Increased Carotid Artery Intima-Media Thickness Is Associated With a Novel Mutation of Low-Density Lipoprotein Receptor Independently of Major Cardiovascular Risk Factors

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The current study sought to investigate the role of low-density lipoprotein receptor (LDLr) mutations in assessing the risk profile of familial hypercholesterolemia (FH) patients, independently of major cardiovascular risk factors. FH due to LDLr mutations is associated with premature atherosclerosis. The variable clinical severity of the disease in heterozygotes has been related to cholesterol levels and the coexistence of other cardiovascular risk factors, but the independent role of different LDLr mutations is still unclear. cDNA of LDL gene was sequenced in 102 patients with clinical features of heterozygous FH. Carotid artery intima-media thickness (IMT) was measured by B-mode ultrasound imaging in all patients. Sixteen different mutations (5 never described) were found in 82 patients (49 families; mean age, 39 years; 53% women). One of the newly described mutations, the 2312-3 C→A, was found in 24 patients (13 families). The mean of maximum thicknesses was significantly higher in the 2312-3 C→A group than in patients with other LDLr mutations ($P = .004$ after adjustment for major cardiovascular risk factors). Similar results ($P = .001$) were obtained in the adjusted comparisons of probands only, and of the patients with similar baseline cholesterol ($P = .002$). This study indicates that the identification of an LDLr mutation can help to assess the risk profile of FH patients independently of the major cardiovascular risk factors.

FAMILIAL hypercholesterolemia (FH) is a dominantly inherited disorder characterized by elevated plasma level of low-density-lipoprotein (LDL) cholesterol; it is due to a mutation of the LDL receptor (LDLr) gene, or to a mutation in the apolipoprotein B-100 gene. FH patients show premature atherosclerosis and have a great increase in cardiovascular mortality.[1]

The relationship between different LDLr mutations and the clinical expression of the disease is still unclear.[2-4] A more or less severe clinical expression of the disease has been related to plasma total and LDL cholesterol levels, which in turn are related to the percentage of residual LDLr activity;[5] however, other investigators have failed to show a relationship between null allele mutations and cardiovascular disease (CVD) mortality.[6] Other reports have stressed the importance of associated and traditional CVD risk factors,[7] and the coexistence of other lipid-related risk factors. However, in this search for the identification of risk factors able to explain the clinical heterogeneity of FH patients, it is still unclear whether the different LDLr mutations play an independent role as risk factor, in view of the dominant role of the cholesterol burden and of the other major CVD risk factors.[7,8]

The aim of the present study was to address the question of whether the identification of an LDLr mutation plays a role in the assessment of the CVD risk profile independently of the major risk factors. In this pursuit, we performed (1) cDNA sequencing in a comprehensive search for old and new highly prevalent LDLr mutations in a local sample of patients with clinical features of heterozygous FH; (2) a clinical evaluation of patients with different mutations; and (3) high-resolution B-mode ultrasound examination of carotid arteries in patients with different LDLr mutations, to noninvasively assess intima-media thickness (IMT). IMT is a standardized and reproducible marker of preclinical atherosclerosis, is significantly increased in heterozygous and homozygous FH patients,[9,10] and has been proven to be a powerful predictor not only of cerebrovascular events but also of ischemic heart disease.[11,12]

MATERIALS AND METHODS

Clinical Diagnosis of FH

One hundred two patients having the clinical features of heterozygous FH, consecutively admitted to the outpatient Lipid Clinic of the "Federico II" University of Naples, were asked to participate in the study. All patients accepted and signed an informed consent.

A "definite" diagnosis of FH was considered when patients fulfilled the Simon Broome Register criteria[13] (13 patients). The diagnosis was considered as "possible" if baseline plasma cholesterol was above 7.49 mmol/L or LDL cholesterol above 4.91 mmol/L (total cholesterol $>$ 6.46 mmol/L if age $<$ 16 years) in the proband, and at least in 1 first-degree relative, with plasma triglyceride below 2.25 mmol/L in all family members (89 patients).

