

# Impact of genetic defects on atherosclerosis in patients suspected of familial hypercholesterolaemia

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## Abstract

**Background** Among patients with severe hypercholesterolaemia and a family history of early cardiovascular disease, we assessed whether patients with mutations of low-density lipoprotein (LDL) receptor and apolipoprotein B genes related to familial hypercholesterolaemia (FH) have a different degree of atherosclerosis than those without such mutations.

**Method** In our lipid clinics, 273 patients were selected on the basis of a severe hypercholesterolaemia (cholesterol above 95th percentile) and a family history of early cardiovascular disease. By molecular genetic test, 122 patients were classified as FH. Atherosclerosis was evaluated by the ultrasonographic measurement of intima-media thickness (IMT) in the carotid and femoral arteries.

**Result** Despite the fact that non-FH individuals had a higher prevalence of obesity, hypertension, diabetes and hypertriglyceridaemia, FH individuals had significantly greater carotid and femoral IMT than non-FH patients: difference between carotid and femoral IMT, respectively, 0.19 mm (95% CI, 0.08–0.29;  $P < 0.001$ ) and 0.20 mm (95% CI, 0.09–0.35;  $P = 0.001$ ), respectively. These differences remained statistically significant after adjustment for the various risk factors as well as in sub-analysis restricted to the patients with LDL-cholesterol between 240 and 300 mg dL<sup>-1</sup> (range with similar distribution in the two groups). When classified according to the severity of their mutations, FH individuals with null LDL receptor allele tended to have thicker carotid IMT than FH individuals carrying the LDL receptor-defective allele.

**Conclusion** Among patients with severe hypercholesterolaemia and a family history of early cardiovascular disease, the presence of a genetically ascertained FH is associated with a higher degree of atherosclerosis. This suggests that molecular genetic identification of FH may be helpful to evaluate better the coronary heart disease risk in these patients.

**Keywords** Carotid artery, familial hypercholesterolemia, femoral artery, intima-media thickness, low-density lipoprotein receptor mutation.

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## Introduction

In the presence of hypercholesterolaemia, the risk of cardiovascular disease (CVD) is higher among patients with a family history of early CVD and these patients

should receive appropriate treatment [1]. There is, however, no consensus as to whether the decision should take into account the genetic defect underlying the familial aggregation of early CVD and/or hypercholesterolaemia. To date, few hereditary defects in lipid metabolism have been identified at the molecular level, with familial hypercholesterolaemia (FH) the best known. FH is an autosomal dominant disorder with a prevalence of approximately 1 in 500 and is caused by mutant alleles of the low-density lipoprotein receptor (LDL-R) gene [2] or of the apolipoprotein B (Apo B) gene (this last form of FH is also called familial defective Apo B or FDB) [3].

With the emerging ability to identify FH using relatively simple molecular tests [4,5] a critical issue for the introduction of such tests into routine practice is whether determination of the molecular defect is of any help in

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prevention and management. There is no doubt that cardiovascular events [2] and atherosclerosis [6–10] develop more frequently and earlier in patients with FH in comparison with normolipidaemic patients. Before deciding to implement expensive genetic tests in clinical practice, however, the question is really whether FH individuals have more severe atherosclerosis in comparison to non-FH individuals who appear clinically difficult to distinguish from FH. To address the problem, we evaluated atherosclerosis in series of patients characterized by a severe hypercholesterolaemia and a family history of early cardiovascular diseases and classified as having FH or not having FH on the basis of molecular screening for LDL-R and Apo B mutations. Atherosclerosis was evaluated in the carotid and femoral arteries by the measurements of intima-media wall thickness (IMT) using high-resolution B-mode ultrasound. These measurements not only provide information on the local atherosclerotic vascular burden but also have clinical significance as it has been demonstrated that increased IMT is associated with a greater occurrence of cardiovascular events in the general population [11] as well as in the FH population [6,12].

