European Heart Journal (2001) **22,** 465–471
doi:10.1053/euhj.2000.2408, available online at http://www.idealibrary.com on IDEAL®

Importance of HDL cholesterol levels and the total/HDL cholesterol ratio as a risk factor for coronary heart disease in molecularly defined heterozygous familial hypercholesterolaemia

J. T. Real[1], F. J. Chaves[2], I. Martínez-Usó[1], A. B. García-García[2], J. F. Ascaso[1] and R. Carmena[1]

[1]*Service of Endocrinology and Nutrition, Department of Medicine, Hospital Clínico Universitario, University of Valencia, Valencia, Spain;* [2]*Instituto de Investigaciones Citológicas, Valencia, Spain*

Aims To assess the relationship of the lipid profile to coronary heart disease in a group of heterozygous familial hypercholesterolaemic subjects with similar age, sex, body mass index, prevalence of angiotensin converting enzyme DD genotype and type of low density lipoprotein receptor mutation.

Methods and Results A total of 66 molecularly defined heterozygous familial hypercholesterolaemic subjects, 33 of whom had coronary heart disease, were studied. Clinical features, cardiovascular risk factors and lipid parameters were compared in both groups. Familial hypercholesterolaemic patients with coronary heart disease showed significantly lower values of mean plasma HDL cholesterol and a higher total/HDL cholesterol ratio as compared with familial hypercholesterolaemic subjects free of coronary heart disease. Total and LDL cholesterol concentrations were higher in patients with coronary heart disease, without reaching statistical significance. No differences in plasma lipoprotein(a) levels on absolute and log-transformed values were observed between the two groups. In the whole familial hypercholesterolaemia group, plasma HDL cholesterol levels were related to plasma triglyceride values and to LDL receptor gene 'null mutations'.

Conclusions In familial hypercholesterolaemic subjects of similar age, gender, body mass index, systolic and diastolic blood pressure, and genetic factors that could influence coronary heart disease risk, plasma HDL cholesterol values and total/HDL cholesterol ratios are two important coronary risk factors. Hence, treatment of familial hypercholesterolaemia should focus not only on lowering total and LDL cholesterol levels, but also on increasing HDL cholesterol values for coronary heart disease prevention. More prospective and intervention trials should be conducted to establish the relationship of HDL cholesterol levels and coronary heart disease in familial hypercholesterolaemia.
(Eur Heart J 2001; 22: 465–471, doi:10.1053/euhj.2000.2408)

Key Words: Heterozygous familial hypercholesterolaemia, HDL cholesterol levels, coronary heart disease, angiotensin converting enzyme DD genotype, low density lipoprotein receptor mutations.

See page 442 for the Editorial comment on this article

Introduction

Familial hypercholesterolaemia is an autosomal dominant disease defined at the molecular level by one of a number of mutations in the low density lipoprotein (LDL) receptor gene and characterized by markedly elevated LDL cholesterol levels, tendon xanthomata, and increased risk of premature coronary heart disease[1,2]. Despite its hereditary nature, familial hypercholesterolaemia shows great variability in phenotypic expression that is dependent on environmental and genetic factors[3]. Considerable variation in the severity of atherosclerotic complications among families with familial hypercholesterolaemia has long been recognised[4–6]. The expression of coronary heart disease

Revision submitted 1 August 2000, and accepted 2 August 2000.

Supported by grants from Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, FIS (FIS 96/2063 & 99/0008).

Correspondence: Dr Rafael Carmena, Department of Medicine, Hospital Clínico Universitario, Avda. Blasco Ibáñez 17, E-46010 Valencia, Spain.

0195-668X/01/060465+07 $35.00/0

among familial hypercholesterolaemic patients may be influenced by other risk factors, such as age, male sex, smoking, visceral obesity, high total cholesterol levels, low HDL cholesterol levels, and high lipoprotein(a) levels[3–6]. In addition, the prevalence of coronary heart disease in familial hypercholesterolaemia has been associated with the type of LDL receptor mutation[7] and the angiotensin converting enzyme (ACE) DD genotype[8].