Demographics and prevalence of major cardiovascular risk factors in the study participants are shown in Table 1. Fourteen patients (14%) had a history of coronary heart disease. Eleven patients (9 males and 2 females) were myocardial infarction survivors; 9 had experienced premature ($<$55 years, all males) myocardial infarction. Two patients had undergone a coronary bypass graft surgery and 1 had stable angina. One patient underwent carotid endarterectomy. Twenty-five patients had blood pressure higher than 140/90 mm Hg. No patient was diabetic.

LDLr Mutations

cDNA of LDLr gene was sequenced by a rapid automated method, as elsewhere described.[14] Sixteen different mutations affecting the LDLr coding region, including the 5 new mutations, were found and

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Table 1. Cardiovascular Risk Factors and Tendon Xanthomas in the 102 Study Patients According to LDLr Mutation Status

	Mutation-Positive (n = 82)	Mutation-Negative (n = 20)
Age (yr)	37 ± 2	45 ± 4
Gender (% women)	49	65
Body mass index (kg/m^2)	25.4 ± 0.5	24.8 ± 0.7
Baseline total cholesterol (mmol/L)	9.7 ± 0.2	10.7 ± 0.7
Triglycerides (mmol/L)	1.3 ± 0.1	1.8 ± 0.3
HDL cholesterol (mmol/L)	1.2 ± 0.04	1.2 ± 0.06
Triglycerides/HDL cholesterol	1.09 ± 0.09	1.86 ± 0.45
Cholesterol-year score (mmol/L year)	382 (248-473)	410 (270-542)
Systolic blood pressure (mm Hg)	127 ± 3	129 ± 4
Diastolic blood pressure (mm Hg)	77 ± 1	81 ± 3
Cigarette smoking (%)	18	16
Hypertension (%)	23	37
Cardiovascular events (%)	14	16
Tendon xanthomas (%)	16	5
Meanmax IMT	0.90 ± 0.04	0.91 ± 0.07

NOTE. Values are mean ± SEM, median and interquartile range for cholesterol-year score.

have been reported elsewhere.[14] One of them, a IVS15-3C→A mutation, damages the 3′ acceptor splice site of intron 15 and results in the elimination of exon 16 from the mature mRNA. This deletion produces a receptor protein with an in-frame deletion of 26 amino acids between the position Q^{749} and V^{776} of the membrane-spanning domain, and is likely to produce a soluble LDLr.[14] Hereafter, this mutation will be indicated as 2312-3 C→A.

Cholesterol Burden Calculation

Since all patients, including children, were on lipid-lowering treatment, a cholesterol-year score was calculated, taking into account variations in cholesterol level according to treatment.[15] Each patient's clinical record was examined and all lipid measurements preceding carotid ultrasound scans were recorded. Plasma lipid concentrations before lipid-lowering diet and/or drug were taken as baseline plasma lipid levels. Each patient's baseline total cholesterol level (mmol/L) was multiplied by patient age at diagnosis (on average 35.6 years in the group with the 2312-3 C→A mutation *v* 35.2 in the group with the other mutations; difference not significant [NS]). Mean total cholesterol level on diet and/or on drug was multiplied by the years of treatment. The mean number of years of treatment was 4.42 (range, 1 to 15). The mean number of previous lipid measurements was 8.55 (range, 2 to 26). The pretreatment and post-treatment cholesterol-years (mmol/L year) were added to each other to get the total cholesterol-year score.

Environmental Risk Factors Evaluation

A 7-day record of dietary habits was filled out by 85% of patients. Average daily calories were 1,800 and 1,850 in the 2 groups (new mutation and other mutations, respectively). Percentage of saturated fat was approximately 11% in both groups, with a cholesterol content of about 210 mg/d in both groups. A questionnaire for assessment of physical activity at work and in leisure time did not indicate significant differences between the 2 groups.

Laboratory Analyses

After an overnight fast, blood samples were obtained by venipuncture from all patients. Serum total cholesterol and triglyceride levels were determined using an enzymatic method. High-density lipoprotein (HDL) cholesterol was determined after precipitation by sodium fosfotungstate. LDL cholesterol was calculated by Friedewald's formula.