## Methods

### Subjects

Patients with severe hypercholesterolaemia and a family history of early cardiovascular disease were recruited in our lipid clinic to participate in the present observational study. The patients were invited to take part in a complete cardiovascular screening operation including a questionnaire and a medical examination seeking traditional cardiovascular risk factors, B-mode ultrasound of carotid and femoral arteries and a genetic test to diagnose FH. Severe hypercholesterolaemia was defined as a cholesterol level above the 95th percentile for age and sex [13] and a family history of early cardiovascular disease was defined as at least one first-degree relative with early cardiovascular disease before the age of 55 years in men or 65 years in women. Patients with a previous history of cardiovascular disease and patients with hyperlipoproteinaemia type III were excluded in this study, because of their specific cardiovascular risk.

### Molecular genetic identification of FH

The R3500Q-mutation in the Apo B gene was sought first [14]. LDL-R gene mutations were then identified using denaturing gradient gel electrophoresis [15] and DNA sequencing of the abnormal exon. When these analyses were negative, DNA were subjected to long-range polymerase chain reaction (PCR) according to a previous study [16]. For a new mutation, the pathological significance of the mutation was confirmed by the co-segregation in at least two hypercholesterolaemic and in two normocholesterolaemic relatives of the patient or by

analysis of defective LDL-R function in T lymphocytes [17]. We attempted to classify the mutations according to their predicted severity. Except for large rearrangements, nonsense mutations or mutations causing frameshift, the mutation's effect on receptor function (usually defined by class: 1, 2a, 2b, 3, 4a, 4b, 5) is not easily predictable based solely on the knowledge of the corresponding amino acid change. Moreover, the semiquantitative method used to estimate LDL-R function in lymphocytes in some of our patients was not sensitive enough to discriminate between the mutation's classes. We therefore simply considered as LDL-R null alleles, the nonsense mutations or the mutations resulting in a frameshift, and, as defective alleles, the mis-sense mutations and the mutations located at a splicing site.

### B-mode ultrasound of carotid and femoral arteries

Duplex scans of carotid and femoral arteries were carried out using an ultrasound scanner (ATL HDI-3000) with a 4–7 Mhz linear transducer (aperture of 38 mm). The sonographer was unaware of the genetic status of the patient and used a classical protocol [18–22]. The carotid and femoral arteries were investigated bilaterally at various segments (three per artery) for real-time measurements of maximal IMT: the proximal 1 cm of the internal carotid artery, the carotid bifurcation, the distal 1 cm of the common carotid artery; the 1 cm proximal, the 1 cm distal and the 10 cm distal to the site where the artery divides into the superficial and the profound femoral artery.

A carotid and femoral artery 'score' was calculated as the mean of the six measurements of the maximum IMT (mean-max IMT) obtained in the three segments at each side for each artery [19–23]. The intraobserver variability of the real-time measurement of maximal femoral and carotid IMT at the various sites was studied in 10 patients who were invited to our center on two occasions 2 weeks apart. The means and standard deviations of the absolute difference between 60 paired readings (10 patients  $\times$  6 sites) of maximal IMT were  $0.10 \pm 0.08$  mm in the carotid arteries and  $0.11 \pm 0.10$  mm in the femoral arteries. The correlation coefficient between the two measurements was 0.98 in the carotid arteries and 0.96 in the femoral arteries. The means and standard deviations of the absolute difference between 10 paired mean-max IMT were  $0.06 \pm 0.05$  mm in the carotid arteries and  $0.07 \pm 0.05$  mm in the femoral arteries. These mean absolute differences between repeated visits were small compared to the values of mean-max IMT:  $1 \pm 0.53$  mm and  $1.1 \pm 0.63$  mm in the carotid and femoral arteries, respectively. These values of reproducibility are similar to those found in the quality control data of other studies [19–23].

### Statistical analysis

Continuous variables were compared using Student's *t*-test and categorical variables were compared using the  $\chi^2$  test. To correct the difference of IMT between groups for the

confounding effect of risk factors, we carried out an analysis of covariance in which the nominal variable was the FH status and the covariates were various combinations of risk factors (see legend to Fig. 2). All statistical analyses were performed using the statistical package for social sciences (SPSS 8.0; Benelux, Leuven, Belgium).

## Results

### Cardiovascular risk factors in FH and non-FH patients

Two hundred and seventy-three patients with severe hypercholesterolaemia and with a family history of early CVD were evaluated. Amongst them, 122 patients were identified as FH patients. Table 1 shows the spectrum of

mutations found in these FH patients, some of which have already been described [4].