The ACE DD genotype is associated with an elevated plasma concentration of ACE, which results in an increase of angiotensin II, and an elevated influx of ^{125}I-labelled LDL into the arterial wall of rabbits[9]. Therefore, the ACE gene polymorphism could be a potential coronary risk factor in primary hyperlipaemias. Recently, O'Malley *et al*. have demonstrated an association between coronary heart disease and the ACE DD genotype in a familial hypercholesterolaemia population[8].

On the other hand, LDL receptor gene 'null mutations' that generate no LDL receptor protein have been associated with higher LDL cholesterol levels, poor response to statin treatment and coronary heart disease in familial hypercholesterolaemic subjects[7,10–13].

The present case/control study was undertaken to assess what component of the lipid profile correlated best with coronary heart disease prevalence in a group of heterozygous familial hypercholesterolaemic subjects with similar age, sex distribution, body mass index, prevalence of ACE DD genotype and LDL receptor class mutations.

Patients and methods

Patients

The case/control study consisted of 66 heterozygous familial hypercholesterolaemic subjects (33 coronary heart disease+ cases and 33 coronary heart disease− controls) selected by matching criteria (age, sex distribution, body mass index, prevalence of ACE DD genotype and LDL receptor class mutations) from a total of 280 heterozygous familial hypercholesterolaemia subjects attending our Lipid Clinic. The 280 heterozygous familial hypercholesterolaemic subjects were diagnosed based on clinical criteria for familial hypercholesterolaemia. In 230, a mutation at the LDL receptor gene could be detected (82%). In 40 molecularly defined familial hypercholesterolaemic patients, coronary heart disease could be confirmed. We randomly selected, among the 240 molecularly defined familial hypercholesterolaemia without coronary heart disease, 33 subjects matched for age, sex, body mass index, DD genotype and LDL receptor gene mutation, with 33 out of the 40 familial hypercholesterolaemic subjects with coronary heart disease.

All subjects were Caucasians and lived in the Autonomous Community of Valencia, Spain. The institutional ethics committee approved the protocol and all subjects gave written informed consent to take part in the study.

Clinical and biochemical criteria for diagnosing familial hypercholesterolaemia included: plasma levels of total and LDL cholesterol higher than the 95th percentile corrected for both age and sex, presence of tendon xanthomas, coronary artery disease in the proband or in a first-degree relative, and bimodal distribution of total cholesterol and LDL cholesterol levels in the family (autosomal dominant pattern of lipid IIa phenotype). Clinical diagnosis of familial hypercholesterolaemia was genetically confirmed in 82% of the familial hypercholesterolaemia subjects by the presence of major rearrangements at the LDL receptor locus detected by Southern blot, or minor LDL receptor mutations detected by polymerase chain reaction single-strand conformation polymorphism analysis. There was considerable molecular heterogeneity in the whole familial hypercholesterolaemia group, i.e. six major rearrangements and 23 minor mutations, 112insA, C95R, and C358Y being the most prevalent.

In all patients, a full medical history was obtained and a complete physical examination was performed. Body mass index was calculated as weight divided by height squared ($kg \cdot m^{-2}$). Cigarette smoking was assessed by self-report, and subjects were classified as current smokers or non-smokers. Blood pressure was measured with a von Recklinghausen sphygmomanometer in the sitting position and after a 5 min rest. The mean value of three measurements was considered. A patient was classified as having diabetes if he or she fulfilled the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[14].

The criteria for positive coronary heart disease were: (a) documented history of myocardial infarction (presence of typical chest pain and electrocardiographic and creatine phosphokinase-MB monitoring during the acute phase, or deep and wide Q-waves as an electrocardiographic manifestation of an old infarction, n=11) or (b) the presence of angina (history of typical exercise-associated chest pain) and positive coronary angiography (70% stenosis on coronary arteriography).