High-Resolution B-Mode Ultrasound

In all patients, carotid artery high-resolution B-mode ultrasound was performed and IMT was measured. Biosound 2000 II s.a. (Biosound Inc, Indianapolis, IN) was used to examine the extracranial carotid arteries bilaterally. This system, equipped with an 8-MHz transducer with pulsed-wave Doppler and spectrum analysis capabilities, provides high-resolution ultrasonic images with 0.3 mm axial resolution and 256 degrees of gray scale. The ultrasound imaging examination was performed by an experienced certified sonographer (F.F.) following a standardized protocol developed by the Division of Vascular Ultrasound Reasearch at Wake Forest University School of Medicine, Winston Salem, NC.[16] The aims of the protocol were to define intra-arterial carotid artery anatomical references (ie, crest at the origin of the bifurcation, and the arch of the flow divider), and specific ultrasonic interfaces on both the near and far wall to provide valid and reliable measurements of IMT at the level of 2 standardized segments: the distal 1.0 cm of the common carotid artery and the carotid bifurcation. Scans were recorded on super VHS videotape for offline analysis. One certified ultrasound reader (M.D.M.), unaware of the patients' genetic condition, reviewed the scans and made IMT quantitative measurements, using a computerized analyzing system based on automated detection of the echo structures, with the option of making manual corrections by the operator. The reader selected the frame for each of the 8 carotid wall segments that contained the thickest IMT. The mean of maximum carotid IMT thicknesses measured on 8 interfaces (4 for each side) was the ultrasound end-point mean max. In the present study, estimation of intra-observer variability for both the sonographer and the reader gave a coefficient of reliability for the mean max IMT of 0.97, comparable with the same parameter measured in the European Lacidipine Study on Atherosclerosis (ELSA).[17]

Statistical Analyses

Data analysis was performed with a commercially available SPSS 10.0 statistical software (SPSS Inc, Chicago, IL).

Parametric results are expressed as means ± SEM. Triglyceride concentrations were log-transformed before analysis. Cholesterol-year score was expressed as median (interquartile range) and Mann-Whitney *U* statistic was used for comparisons of this parameter between groups. Differences between independent means of the other risk factors were analyzed by Student's *t* test for continuous variables, and the distribution of categorical variables was analyzed by chi-square test. Analysis of covariance (ANCOVA) was used to detect differences in carotid IMT after adjustment for covariates.

RESULTS

Eighty-two (80%) of the 102 patients had an LDLr mutation. No significant difference was found between mutation-positive and mutation-negative patients for traditional CVD risk factors and carotid IMT (Table 1). Patients with the 2312-3 C→A mutation (n = 24, 13 families) had higher baseline total cholesterol concentrations than patients with other LDLr mutations. However, the difference in terms of cholesterol-year score did not reach statistical significance (Table 2). No significant differences in the prevalence of other cardiovascular risk factors and events were found in either group, but the prevalence of tendon xanthomas was 3-fold higher in the 2312-3 C→A mutation group than in the other group (P = .027).

Table 2. Cardiovascular Risk Factors and Tendon Xanthomas in FH Patients With New LDLr Mutation Versus Patients With Other LDLr Mutations

	2312-3C→A Mutation (n = 24)	Other Mutations (n = 58)
Age (yr)	38 ± 4	37 ± 2
Gender (% women)	42	52
Body mass index (kg/m^2)	25.9 ± 0.9	25.2 ± 0.6
Total cholesterol (mmol/L)	10.8 ± 0.5*	9.2 ± 0.3
Triglycerides (mmol/L)	1.38 ± 0.17	1.26 ± 0.11
HDL cholesterol (mmol/L)	1.26 ± 0.09	1.23 ± 0.05
Triglycerides/HDL cholesterol	1.28 ± 0.22	1.01 ± 0.09
Cholesterol-year score (mmol/L year)	437 (254-640)	357 (242-424)
Systolic blood pressure (mm Hg)	130 ± 6	126 ± 3
Diastolic blood pressure (mm Hg)	76 ± 2	77 ± 2
Cigarette smoking (%)	17	18
Hypertension (%)	25	22
Cardiovascular events (%)	22	11
Tendon xanthomas (%)	29†	9
Meanmax IMT	1.05 ± 0.08‡	0.82 ± 0.04

NOTE. Values are mean ± SEM, median and interquartile range for cholesterol-year score.

*P = .002.

†P = .027.

‡P = .021.