The distribution of classical risk factors was different in FH and non-FH patients (Table 2). Triglycerides, LDL-cholesterol, and lipoprotein (a) were higher in FH patients whereas non-FH patients had a higher prevalence of hypertension and of current smoking and on average had a higher body-mass index, higher waist-to-hip ratio and higher blood pressure. Compared to FH men, prevalences of diabetes, triglyceride concentration and heart rate were also higher in non-FH men. Non-FH women were on average older than FH women. Overall, non-FH patients aggregated a greater number of classical risk factors as compared with FH patients.

### IMT of carotid and femoral arteries

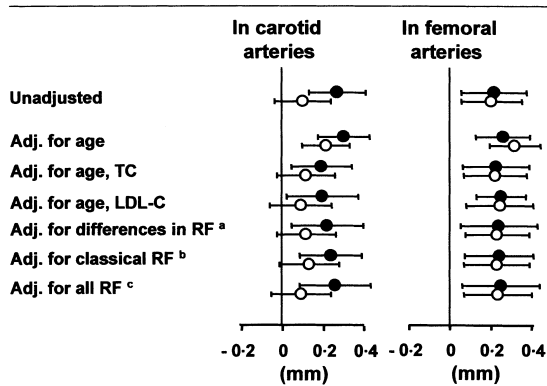
The carotid IMT was greater in FH patients

**Table 1** Mutations in the low-density lipoprotein receptor (LDL-R) and apolipoprotein B (Apo B) genes in the familial hypercholesterolaemia (FH) patients

Mutation	Nucleotide change	Exon	Effect	<i>n</i>
<i>Nonsense</i>				
E10X	G→T at 91	2	Glu→stop 10	4
E92X(FH Paris-5)	G→T at 337	4	Glu→stop 92	2
E119X (FH-Venezuela)	G→T at 418	4	Glu→stop 119	2
C122X (FH-Jolimont)	C→A at 429	4	Cys→stop 122	24
Y398X *	C→A at 1257	9	Tyr→stop 398	2
W599X *	G→A at 1859	13	Trp→stop 599	2
<i>Frameshift, insertion, deletion</i>				
2053del14 *	Deletion of 2053–2066	14	Frameshift/stop	4
2451insAGAA (FH Paris-3)	Insertion AGAA at 2451	17	Frameshift/stop	1
518delG *	Deletion G at 518	4	Frameshift/stop	2
1042insA *	Insertion A at 1042	7	Frameshift/stop	2
<i>Splicing</i>				
1359–1G→A	G↔A at 1359–1	In 10	Splicing alteration	6
1846–1G→A	G↔A at 1846–1	In 13	Reduced mRNA	5
<i>Mis-sense</i>				
E80K (FH-Lancashire)	G→A at 301	3	Glu→Lys 80	3
D200G (FH-Padova-1)	A→G at 662	4	Asp→Gly 200	3
E256K/1402T * (double mutant allele)	G→A at 829/T→C at 1268	6 and 9	Glu→Lys 256 and Ile→Thr 402	9
K290R/C292W * (double mutant allele)	A→G at 932/C→G at 939	6	Lys→Gly 290 and Cys→Trp 292	17
I402P *	T→C at 1268	9	Ile→Thr 402	2
T413R *	C→G at 1301	9	Thr→Arg 413	3
D471N *	G→A at 1474	10	Asp→Asn 471	2
V502M	G→A at 1567	10	Val→Met 502	2
G571E (FH Naples-4)	G→A at 1775	12	Gly→Glu 571	3
R574W *	C→T at 1782	12	Arg→Trp 574	2
P664L	C→T at 2054	14	Pro→Leu 664	4
H635Q *	C→G at 1968	13	His→Gln 635	2
<i>Large rearrangement</i>				
Del 3 kb, e7–8 *	Deletion of 3 kb, exon 7 + 8	7–8		2
Familial defective Apo B R3500Q (ApoB3500) *	G→A at 10 699	26	Arg→Glu 3500	14
Total				122

\*Novel mutations, the other mutations are already described elsewhere [4].

