Nutrition, Metabolism & Cardiovascular Diseases (2019) **29**, 1068–1076

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Polyvascular subclinical atherosclerosis in familial hypercholesterolemia: The role of cholesterol burden and gender

Alessandro Mattina [a,b,*], Antonina Giammanco [a], Philippe Giral [c,d], David Rosenbaum [c,d,g], Alain Carrié [d,e], Philippe Cluzel [d,f,g], Alban Redheuil [d,f,g], Randa Bittar [h], Sophie Béliard [i,j], Davide Noto [a], Angelo Quartarone [b], Maurizio Averna [a], Éric Bruckert [c,d], Antonio Gallo [c,d,g]

[a] *Department of Biomedicine, Internal Medicine and Medical Specialties, Division of Internal Medicine and Metabolic diseases, University of Palermo, Via del Vespro, 129, 90127 Palermo, Italy*
[b] *IRCCS Centro Neurolesi "Bonibo-Pulejo", Via Provinciale Palermo, Contrada Casazza, 95124 Messina, Italy*
[c] *Cardiovascular Prevention Unit, Endocrinology and Metabolism Service, Assistance Publique Hôpitaux de Paris, Pitié-Salpêtrière Hospital, Paris, France*
[d] *Sorbonne Universite, Inserm, Institute of Cardiometabolism and Nutrition (ICAN), UMR_S1166, Hôpital de la Pitié, Paris, France*
[e] *Assistance publique–Hôpitaux de Paris (APHP), Hôpitaux Universitaires Pitié-Salpêtrière/Charles-Foix, Department of Biochemistry for Endocrinology and Oncology, Obesity and Dyslipidemia Genetics Unit, Paris, France*
[f] *Département d'imagerie cardiovasculaire et de radiologie interventionnelle, Pôle Imagerie – Groupe Hospitalier Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France*
[g] *Imaging Core Lab, Institute of Cardiometabolism and Nutrition, ICAN, Paris, France*
[h] *Biochemistry functional Unit for Metabolic Disease Assistance Publique/Hôpitaux de Paris, Groupe Hospitalier Pitié-Salpêtrière, Paris, France*
[i] *C2VN, INRA, INSERM, Aix-Marseille Univ, Marseille, France*
[j] *Endocrinology Department, APHM, Marseille, France*

Received 8 February 2019; received in revised form 7 June 2019; accepted 17 June 2019
Handling Editor: F. Galletti
Available online 3 July 2019

KEYWORDS
Familial hyper cholesterolemia;
Cholesterol burden;
Calcium score;
Coronary artery calcium;
Atherosclerosis;
Cardiovascular disease;
Cardiovascular risk;
Peripheral arterial atherosclerosis

Abstract *Background and aim:* Heterozygous familial hypercholesterolemia (HeFH) is a genetic disease characterized by a heterogeneous phenotype. The assessment of cardiovascular (CV) risk is challenging for HeFH. Cholesterol burden (CB) allows to estimate the lifelong exposure to high levels of cholesterol. The aim of this study was to analyze the distribution of subclinical atherosclerosis and the relationship between atherosclerosis and the CB in a sample of HeFH patients, focusing on sex-related differences.
Methods and Results: 154 asymptomatic HeFH subjects underwent coronary-artery-calcium score (CACs) and Doppler ultrasound of carotid and femoral arteries. Yearly lipid profiles and HeHF history were obtained from patients' files in order to calculate total CB. Atherosclerotic burden was defined by the presence of CACs > 0 or by the presence of carotid or femoral plaque. Study population was stratified according to gender. The prevalence of CAC, carotid and femoral atherosclerosis was of 62%, 55% and 56%, respectively. Coronary district was the least involved in women, who had a higher prevalence in carotid atherosclerosis. When two vascular districts were affected, women had an increased prevalence of femoral and carotid atherosclerosis whereas men had a higher prevalence of coronary and femoral atherosclerosis. CB correlated to the presence of atherosclerosis in any of the three vascular districts with a significant increasing trend depending on the number of affected areas.

* Corresponding author. Department of Biomedicine, Internal Medicine and Medical Specialties, Division of Internal Medicine and Metabolic diseases. University of Palermo, Via del Vespro, 129, 90127 Palermo, Italy.
E-mail address: alessandromattina@gmail.com (A. Mattina).

https://doi.org/10.1016/j.numecd.2019.06.015

Conclusions: A polyvascular atherosclerotic burden is found in asymptomatic HeFH patients. Gender differences in the territory distribution were observed. The early and lasting exposure to high cholesterol, as expressed by CB, is a major determinant of atherosclerotic burden.

Background

Familial hypercholesterolemia (FH, OMIM #143890) is an autosomal dominant genetic disease characterized by mutations in the genes codifying for the low-density lipoprotein receptor (*LDLR*), the apolipoprotein B (*APOB*), and the pro-protein convertase subtilisin/kexin 9 (*PCSK9*) even though other genes have recently been identified to be candidates for this inherited disease. The clinical phenotype of the heterozygous form (HeFH) is heterogeneous [1] and the prevalence may be as high as 1 in 200 to 1 in 500 people [2]. The assessment of cardiovascular (CV) risk in HeFH patients is challenging. The traditional risk calculators (such as European Society of Cardiology's Heart Score [3] or the Framingham Risk Score [4]) are often not appropriate for HeFH subjects because such individuals are at considerably high risk due to lifelong elevated LDL cholesterol levels.

