-removal in rats. *Biochim Biophys Acta.* 1042 : 150-152.

Tam S-P, Archer TK, Deeley RG. 1986. Biphasic effects of estrogen on apolipoprotein synthesis in human hepatoma cells : Mechanisms of antagonism by testosterone. *Proc Natl Acad Sci USA.* 83 : 3111-3115.

Teng B, Sniderman AD, Soutar AK, Thompson GR. 1986. Metabolic basis of hyperapobetalipoproteinemia. Turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J Clin Invest.* 77 : 663-672.

Teng B, Thompson GR, Sniderman AD, Forte TM, Krauss RM, Kwiterovich PO Jr. 1983. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinaemia, normolipidemia and familial hypercholesterolaemia. *Proc Natl Acad Sci USA.* 80 : 6662-6666.

Teng BB, Burant CF, Davidson NO. 1993. Molecular cloning of an apolipoprotein B messenger RNA editing protein. *Science.* 264 : 1816-1819.

Thrift RN, Drisko J, Dueland S, Trawick JD, Davis RA. 1992. Translocation of apolipoprotein B across the endoplasmic reticulum is blocked in a nonhepatic cell line. *Proc Natl Acad Sci USA.* 89 : 9161-9165.

Tollefson JH, Ravnik S, Albers JJ. 1988. Isolation and characterization of a phospholipid transfer protein (LTP-II) from human plasma. *J Lipid Res.* 15 : 1593-1602.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Utermann G, Hardewig A, Zimmer F. 1984b. Apolipoprotein E phenotypes in patients with myocardial infarction. *Hum Genet.* 65 : 237-241.

Utermann G, Kindermann I, Kaffarink H, Steinmetz A. 1984a. Apolipoprotein E phenotypes and hyperlipidemia. *Hum Genet.* 65 : 232-236.

Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J. 1993. Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler Thromb.* 13 : 1110-1118.

Warnick GR, Mayfield C, Albers JJ, Hazzard WR. 1979. Gel isoelectric focusing method for specific diagnosis of familial hyperlipoproteinemia type 3. *Clin Chem.* 25 : 279-284.

Webb JC, Patel DD, Jones MD, Knight BL, Soutar AK. 1994. Characterization and tissue-specific expression of the human very low density lipoprotein (VLDL) receptor mRNA. *Hum Mol Genet.* 3 : 531-537.

Weisgraber KH, Innerarity TL, Mahley RW. 1982. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J Biol Chem.* 257 : 2818-2821.

Weng W, Breslow JL. 1996. Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. *Proc Natl Acad Sci USA.* 93 : 14788-14794.

Wetterau JR, Aggerbeck LP, Bouma ME, Eisenberg C, Munck A, Hermier M, Schmitz J, Gay G, Rader DJ, Gregg RE. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science.* 258 : 999-1001.

Wijsman EM, Motulsky AG, Guo SW, Yang M, Austin MA, Brunzell JD, Deeb S. 1992. Evidence against linkage of familial combined hyperlipidemia to the apoAI-CIII-AIV gene complex. *Circulation.* 86, suppl. 1 : 1420.

Williams KJ, Petrie KA, Brocia RW, Swenson TL. 1991. Lipoprotein lipase modulates net secretory output of apolipoprotein B *in vitro* . *J Clin Invest.* 88 : 1300-1306.

Williams RR, Hunt SC, Hopkins PN, Stults BM, Wu LL, Hasstedt SJ, Barlow GK, Stephenson SH, Lalouel J-M, Kuida H. 1988. Familial dyslipemic hypertension : Evidence from 58 Utah families for a syndrome present in approximately 15% of patients with essential hypertension. *JAMA.* 259 : 3579-3586.

UNIVERSITAT ROVIRA I VIRGILI
ASPECTES METABÒLICS I PAPER DE L´AGREGAT GENÈTIC AL-CIII-AIV EN LA HIPERLIPEMIA FAMILIAR COMBINADA
Josep Ribalta Vives
ISBN:978-84-691-1879-5/DL:T-341-2008

Willnow E, Sheng Z, Ishibashi S, Herz J. 1994. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science.* 264 : 1471-1474.

Wojciechowski AP, Farrall M, Cullen P, Wilson TME, Bayliss JD, Farren B, Griffin BA, Caslake MJ, Packard CJ, Shepherd J, Thakker R, Scott J. 1991. Familial combined hyperlipidaemia linked to the apolipoprotein AI-CIII-AIV gene cluster on chromosome 11q23-q24. *Nature.* 349 : 161-164.

Xu CF, Angelico F, Del Ben M, Humphries S. 1993. Role of genetic variation at the apo AI-CIII-IAV gene cluster in determining plasma apo AI levels in boys and girls. *Genet Epidemiol.* 10 : 113-122.

Xu CF, Talmud P, Schuster H, Houlston R, Miller G, Humphries S. 1994. Association between genetic variation at the apo AI-CIII-AIV gene cluster and familial combined hyperlipidaemia. *Clin Genet.* 46 : 385-397.

Yen FY, Deckelbaum RJ, Mann CJ, Marcel YL, Milne RW, Tall AR. 1989. Inhibition of cholestery ester transfer protein activity by monoclonal antibody : Effects on cholesteryl ester formation and neutral lipids mass transfer in human plasma. *J Clin Invest.* 83 : 2018-2024.

Zannis VI, Breslow JL. 1980. Characterization of a unique human apolipoprotein E variant associated with type III hyperlipoproteinemia. *J Biol Chem.* 255 : 1759-1762.