Mean differences of IMT between FH and non-FH individuals in Men (●) and in Women (○)



**Figure 1** Difference of intima-media thickness (IMT) in carotid and femoral arteries in familial hypercholesterolaemia (FH) and non-FH patients after adjustment for various combinations of risk factors (RF).<sup>a</sup>The risk factors with the greatest differences between FH and non-FH individuals: age, triglycerides (TC), waist-to-hip ratio (WHR), log lipoprotein (a) [lp(a)], log(TG) and glycaemia in women; age, TC and systolic blood pressure (SBP) in men. <sup>b</sup>Classical risk factors: age, TC, HDL-C, smoking, SBP, diabetes and LVH. <sup>c</sup>All risk factors: the classical risk factors plus BMI, log(TG) and log[lp(a)].

( $1.16 \pm 0.47$  mm) than in non-FH patients ( $0.97 \pm 0.37$  mm), with a statistically significant difference of 0.19 mm (95% CI, 0.08–0.29;  $P < 0.001$ ). The femoral IMT was also greater in FH patients ( $1.18 \pm 0.52$  mm) than in non-FH patients ( $0.98 \pm 0.43$  mm), with a statistically significant difference of 0.20 mm (95% CI, 0.09–0.31;  $P < 0.001$ ). The difference of IMT varied in men and women (Table 3): femoral artery IMTs were significantly greater in FH men and women compared to the IMTs of non-FH subjects whereas the IMTs of carotid arteries were only significantly greater in FH men compared to non-FH men.

### Multivariate analysis

The carotid IMTs were significantly greater in FH patients compared to non-FH patients after all adjustments in men had been made but after only adjustment for age in women (Fig. 1). In men and women, the femoral IMTs were significantly greater in FH patients compared to non-FH patients after adjustments. In summary, the difference of carotid and femoral IMTs adjusted for sex, age and cholesterol level were 0.18 mm (95% CI, 0.05–0.26;

**Table 2** Clinical and biological features of the patients with or without familial hypercholesterolaemia (FH)

	FH men	Non-FH men	<i>P</i>	FH women	Non-FH women	<i>P</i>
<i>n</i>	63	87		59	64	
Age (years)	44.8 ± 10.8	46.6 ± 9.3	NS	46.0 ± 11.9	51.5 ± 11.0	0.01
Body mass index (kg m <sup>-2</sup> )	27.2 ± 4.0	28.6 ± 3.9	0.03	26.1 ± 4.0	27.8 ± 5.0	0.05
Waist to hip ratio	0.90 ± 0.07	0.94 ± 0.07	0.0001	0.78 ± 0.08	0.81 ± 0.07	0.04
Obesity (BMI > 30 kg m <sup>-2</sup> )	22%	21%	NS	19%	31%	0.004
Current smoker percentage	19%	33%	0.04	12%	20%	0.05
Systolic BP (mmHg)	134.3 ± 25	142.8 ± 22	0.03	136.6 ± 18.7	143.2 ± 28	NS
Diastolic BP (mmHg)	82.3 ± 9.5	84.3 ± 11.2	NS	78.1 ± 13.9	84.5 ± 12	0.01
Heart rate (min <sup>-1</sup> )	74.8 ± 10.3	80.5 ± 14.8	0.02	81.1 ± 13.4	81.9 ± 15	NS
Hypertension (%)	16%	34%	0.01	20%	28%	0.02
Left ventricular hypertrophy (%)	0%	3%	NS*	0%	3%	NS*
Fasting glycemia, (mM)	93 ± 9	108 ± 38	0.004	91 ± 14	98 ± 41	NS
Diabetes (%)	3%	15%	0.02*	2%	8%	0.20*
Total cholesterol (mg dL <sup>-1</sup> )	408 ± 98	315 ± 32	0.0001	407 ± 85	316 ± 35	0.0001
HDL-cholesterol, mg dL <sup>-1</sup>	49 ± 12	46 ± 15	NS	64 ± 17	60 ± 20	NS
Triglycerides, mg dL <sup>-1</sup>	194 ± 163	303 ± 227	0.0001	149 ± 84	198 ± 148	NS
LDL-cholesterol, mg dL <sup>-1</sup>	320 ± 78	220 ± 29	0.0001	313 ± 83	221 ± 33	0.0001
Lp(a), mg dL <sup>-1</sup>	51 ± 48	21 ± 28	0.0001	42 ± 41	36 ± 46	NS
HyperLp(a) (> 30 mg dL <sup>-1</sup> ),%	56%	21%	0.0001	49%	30%	0.03
Number of risk factors†	5.1 ± 1.2	6.0 ± 1.3	< 0.0001	3.8 ± 1.1	4.5 ± 1.2	< 0.0001

BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein (a); NS, not statistically significant. Hypertension was defined if under antihypertensive drug therapy, or if the systolic or diastolic pressures at three successive visits were above 160 mmHg or 95 mmHg, respectively. Diabetes was defined if under antidiabetic drug therapy or if the fasting plasma glucose concentrations at three successive visits were above 126 mg dL<sup>-1</sup>. Lipid levels were determined after at least 6 weeks of withdrawal of any lipid-lowering therapy. Mean ± SD values compared by *t*-test between FH and non-FH. For TG and lp(a), untransformed data are given but the statistical significance was evaluated on log-transformed data. Categorical parameters compared by  $\chi^2$  test or by Fisher's exact test (\*). † Including male gender, age > 50 years in men and age > 60 years in women, TC > 200 mg dL<sup>-1</sup>, HDL-C < 35 mg dL<sup>-1</sup>, TG > 200 mg dL<sup>-1</sup>, current smoking, diabetes, hypertension, body mass index > 30 kg m<sup>-2</sup>, family history of early CVD.

**Table 3** Comparison of carotid and femoral IMT between FH and non-FH-patients

	FH patients	Non-FH patients	Difference:	Mean (9.5% CI)	<i>P</i> (unadjusted)
<b>Men</b>					
Carotid artery IMT (mm)	1.27 ± 0.47	1.00 ± 0.40	+ 0.27	(+ 0.13 to + 0.41)	< 0.001
Femoral artery IMT (mm)	1.30 ± 0.53	1.08 ± 0.46	+ 0.22	(+ 0.05 to + 0.38)	0.01
<b>Women</b>					
Carotid artery IMT (mm)	1.04 ± 0.45	0.93 ± 0.33	+ 0.10	(- 0.04 to + 0.24)	0.15
Femoral artery IMT (mm)	1.05 ± 0.49	0.84 ± 0.32	+ 0.20	(+ 0.05 to + 0.35)	0.01

*P* = 0.003) and 0.23 mm (95% CI, 0.12–0.35; *P* < 0.001), respectively.

adjusted difference: - 0.03 mm; 95% CI, - 0.27–0.26; *P* = 0.96).

### Analysis of subgroups with comparable LDL-cholesterol

The distribution of LDL-cholesterol overlapped substantially in FH and non-FH subjects (data not shown). This overlap offered an opportunity to explore further the difference of IMTs between the two groups. The overlapping range of 240–300 mg dL<sup>-1</sup> included an approximately equal distribution of subjects of each group (16 FH men vs. 14 non-FH men and 15 FH women vs. 12 non-FH women). All risk factors examined were similar in these subgroups except in women, in whom age was, on average, higher in non-FH women (56 ± 10 years) than in FH women (42 ± 11 years). FH men demonstrated a significantly greater carotid IMT with a mean difference of 0.24 mm (95% CI, 0.01–0.48; *P* = 0.04) and a greater femoral IMT with a mean difference of 0.46 mm (95% CI, 0.13–0.78; *P* = 0.008). In women, femoral IMT was shown to be significantly greater in FH women with a mean age-adjusted difference of 0.46 mm (95% CI, 0.19–0.74; *P* = 0.002) whereas carotid IMT failed to show any difference even after adjustment for age (mean age-

### Relationship between mutation type and IMT

FH men and women with LDL-R null alleles (nonsense mutations or mutations causing frameshift) tended to have thicker IMT than FH individuals with defective alleles (mis-sense mutations or splice-site mutations). However, the difference was only statistically significant for carotid IMT in women (Table 4).

There was a trend toward a smaller carotid IMT in men carrying Apo B-R3500Q compared to men carrying LDL-R mutations. These differences were not observed in women. LDL-cholesterol was, on average, the greatest in LDL-R null allele carriers and the lowest in R3500Q Apo B carriers. These various groups did not differ with regards to distribution of age, high-density lipoprotein cholesterol and triglycerides (data not shown).