Laboratory methods

Measurement of lipids and lipoproteins

After a 12–14 h fast, blood samples were collected in tubes containing EDTA and were centrifuged within 4 h. Plasma was stored at 4 °C for a maximum of 3 days. DNA extraction was performed by standard procedures. Cholesterol and triglyceride levels were measured by enzymatic techniques[15,16]. HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with polyanions[17] and VLDL cholesterol after separation of VLDL ($d<1{\cdot}006\ g \cdot ml^{-1}$) by ultracentrifugation[18]. The LDL cholesterol was calculated by subtracting VLDL and HDL cholesterol from total cholesterol. Total plasma apoB was measured by immunoturbimetry[19]. Lipoprotein(a) levels were determined

Table 1 Clinical parameters of 66 heterozygous familial hypercholesterolaemia patients with and without coronary heart disease (CHD)

Data	CHD+ (n=33)	CHD − (n=33)	*P* value
Age, years	56·5 (9·3)	53·9 (9·8)	ns
Male patients, n	14	14	ns
Body mass index, kg . m^{-2}	26·1 (2·9)	26·3 (3·7)	ns
Systolic blood pressure, mmHg	134·4 (17·3)	133·5 (20·8)	ns
Diastolic blood pressure, mmHg	76·9 (9·7)	78·1 (9·5)	ns
Diabetes mellitus, n	3 (9)	2 (6)	ns
Essential hypertension, n	5 (15)	5 (15)	ns
Current smokers, n	2 (6)	3 (9)	ns
ACE DD genotype, n	15 (45)	15 (45)	ns
LDL null mutations, n	18 (54)	18 (54)	ns
Xanthomas %	24 (72)	9 (27)	0·03

Data as mean (SD), n or (percentages).

by a commercially available ELISA (SDI Strategic Diagnosis, Delaware, U.S.A.).

Genetic methods

The genetic diagnosis of familial hypercholesterolaemia was established by identifying different LDL gene mutations. Major rearrangements were detected by Southern blot analysis of *Bgl*II and *Kpn*I plus *Xba*I digested genomic DNA. Small and minor mutations were detected by polymerase chain reaction-SSCP analysis and semi-automated sequencing. The running conditions for SSCP analysis and the oligonucleotides used were performed according to Lombardi *et al.*[20] and Leren *et al.*[21], with minimal modifications.

An LDL receptor gene 'null mutation' was considered in the case of: (a) major rearrangements affecting the promotor region, (b) or small mutations that create a stop codon (W-18X) or generate a truncated protein (deletion of ATGA at nucleotide 790 in exon 5). Subjects with null mutations included: 112insA, deletion promotor to exon 1, deletion promoter to exon 2, deletion 790 and W-18X (as described by Day[22] and Table 2).

Insertion/deletion polymorphism of the ACE gene was determined by amplification of intron 16 following the method of Redon *et al.*[23], with primers 5′CTGGA GACCACTCCATCCTTTCT3′ and 5′GATGTGGCC ATCACATTCGTCAGAT3′, 1 µg of target DNA, 1 U of *Taq* DNA polymerase, and reaction mixture containing 10 mM Tris-HCl (pH 8·4), 3 mM $MgCl_2$, 50 mM KCl, and 0·1 mg . ml^{-1} gelatin. The fragments obtained were analysed by electrophoresis on agarose gel.

Statistical analysis

Data were analysed with the Statistical Package for the Social Sciences (SPSS)[24] and expressed as mean ± standard deviation (SD). Mean values of quantitative variables were compared with the Student's t-test for unpaired data except for plasma levels of triglycerides and lipoprotein(a) values, which were analysed with the Mann–Whitney U test. The distribution of both triglyceride and lipoprotein(a) values were highly skewed, and a logarithmic transformation was therefore applied to produce approximately normal distribution. Proportions were compared with contingency tables and the chi-square test or the Fisher's exact test. Pearson's statistics were used to assess the relationship between variables. Multiple regression analysis was used to determine the variables that were associated with coronary heart disease and to calculate adjusted odds ratios. Age and body mass index were included in the logistic model as covariates. For each odds ratio we estimated two-tail *P*-values and 95% confidence intervals.