Carotid mean max IMT was significantly higher in patients with the 2312-3 C→A mutation than in patients with the other LDLr mutations (P = .021) (Table 2). The same was true in the multivariate model (adjusted for age, gender, and cholesterol-year score; P = .002), and in the extended multivariate model (including cholesterol-year score, age, and gender distribution, systolic blood pressure, smoking habits; P = .004) (Fig 1). If baseline cholesterol concentration was used instead of cholesterol-year score in the 2 multivariate models, significance levels were 0.002 and 0.003, respectively. Addition of baseline triglyceride/HDL cholesterol ratio to the model did not change significance (P = .004) (Fig 1).

The analysis carried out in probands (2312-3 C→A mutation *v* other mutations; Table 3 and Fig 2) generated the same pattern observed in the comparisons between whole groups. This subanalysis confirmed statistically significant differences in baseline cholesterol and prevalence of xanthomas, while cholesterol-year score was not significantly different. Carotid meanmax IMT in probands with the 2312-3 C→A mutation was higher than in probands with the other LDLr mutations: P = .007 unadjusted, P < .001 in the multivariate model, and P = .001 in the extended multivariate model. Using baseline cholesterol in the model instead of cholesterol-year score, the levels of significance in the 2 multivariate models were .001 and .003, respectively.

Another subgroup analysis was performed in patients with plasma total cholesterol ≥ 9.0 mmol/L (means ± SEM were 10.9 ± 0.48 in the 2312-3 C→A group and 11.0 ± 0.36 in the group with the other mutations, P = 0.883). Carotid meanmax IMT was significantly higher in the 2312-3 C→A group (n = 23; mean IMT, 1.05 ± 0.83 mm) than in the group with other mutations (n = 19; mean IMT, 0.76 ± 0.39 mm), (P = .003 unadjusted, P < .001 in the multivariate model, P = .002 in the extended multivariate model).

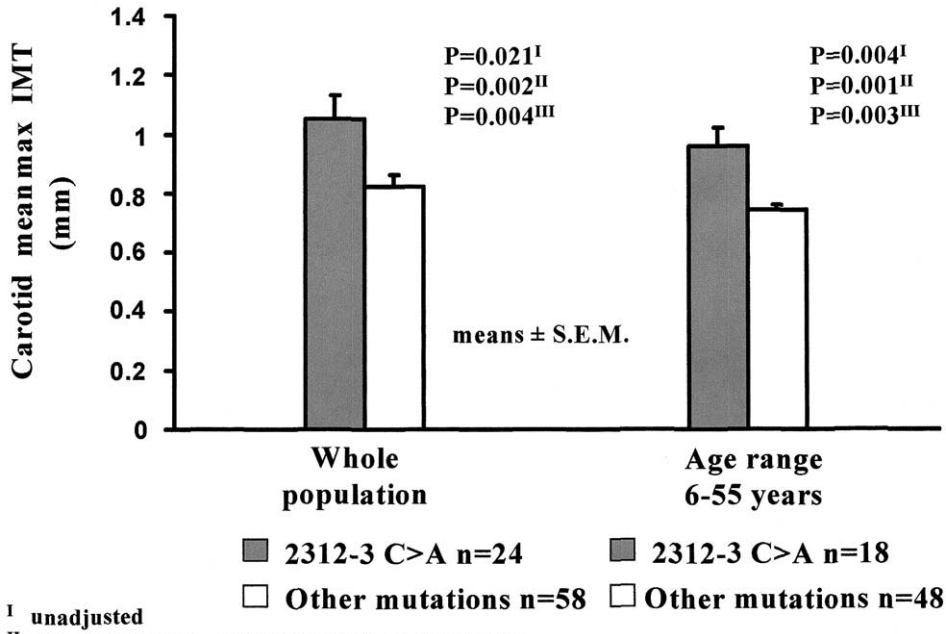

Fig 1. Carotid meanmax IMT in FH patients with 2312-3 C→A mutation *v* patients with other LDLr mutations.