### Discussion

An analysis of a cohort of patients with severe

**Table 4** Relationship of low-density lipoprotein (LDL) cholesterol or intima-media thickness (IMT) with the type of mutation causing familial hypercholesterolaemia (FH)

	LDL-R null allele carriers (Group A)	LDL-R defective allele carriers (Group B)	ApoB-R3500Q carriers (Group C)	<i>P</i> A vs. B	<i>P</i> C vs. A and B
<b>Men</b>					
<i>n</i>	21	35	7		
Carotid IMT	1.43 ± 0.55	1.23 ± 0.39	0.97 ± 0.37	0.10	0.08
Femoral IMT	1.30 ± 0.45	1.33 ± 0.57	1.09 ± 0.57	0.82	0.29
LDL cholesterol	346 ± 90	312 ± 68	260 ± 58	0.12	0.04
<b>Women</b>					
<i>n</i>	26	26	7		
Carotid IMT	1.15 ± 0.55	0.90 ± 0.27	1.19 ± 0.57	0.05	0.36
Femoral IMT	1.15 ± 0.55	0.96 ± 0.30	1.02 ± 0.60	0.15	0.97
LDL cholesterol	346 ± 89	288 ± 66	294 ± 88	0.02	0.52

hypercholesterolaemia and a family history of early cardiovascular diseases indicated that patients with genetically ascertained FH had a higher degree of atherosclerosis than non-FH patients in the carotid and femoral arteries.

We suggest several logical elements to explain this finding. First, FH patients probably experience an earlier and longer exposure to higher LDL-cholesterol concentrations than non-FH patients like those presented in this study. It is known that, in FH subjects, high LDL-cholesterol is already present at birth whereas it only expresses itself later at adulthood in other lipid (even though genetic) disorders. As a matter of fact, the cholesterol-years score, a measure of the lifetime cholesterol levels evaluated by the product of age with cholesterol level is considered as the parameter best correlated to IMT and CVD occurrence in FH subjects [6–8,24]. Second, FH promotes increased plasma lipoprotein (a) concentrations, and this has been linked to the progression of atherosclerotic lesions, i.e. intima-media thickening in carotid and femoral arteries and premature cardiovascular events [25]. Third, in known FH families, severe hypercholesterolaemia almost automatically designates the patient as FH, and thus signifies a very high cardiovascular risk for the patient. In contrast, in non-FH families, hypercholesterolaemia may not be the best predictor of cardiovascular disease. Indeed, in these families, the lipid factors may not be the main contributor to CVD as in FH. Rather, CVD may only occur in the peculiar individuals who cumulated multiple genetic and environmental factors that affect not only lipid metabolism but also other processes such as haemostasis, glucose metabolism, endothelium function etc, and that only produce early CVD by the fact of interactions.

The current study underpins and reinforces the idea that the precise identification of FH, preferably by molecular DNA diagnosis, may be very useful in proper cardiovascular risk assessment, and especially in primary prevention. Several previous studies have shown greater carotid IMT [6–10], femoral IMT [10] in FH patients as compared with normolipidaemic controls studies. However, the crucial question, before applying genetic tests in clinical practice is whether genetic testing for FH diagnosis could help determine the patients with greater CVD risk among clinically indistinguishable patients. To the best of our knowledge, this study is the first to compare FH patients with hypercholesterolaemic patients likely to be confounded in routine practice. Remarkably, the group of non-FH patients we selected accumulated more risk factors than the FH patients, i.e. higher values of body-mass index, waist-to-hip ratio, glycaemia, blood pressure, triglycerides and heart rate, as well as higher prevalence of smoking, hypertension, diabetes and obesity. In routine practice, these risk factors are readily assessed as opposed to the genetic status and the accumulation of risk factors in a patient is often experienced as a greater concern for most physicians than the possible genetic nature of the lipid disorder. Therefore, in clinical practice more attention could be given to non-FH patients, with more risk factors,