Results

The clinical characteristics of coronary heart disease+ and coronary heart disease − familial hypercholesterolaemic subjects are shown in Table 1. Coronary heart disease+ patients showed a significantly higher prevalence of xanthomas. Both groups showed similar age, sex distribution, body mass index, systolic and diastolic blood pressure, and prevalence of diabetes mellitus, of LDL receptor 'null mutations' and of ACE DD genotype distribution.

Table 2 shows the molecular characteristics of LDL receptor gene mutations included in the study. LDL receptor gene null mutations were: (a) major rearrangements affecting the promotor region (deletion of promotor to exon 1 and deletion of promotor to exon 2), (b) W-18X a G to A change at nucleotide 11 that creates a stop codon, 112insA — an insertion of A in exon 2 that generates a stop codon, and a small deletion of ATGA at nucleotide 790 in exon 5 that creates a stop codon and a truncated protein. LDL receptor gene defective mutations included mutations of aminoacids at different positions: C358Y (G to A at nucleotide 1146, Cys to Tyr), T413R (C to G at nucleotide 1301, Thr to Arg), R395W (C to T at nucleotide 1246, Arg to Pro), C68W (C to G at nucleotide 267, Cys to Trp), G642E (G to A

Table 2 Molecular characteristics of LDL mutations included in the study

Mutation	N	Type	Exon (nucleotide)	Change
112insA	16	Null	2 (11)	Ins A→stop
Deletion promotor to exon 1	4	Null	Promotor-1	Deletion
Deletion promoter to exon 2	4	Null	Promotor-2	Deletion
Deletion 790	2	Null	5 (790)	Del ATGA stop
W-18X	10	Null	1 (11)	G→A stop
C358Y	8	Defective	8 (1146)	G→A
T413R	6	Defective	9 (1301)	C→G
R395W	2	Defective	9 (1246)	C→T
C68W	2	Defective	3 (267)	C→G
G642E	2	Defective	14 (1998)	G→A
C95R	4	Defective	4 (346)	T→C
E256K	6	Defective	6 (829)	G→A

Table 3 Lipids and lipoproteins in 66 subjects with heterozygous familial hypercholesterolaemia (33 with CHD)

Data	CHD+ (n=33)	CHD− (n=33)	*P* value
Total cholesterol, mmol . l^{-1}	9·7 (1·7)	9·1 (2·0)	ns
Triglycerides, mmol . l^{-1}	1·96 (2·2)	1·34 (0·6)	ns
$Log_{(10)}$ triglycerides	2·14 (0·28)	2·04 (0·21)	ns
HDL cholesterol, mmol . l^{-1}	1·15 (0·33)	1·37 (0·39)	0·03
Total cholesterol/HDL cholesterol	8·9 (2·7)	7·2 (2·9)	0·02
LDL cholesterol, mmol . l^{-1}	7·54 (1·68)	7·0 (2·1)	ns
VLDL, mmol . l^{-1}	0·75 (0·4)	0·62 (0·3)	ns
Apo B, gl . l^{-1}	1·66 (0·5)	1·69 (0·42)	ns
$Log_{(10)}$ Lp(a)	1·14 (0·45)	1·12 (0·55)	ns
Lp(a) $\geq$20 mg . dl^{-1},* %	33	23	ns

Data as mean (SD) or percentages.
*Cutpoint for high cardiovascular risk.

at nucleotide 1988, Gly to Glu), C95R (T to C at nucleotide 346, Cys to Arg) and E256K (G to A change at nucleotide 829, Glu to Lys).

Table 3 shows plasma lipid values in both familial hypercholesterolaemia groups. Plasma HDL cholesterol values were significantly lower and total/HDL cholesterol ratios significantly higher in familial hypercholesterolaemia coronary heart disease+ ($1{\cdot}16 \pm 0{\cdot}34$ vs $1{\cdot}37 \pm 0{\cdot}39$ $P=0{\cdot}03$; $8{\cdot}9 \pm 2{\cdot}7$ vs $7{\cdot}2 \pm 2{\cdot}9$ $P=0{\cdot}02$). The triglyceride/HDL cholesterol ratio for the group with familial hypercholesterolaemia and coronary heart disease was 1·7, compared with 0·98 for the non-coronary heart disease group, $P<0{\cdot}02$. The odds ratios for coronary heart disease were 3·92 (CI 95% 1·21–12·64, $P=0{\cdot}018$) for HDL cholesterol <1·03 mmol . l^{-1} and 0·28 (CI 95% 0·1–0·8, $P=0{\cdot}016$) for the total cholesterol/HDL cholesterol ratio <6·5. Total and LDL cholesterol concentrations were higher in patients with coronary heart disease, but the differences did not reach statistical significance. There were no differences in lipoprotein(a) levels on absolute and log-transformed values among patients with and without coronary heart disease.