Table 3. Cardiovascular Risk Factors and Tendon Xanthomas in FH Probands With New LDLr Mutation Versus Probands With Other LDLr Mutations

	2312-3C→A Mutation (n = 13)	Other Mutations (n = 36)
Age (yr)	35 ± 4	37 ± 2
Gender (% women)	46	61
Body mass index (kg/m^2)	26.3 ± 1.4	24.1 ± 0.7
Total cholesterol (mmol/L)	10.4 ± 0.5*	9.2 ± 0.3
Triglycerides (mmol/L)	1.24 ± 0.22	1.15 ± 0.09
HDL cholesterol (mmol/L)	1.22 ± 0.13	1.29 ± 0.06
Triglycerides/HDL cholesterol	1.21 ± 0.30	0.98 ± 0.1
Cholesterol-year score (mmol/L year)	334 (223-440)	339 (240-394)
Systolic blood pressure (mm Hg)	128 ± 5	126 ± 3
Diastolic blood pressure (mm Hg)	77 ± 2	77 ± 2
Cigarette smoking (%)	8	17
Hypertension (%)	15	20
Cardiovascular Events (%)	15	9
Tendon xanthomas (%)	39†	6
Meanmax IMT	1.02 ± 0.09‡	0.79 ± 0.04

NOTE. Values are mean ± SEM, median and interquartile range for cholesterol-year score.

*P = .028.

†P = .004.

‡P = .007.

DISCUSSION

The present study provides evidence that a mutation of LDLr, compared with other LDLr mutations, independently contributes to an increased prevalence of carotid atherosclerosis, together with plasma cholesterol levels and major CVD risk factors.

In the present study, criteria for the "definite" clinical diagnosis of heterozygous FH proved accurate (ie, patients were found to be LDLr mutation–positive) in 8 of the 9 patients who met them. Criteria for the "possible" clinical diagnosis of heterozygous FH, based also on the evaluation of first-degree relatives, were those suggested by the Italian National Committee for the standardization of diagnosis and treatment of familial dyslipidemias and proved effective in 80% of the patients. In this FH sample, the presence of new mutations in the promoter region was not explored. However, in a comprehensive review of Italian FH mutations,[5] patients with these mutations accounted for only 5% of cases. The newly discovered mutation destroys the 3′ acceptor splice site of intron 15 and results in the elimination of exon 16 from the mature mRNA, with consequent damage of the membrane spanning domain and possible production of a soluble LDLr (possible class 4). This new LDLr mutation was the single most prevalent one in the present sample and patients bearing it were compared to the other mutation positive patients. The numbers of patients with the other mutations were too small for comparisons of each mutation to the 2312-3 C→A, but this study is not a head-to-head comparison between individual mutations. It actually indicates that the detection of a highly prevalent LDLr mutation carrying more severe preclinical atherosclerosis allows the identification of FH patients at higher risk than other FH patients.

Common risk factors were taken into account on the basis of previous investigations[5] where LDLr activity was also measured. Age and arterial hypertension were the strongest cardiovascular predictors, whereas LDL cholesterol (and apolipoprotein E genotype) was not found to be a good predictor, at least in receptor-negative patients, thus suggesting the need for additional indicators of risk. In another study aiming at a comprehensive evaluation of coronary artery disease predictors in