than to FH patients whose genetic status is generally unknown. Nevertheless, as is demonstrated by our results, atherosclerosis, defined by the thickening of carotid and femoral IMT, is far more severe in FH than in non-FH patients. Given the association between the intima-media thickening and the occurrence of clinical events demonstrated in the general population [11] as well as in the FH population [6,14], this may suggest that, among patients clinically indistinguishable with regard to cholesterol level and familial history of early coronary heart disease (CHD), FH patients could have a greater risk for CHD than non-FH patients. Such a conclusion should be confirmed by a study designed to compare clinical endpoints between two such groups. Nevertheless, given the fact that it becomes unethical to leave patients suspected of being at high risk (with or without FH) without treatment, such a study may appear hard to perform today and comparison between groups of individuals at high risk would have to rely on very early signs of atherosclerosis, such as IMT, discovered at the first visit and before any treatment is prescribed.

Very few papers have addressed the influence of the nature of the mutation causing FH on the degree of atherosclerosis measured by IMT. In our study, carriers of null alleles (nonsense mutations or mutations causing frameshift) tended to have greater IMT in comparison to the patients carrying a defective allele (mis-sense mutation or mutation located at the splicing site). In a recent study [8], Tonsstad *et al.* also found variation in IMT: FH subjects with a class 2B mutation (considered as defective alleles) had a tendency towards a decreased common carotid artery IMT in comparison to subjects with mutations belonging to the other classes (mostly null alleles). The observation of an effect of the type of mutation on IMT are consistent with the findings of Vohl [26] and Gaudet [27] who have compared the effect of two LDL-R class mutations on CHD. In these studies, coronary artery disease (CAD) event was an earlier event [26,27] in the null allele carriers than in the defective allele carriers. It was also found that the mean number of diseased vessels with > 50% stenosis was higher in null allele carriers than defective allele carriers [26]. In our study, the number of individuals carrying the R3500Q Apo B mutation (Familial defective apolipoprotein) was too small to clearly assess the difference of IMT in comparison with FH individuals carrying LDL-R mutations. However the tendency towards a smaller carotid IMT in men carrying Apo B-R3500Q compared to those who were LDL-R mutation carriers is in agreement with the previous observations that Apo B-R3500Q carriers have a lower risk of CVD than do LDL-R mutation carriers [28,29].

Although all previous studies about IMT in FH aggregated the data of both genders in their analysis, it might be important to separate men and women as suggested by the data of our present study. For instance, the difference between FH and non-FH subjects was more marked in men than women in the carotid arteries whereas the difference was similar in

both genders in the femoral arteries. Such an observation may suggest that the interactions between associated risk factors and the FH status may vary not only as a function of gender but also as a function of the anatomical site. A previous study [12] has already shown some difference regarding atherosclerosis between femoral and carotid arteries. In this study, Wittehoek *et al.* compared carotid and femoral IMT in FH patients with or without CVD and observed that the largest absolute difference in IMT between these groups was in the femoral artery.

Some of the methodological issues require additional comments.

First, as the patients were recruited from our lipid clinic which was originally designed for primary prevention, this cohort may not include the patients with the most advanced atherosclerotic disease already complicated by clinical events. Thickening of the intima-media is assumed to be a continuous process with a threshold above which the probability of clinical complications increases abruptly. So by excluding symptomatic patients, the current cohort of patients may not represent all stages of the natural evolution of the atherosclerotic process. Nevertheless, unless clinical events are not related to atherosclerosis, which is in contradiction with the actual theory, such a selection bias is likely to occur equally in FH and non-FH patients. Therefore, the differences observed between our groups must be close to the difference that would have been observed in the entire (i.e. symptomatic and asymptomatic) populations of FH and non-FH patients.

Second, the implication of genes other than LDL-R or of mutations in the LDL-R gene that escaped detection by current methods may have misclassified some FH patients as non-FH patients. Nevertheless, given our result, the presence of FH patients amongst the non-FH cohort would have contributed to reducing the differences observed between the two groups. Therefore, it may be anticipated that the actual differences are greater than reported here.

In summary, the present study demonstrates that, among patients with severe hypercholesterolaemia and a family history of early cardiovascular disease, those with FH have greater IMT in the carotid and femoral arteries than those without FH. Given the fact that greater IMTs have been associated with a greater occurrence of CHD events, we suggest that molecular genetic identification of FH may be of great interest in the evaluation of CHD risk in these patients.

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