In the whole familial hypercholesterolaemia group using Pearson's correlation coefficient, plasma HDL cholesterol levels were related to plasma triglyceride values ($-0{\cdot}24$ $P=0{\cdot}024$) and LDL receptor gene null mutations (0·57 $P=0{\cdot}01$).

The multiple regression analysis including as independent variables total cholesterol, log of triglycerides, log of lipoprotein(a), apolipoprotein B and HDL cholesterol values, showed that only HDL cholesterol values were associated with coronary heart disease (beta $2{\cdot}32 \pm 0{\cdot}9$, $P=0{\cdot}015$).

Discussion

In heterozygous familial hypercholesterolaemia, coronary heart disease has been associated with age, male sex, smoking habits, visceral obesity, elevated total and LDL cholesterol and low HDL cholesterol plasma levels[4–6]. These data indicate that there may be different interactions between lipid fractions and lipoproteins in relation to cardiovascular risk in familial hypercholesterolaemic subjects. Previous studies[5,25] have shown a lack of association between higher lipoprotein(a) levels and coronary heart disease in a population with >10-kilobase deletion of the LDL receptor gene, although this finding has not been reported in other familial

hypercholesterolaemia populations[26]. The study of Ferrières *et al*.[4] also demonstrated a gender-specific lipoprotein influence on coronary heart disease in a large sample of familial hypercholesterolaemic patients carrying the same LDL receptor gene defect. In our study, carried out in a familial hypercholesterolaemia group of similar age, sex distribution, body mass index, ACE DD genotype distribution, and LDL receptor null mutations distribution, we found that only low HDL cholesterol levels and high total/HDL cholesterol ratios are associated with coronary heart disease.

In addition, Hausmann *et al*.[27] have shown by stepwise multiple regression analysis both in heterozygous familial hypercholesterolaemia and in familial combined hyperlipidaemia, that the strongest predictor of plaque burden in the left anterior descending coronary artery was the level of HDL cholesterol and the total/HDL cholesterol ratio. Sudhir *et al*.[28] have also demonstrated a strong association between plasma HDL cholesterol levels and coronary ectasia in heterozygous familial hypercholesterolaemia.

These findings underline the importance of plasma HDL cholesterol values, and probably reverse cholesterol transport, as coronary risk factors in familial hypercholesterolaemic patients. In the general population, several epidemiological studies have demonstrated the importance of low HDL cholesterol plasma levels as an independent coronary risk factor[29–31]. Familial hypercholesterolaemic subjects with alterations in HDL transport will have lower HDL cholesterol levels and more severe cholesterol deposits in atherosclerotic plaques, with a higher risk of myocardial infarction. In our study, familial hypercholesterolaemic subjects with coronary heart disease had lower HDL cholesterol levels and a higher prevalence of xanthomas, indicating higher cholesterol deposits in different tissues.

The observed differences in plasma HDL cholesterol levels among familial hypercholesterolaemia populations[4,27,28] could be explained by interactions of environmental factors (i.e. obesity, diet, exercise) with LDL receptor status, by a direct effect of the type of LDL receptor mutations, or by a gene–gene interaction of LDL receptor mutations and other candidate genes.