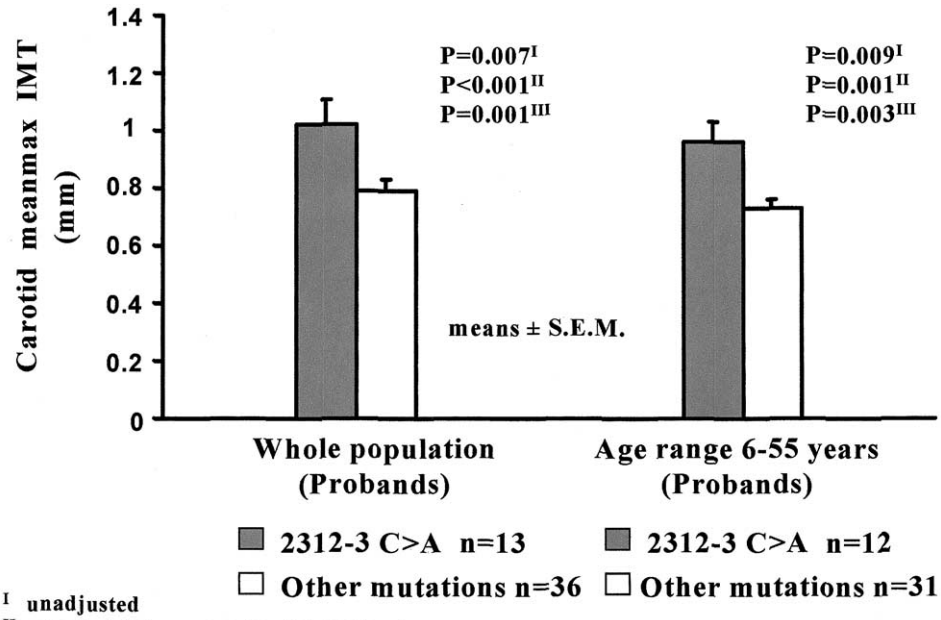

Fig 2. Carotid meanmax IMT in proband FH patients with 2312-3 C→A mutation *v* proband FH patients with LDLr mutations.

FH,[7] age, gender, cigarette smoking, and LDL composition (small LDL) were confirmed to be the most powerful predictors of cardiovascular risk, while lipoprotein(a) and presence of xanthoma played a role only in premature coronary cases, whereas insulin, fibrinogen, homocysteine, C-reactive protein, and angiotensin-converting enzyme insertion/deletion polymorphism seemed to have no role.

Although the prevalence of tendon xanthomas was higher in the 2312-3 C→A group, inclusion of xanthomatosis in the multivariate analysis did not change levels of statistical significance. The presence of xanthomas is also age-related, and this criterion could fail to identify high risk FH children, while detection of a high-risk mutation would be effective.

In the present analysis, we chose B-mode ultrasound carotid IMT as an indicator of cardiovascular disease, based on the extensive and prospective evidence that IMT predicts coronary events.[18] In addition, the mean max IMT proved to be a reliable outcome end-point in large intervention studies with cholesterol-lowering drugs[19,20] and is commonly regarded as an indicator of the extent of the atherosclerotic process. A significant relationship exists between carotid IMT and the most common cardiovascular risk factors.[10] However, the differences in meanmax IMT between mutation 2312-3 C→A and other LDLr mutations remained significant after adjustments for the major cardiovascular risk factors and for the triglycerides-to-HDL cholesterol ratio which is an indicator of LDL particle size.[21] Possible inflation of statistical significance due to the influence of familial factors or family aggregation of risk was also taken into account by performing a separate analysis in probands, which yielded the same results as the analysis of the whole group. Since baseline plasma cholesterol was significantly higher in the 2312-3 C→A group than in the group with the other mutations, a subgroup analysis of carotid IMT in patients with baseline plasma cholesterol above 9.0 mmol/L was performed to allow comparison of groups with similar baseline cholesterol. Unadjusted and multivariate models confirmed the independent role of the 2312-3 C→A mutation as risk factor for increased carotid IMT as compared to the other mutations with similar cholesterol levels.

Although families with the 2312-3C→A mutation were not aware of possible kinships and came from different areas of southern Italy, a founder effect cannot be ruled out. However, the rationale of the present study was to ascertain if the detection of an LDLr mutation could identify FH subjects at particularly high cardiovascular risk as compared to other FH subjects. Whether this mutation is by itself an independent cause of increased atherosclerosis or is a marker (or a causal component) of a founder effect, does not diminish the importance of the identification of the mutation, particularly in children. In fact, in FH children, as compared to other FH children, the evidence of other cardiovascular risk factors, including high carotid IMT, xanthomas, lipoprotein(a), homocysteine, and even very high levels of plasma cholesterol can be lacking or less pronounced until adulthood. But the identification of high-risk mutation could allow early and intensive intervention.

In conclusion, this study indicates that the detection of LDLr mutations can be useful to identify FH patients at particularly high atherosclerotic risk. Since the 2312-3 C→A mutation damages the section of the LDLr anchored to the cell membrane, further studies are needed to evaluate the possibility of an atherogenic role of a soluble LDLr[14]—with attached LDL particle released in the circulation.