Visceral fat accumulation, with low HDL cholesterol and high plasma triglyceride levels, was reported as a coronary risk factor in familial hypercholesterolaemic subjects in Japan[32] and in French Canadian familial hypercholesterolaemic patients[6]. Unfortunately, in our case/control study, we did not measure waist and hip circumferences. Visceral fat accumulation can be assessed by the waist/hip ratio or by computed tomography. We can speculate that our familial hypercholesterolaemic subjects would behave like French Canadian familial hypercholesterolaemics, whose visceral fat deposits were related to HDL cholesterol and triglyceride plasma values[6]. Moreover, as suggested by Gaudet *et al*., a better assessment of coronary heart disease risk in familial hypercholesterolaemia should include fasting plasma insulin levels in addition to the waist to hip ratio[6].

The effect of the LDL receptor on the clearance of chylomicron remnants and VLDL particles could directly influence plasma HDL cholesterol levels in familial hypercholesterolaemic subjects. Bowler *et al*. have demonstrated a 50% reduction in the clearance of chylomicron remnants in WHHL rabbits[33], although Kita *et al*. showed normal clearance[34]. In humans, a previous study from Cape Town[35] in five homozygous familial hypercholesterolaemia subjects showed a normal clearance of chylomicron remnants. A more recent study in six Japanese homozygote familial hypercholesterolaemia subjects, a markedly delayed clearance of retinol-labelled triglyceride-rich lipoproteins following an oral fat load was demonstrated. In addition, the binding and clearance of chylomicron remnants by fibroblasts was substantially decreased compared with a control group[36]. Also, Castro-Cabezas *et al*.[37] reported, in heterozygous familial hypercholesterolaemia, a two-fold delay in the area under the curve for clearance of remnant particles. These differences in experimental and human studies could be explained by methodological differences, differences in LDL receptor mutations and/or as a result of the effects of genetic variations in other receptors or pathways involved in chylomicron remnant clearance. Thus, it is possible that familial hypercholesterolaemic subjects with null mutations could have a lower clearance of remnant particles (VLDL and LDL) and, as a result, higher levels of plasma triglycerides together with lower HDL cholesterol concentrations. In support of this hypothesis, the subgroup of familial hypercholesterolaemic subjects with null mutations studied by Vohl *et al*.[7] presented the highest coronary heart disease risk. However, the hypothesis that familial hypercholesterolaemic subjects with null mutations have lower clearance rates of chylomicron and VLDL particles has not yet been supported by kinetic data.

Moreover, an interaction between LDL receptor mutations and the recently described proteins, SB-RI and ABC1, responsible for reverse cholesterol transport could, theoretically, explain differences in HDL cholesterol plasma values among familial hypercholesterolaemic subjects[38,39]. In some familial hypercholesterolaemic patients, low HDL cholesterol levels can be explained by additional heterozygous inheritance of a mutant lipoprotein lipase gene[40].

Our study has shown a relationship between LDL receptor gene null mutations and low HDL cholesterol values, as well as with triglyceride levels. The fact that familial hypercholesterolaemia with coronary heart disease showed significantly lower plasma HDL cholesterol values and a higher total cholesterol to HDL cholesterol ratio may carry therapeutic implications. In a recent secondary prevention trial in a high risk group of men with low plasma HDL cholesterol values, a moderate elevation of 6% in HDL cholesterol together with a 31% reduction in triglyceride plasma values with gemfibrozil was associated with a 22% decreased relative risk for coronary heart disease[41].

In conclusion, in familial hypercholesterolaemic subjects with similar clinical and genetic factors that could influence coronary heart disease risk, plasma HDL cholesterol values and total/HDL cholesterol ratios are two important coronary risk factors. Hence, treatment of familial hypercholesterolaemia should not only focus on lowering total and LDL cholesterol levels, but also on increasing HDL cholesterol for the prevention of coronary heart disease in this high risk group. More prospective and intervention trials should be conducted to establish the relationship between HDL cholesterol levels and coronary heart disease in familial hypercholesterolaemia.

References

[1] Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986; 232: 34–47.

[2] Hobbs H, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat 1992; 1: 445–66.

[3] Hoeg JM. Homozygous familial hypercholesterolemia: a paradigm for phenotypic variation. Am J Cardiol 1993; 72: 11D–14D.

[4] Ferrières J, Lambert J, Lussier-Cacan S, Davignon J. Coronary artery disease in heterozygous familial hypercholesterolemia patients with the same LDL receptor gene mutation. Circulation 1995; 92: 290–5.

[5] Utermann G, Hoppichler F, Dieplinger H, Seed M, Thompson G, Boerwinkle E. Defects in the low density lipoprotein receptor gene affect lipoprotein(a) levels: multiplicative interaction of two gene loci associated with premature atherosclerosis. Proc Natl Acad Sci USA 1989; 86: 4171–4.

[6] Gaudet D, Vohl MC, Perron P *et al*. Relationships of abdominal obesity and hyperinsulinemia to angiographically assessed coronary artery disease in men with known mutations in the LDL receptor gene. Circulation 1998; 97: 871–7.

[7] Vohl MC, Gaudet D, Moorjani S *et al*. Comparison of the effect of two low-density lipoprotein receptor class mutations on coronary heart disease among French-Canadian patients heterozygous for familial hypercholesterolemia. Eur J Clin Invest 1997; 27: 366–73.

[8] O'Malley, Maslen CL, Illinworth DR. Angiotensin-converting enzyme DD genotype and cardiovascular disease in heterozygous familial hypercholesterolemia. Circulation 1998; 97: 1780–3.

[9] Nielsen L, Stender S, Kjeldsen K, Nordestgaard B. Effect of angiotensin and enalapril on transfer of low density lipoprotein into aortic intima in rabbits. Circ Res 1994; 75: 63–9.

[10] Kotze MJ, de Villiers WJS, Stein K *et al*. Phenotypic variation among familial hypercholesterolemic heterozygous for either one of two Afrikaner founder LDL receptor mutations. Arterioscler Thromb 1993; 13: 1460–8.

[11] Gudnason V, Day INM, Humphries SE. Effect on plasma lipid levels of different classes of mutations in the low-density lipoprotein receptor gene in patients with familial hypercholesterolemia. Arterioscler Thromb 1994; 17: 1717–22.

[12] Vuorio AF, Ojala JP, Sarna S, Turtola H, Tikkanen MJ, Kontula K. Heterozygous familial hypercholesterolaemia: the influence of the mutation type of the low-density-lipoprotein receptor gene and PvuII polymorphism of the normal allele on serum lipid levels and response to lovastatin treatment. J Intern Med 237: 1: 43–8.

[13] Leren TP, Hjermann I. Is responsiveness to lovastatin in familial hypercholesterolaemia influenced by the specific mutation in the low density lipoprotein receptor gene? Eur J Clin Invest 1995; 25: 967–73.

[14] Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997; 20: 1183–97.

[15] Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974; 20: 470–5.

[16] Ter Welle HF, Baartscheer T, Fiolet JWT. Influence of free glycerol on enzymatic evaluation of triglycerides. Clin Chem 1984; 30: 1102–3.

[17] Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970; 11: 583–95.

[18] Havel RJ, Eder HJ, Bragdon JH. The distribution and chemical composition of centrifugally separated lipoproteins in human serum. Eur J Clin Invest 1995; 34: 1345–54.

[19] Rosseneu M, Vercaemst R, Steinberg KK, Cooper GR. Some considerations of methodology and standarization of apolipoprotein B immunoassays. Clin Chem 1983; 29: 427–33.

[20] Lombardi P, Sijbrands EJC, van de Giessen K *et al*. Mutations in the low density lipoprotein receptor gene of familial hypercholesterolemic patients detected by denaturing gel electrophoresis and direct sequencing. J Lipid Res 1995; 36: 860–5.

[21] Leren TP, Solberg K, Kodningen OK, Ose L, Tondstad S, Berg K. Evaluation of running conditions for SSCP for detection of point mutations in the LDL receptor gene. PCR Methods and Applications 1993; 3: 159–62.

[22] Day I. The low density lipoprotein receptor (LDL receptor) gene in familial hypercholesterolemia. http:\\www. ucl.ac.uk\FH\Ian Day.

[23] Redon J, Chaves FJ, Liao Y *et al*. Influence of I/D polymorphism of the angiotensin converting enzyme gene on the outcome of microalbuminuria in essential hypertension. Hypertension 2000; 35: 490–5.