REFERENCES

1. Scientific Steering Committee on behalf of the Simon Broome Register Group: Mortality in treated heterozygous familial hypercholesterolemia: implications for clinical management. Atherosclerosis 142:105-112, 1999

2. Moorjani S, Roy M, Torres A, et al: Mutations of low-density-lipoprotein-receptor gene, variation in plasma cholesterol, and expression of coronary heart disease in homozygous familial hypercholesterolemia. Lancet 341:1303-1306, 1993

3. Graham CA, McLean E, Ward AJM, et al: Mutation screening and genotype: phenotype correlation in familial hypercholesterolemia. Atherosclerosis 147:309-316, 1999

4. Gaudet D, Vohl MC, Couture P, et al: Contribution of receptor negative versus receptor defective mutations in the LDL-receptor gene to angiographically assessed coronary artery disease among young (25-49 years) versus middle-aged (50-64 years) men. Atherosclerosis 143:153-161, 1999

5. Bertolini S, Cantafora A, Averna M, et al: Clinical expression of familial hypercholesterolemia in clusters of mutations of the LDL receptor-defective or receptor-negative phenotype. Arterioscler Thromb Vasc Biol 20:E41-E52, 2000

6. Sijbrands EJ, Westendorp RG, Lombardi M P, et al: Additional risk factors influence excess mortality in heterozygous familial hypercholesterolemia. Atherosclerosis 149:421-425, 2000

7. Hopkins PN, Stephenson S, Wu LL, et al: Evaluation of coronary risk factors in patients with heterozygous Familial Hypercholesterolemia. Am J Cardiol 87:547-553, 2001

8. Descamps OS, Gibeau JP, Leysen X, et al: Impact of genetic defects on atherosclerosis in patients suspected of familial hypercholesterolemia. Eur J Clin Invest 31:958-965, 2001

9. Pauciullo P, Iannuzzi A, Sartorio R, et al: Increased intima-media thickness of the common carotid artery in hypercholesterolemic children. Arterioscler Thromb 14:1075-1079, 1994

10. Rubba P, Mercuri M, Faccenda F, et al: Premature carotid atherosclerosis: Does it occur in both familial hypercholesterolemia and homocystinuria?—Ultrasound assessment of arterial intima-media thickness and blood flow velocity. Stroke 25:943-950, 1994

11. Smith SC, Greenland P, Grundy SM: Beyond secondary prevention: Identifying the high-risk patient for primary prevention. Circulation 101:111-116, 2000

12. Nowak J, Nilsson T, Sylvèn C, et al: Potential of carotid ultrasonography in the diagnosis of coronary artery disease. A comparison with exercise test and variance ECG. Stroke 29:439-446, 1998

13. Simon Broome Register Group: Risk of fatal coronary heart disease in familial hypercholesterolemia. BMJ 303:893-896, 1991

14. Liguori R, Bianco AM, Argiriou A, et al: LDL receptor cDNA sequence analysis in familial hypercholesterolemia patients: 5 novel mutations with high prevalence in families originating from Southern Italy. Hum Mutat 17:433-437, 2001

15. Tonstad S, Joakimsen O, Bugge-Stensland E, et al: Carotid intima-media thickness and plaque in patients with familial hypercholesterolemia mutations and control subjects. Eur J Clin Invest 28:971-979, 1998

16. Zanchetti A, Crepaldi G, Bond MG, et al: Systolic and pulse blood pressures (but not diastolic blood pressure) are associated with alterations in carotid intima-media thickness in the moderately hyper-

cholesterolemic hypertensive patients of the Plaque. Hypertension Lipid Lowering Italian Study. J Hypertens 19:79-88, 2001

17. Tang R, Hennig M, Thomasson B, et al: Baseline reproducibility of B-mode ultrasonic measurement of carotid artery intima-media thickness: The European Lacidipine Study on Atherosclerosis (ELSA). J Hypertens 18:197-201, 2000

18. O'Leary D, Polak JF, Kronmal RA, et al: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. N Engl J Med 340:14-22, 1999

19. Mercuri M, Bond MG, Sirtori CR, et al: Pravastatin reduces intima-media thickness progression in an asymptomatic hypercholesterolemic mediterranean population: The Carotid Italian Ultrasound Study. Am J Med 101:627-634, 1996

20. Furberg CD, Adams HP, Applegate WB, et al: Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Circulation 90:1679-1687, 1994

21. Boizel R, Benhamou PY, Lardy B, et al: Ratio of triglycerides to HDL cholesterol is an indicator of LDL particle size in patients with type 2 diabetes and normal cholesterol levels. Diabetes Care 23:1679-1685, 2000