[24] SPSS version 9.1.3. SPSS inc., 1999.

[25] Carmena R, Lussier-Cacan S, Roy M *et al*. Lp(a) levels and hypercholesterolemia sharing the same gene defect. Arterioscler Throm Vasc Biol 1996; 16: 129–36.

[26] Seed M, Hoppichler F, Reaveley D *et al*. Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. N Engl J Med 1990; 322: 1494–9.

[27] Hausmann D, Johnson JA, Sudhir K *et al*. Angiographically silent atherosclerosis detected by intravascular ultrasound in patients with familial hypercholesterolemia and familial combined hyperlipidemia: correlation with high density lipoproteins. J Am Coll Cardiol 1996; 27: 1562–70.

[28] Sudhir K, Ports TA, Amidon TM *et al*. Increased prevalence of coronary ectasia in heterozygous familial hypercholesterolemia. Circulation 1995; 91: 1375–80.

[29] Gordon DJ, Rifklind BM. High density lipoprotein — the clinical implications of recent studies. N Engl J Med 1989; 321: 1311–6.

[30] Goldbourt U, Yaari S, Medalie JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality: a 21-year follow up of 8000 men. Arterioscler Thromb Vasc Biol 1997; 17: 107–13.

[31] Rubins HB, Robins SJ, Iwane MK *et al*. Rationale and desing of the Department of Veterans Affairs High Density lipoprotein cholesterol intervention trial (HIT) for secondary prevention of coronary artery disease in men with low high density lipoprotein cholesterol and desirable low density lipoprotein cholesterol. Am J Cardiol 1993; 71: 45–52.

[32] Nakamura T, Kobayashi H, Yanagi K *et al*. Importance of intra-abdominal visceral fat accumulation to coronary atherosclerosis in heterozygous familial hypercholesterolemia. Int J Obes Relat Metab Disord 1997; 21: 580–6.

[33] Bowler A, Redgrave TG, Mano JCL. Chylomicron remnants clearance in homozygote and heterozygote Watanabe hereditable hyperlipidemic rabbits is defective. Lack of evidence for an independent chylomicron remnant receptor. Biochem J 1991; 276: 381–6.

[34] Kita T, Goldstein JL, Brown MS, Watanabe Y, Hornick CA, Havel RJ. Hepatic up take of chylomicrons remnants in WHHL rabbits: a mechanism genetically distinct from the LDL receptor. Proc Natl Acad Sci USA 1982; 79: 3623–7.

[35] Rubinsztein DC, Cohen JC, Berger GM, van der Westhuyzen DR, Coetzee GA, Gevers W. Chylomicron remnants clearance from the plasma is normal in familial hypercholesterolemic homozygous with defined receptor defects. J Clin Invest 1990; 86: 1306–12.

[36] Mano JCL, Smith D, Yu JCW *et al*. Accumulation of chylomicron remnants in homozygous subjects with familial hypercholesterolemia. Eur J Clin Invest 1998; 28: 379–84.

[37] Castro-Cabezas H, De Bruin TWA, Westerveld KE *et al*. Delayed chylomicron remnants clearance in subjects with heterozygous familial hypercholesterolemia. J Int Med 1998; 244: 299–307.

[38] Krieger M, Kozarsky K. Influence of the HDL receptor SB-RI on atherosclerosis. Curr Opin Lipidol 1999; 10: 491–7.

[39] Rust S, Rosier M, Funke H *et al*. Tangier disease is caused by mutations in the encoding ATP-binding cassette transporter 1. Nature Genet 1999; 22: 352–5.

[40] Pimstone SN, Gagne SE, Gagne C *et al*. Mutations in the gene for lipoprotein lipase. A cause for low cholesterol levels in individuals with heterozygous familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 1995; 15: 1704–12.

[41] Rubins HB, Robins SJ, Collins D *et al*. Gemfibrozil for secondary prevention of coronary heart disease in men with low levels of high density lipoprotein cholesterol. Veterans Affairs High-density lipoprotein cholesterol Intervention Trial Study Group. N Engl J Med 1999; 341: 410–8.