Some authors have proposed various methods for the calculation of the cumulative cholesterol burden (CB) as an estimation of the lifelong exposure to high plasma levels of cholesterol. CB has been associated with the presence of carotid atherosclerosis and carotid intima-media thickness (c-IMT) [5,6], arterial stiffness [7] and coronary and aortic calcifications [8] in small size cohorts of HeFH patients. To our knowledge it is not known whether there is a sex-dependent involvement of a certain vascular district or not.

The aim of this study is to report the prevalence and severity of subclinical atherosclerosis in three different vascular districts (carotid, femoral and coronary), focusing on sex-related differences, and to study the relationship between atherosclerotic burden and the cumulative cholesterol burden in a sample of primary prevention HeFH patients.

Methods

Study population

Patients were recruited consecutively between May 2015 and May 2016 at the Cardiovascular Prevention Unit of the Pitié-Salpêtrière Hospital in Paris, France.

Inclusion criteria were: patients with genetically confirmed HeFH, aged between 20 and 70 years, in regular follow-up since the time of diagnosis, without symptoms or electrocardiographic signs of ischemia. The exclusion criteria were: denial of informed consent, contra-indication to computed tomography (CT), personal history of cardiovascular disease or myocardial infarction, diabetes mellitus, uncontrolled hypertension or triglycerides (TG) $>$ 4.52 mmol/L. Informed consent was obtained from all included patients.

For the description of the population and the distribution of atherosclerosis, patients were stratified by gender.

Evaluation of peripheral atherosclerosis

Ultrasonography measurements of the carotid and femoral arteries were performed using an ACUSON Sequoia ultrasound machine with an 8 MHz transducer. Carotid measurements were performed on the common carotid, the carotid bulb, and the proximal tract of the internal carotid. Three different longitudinal sections of both right and left carotid arteries and cross sections for plaque evaluation were acquired. Plaque was defined as: thickness of the c-IMT $>$ 1.5 mm, irregular shape (protrusion in the lumen and loss of alignment with the border of the adjacent arterial wall), alteration of the wall structure (more echoes than neighboring areas) [9]. C-IMT was measured on an area with no plaques on the common carotid and the mean c-IMT between the right and left c-IMT was calculated for each subject. The percentage of stenosis was assessed using the European Carotid Surgery Trial (ECST) method. Femoral measurements were performed on the common femoral artery and in the proximal sections of superficial and deep femoral artery. Femoral IMT (f-IMT) was measured on the common femoral artery at 1 cm proximal to superficial and deep femoral division. The same criteria used for carotids were used for the identification of femoral plaques and the evaluation of the stenosis percentage. Presence of peripheral atherosclerosis was defined as the presence of carotid/femoral plaque. The maximum stenosis percentage in one of the two carotid or femoral districts, for an overall estimate of total peripheral atherosclerotic burden, was also considered as an additional parameter.

Evaluation of central atherosclerosis

Calcium score was used to detect coronary atherosclerosis, as previously reported [8]. Each patient underwent multidetector CT (Definition Flash, Siemens®) with a total radiation exposure of approximately 0.4 mSv. Imaging of the coronary arteries was performed without contrast medium by ultra-fast high-resolution volumetric tomographic acquisition with 100 ms (radiogenic tube) rotation time, 3 mm layer thickness and 3–5-s apnea. The CAC was quantified on the entire epicardial coronary system using the Agatston scoring method [10] with semi-automated software. Coronary calcium was defined as the presence of a lesion above a 130 Hounsfield Units, with a surface of 3 or more adjacent pixels (at least 1 mm^2). The CAC was

calculated by multiplying the area of calcification (mm^2) by its maximum attenuation value, with the CAC score for each coronary equal to the sum of CAC of all lesions found in the artery. The total CAC score was calculated by summing CAC from the left main, left anterior descending, left circumflex, and right coronary arteries. A radiologist read all CT scans at a central reading center.

The presence of coronary atherosclerosis was defined as CACS > 0.

Calculation of cholesterol burden

Lifelong cholesterol exposure was estimated by calculation of Total Cholesterol Burden (TCB) (mmol-years/L) according to Schmidt et al. [11]. TCB is the addition of cholesterol burden at diagnosis (dCB) and post-diagnosis cholesterol burden (pdCB). dCB was obtained by multiplying the initial serum TC value by the age of the patient at diagnosis. pdCB was calculated by adding the TC values annually measured during follow-up using patients' medical records.

Evaluation of risk factors

The Body Mass Index (BMI) was measured as weight (kg) divided by height (m^2). Arterial hypertension was defined as systolic blood pressure (PAS) $\geq$ 140 mmHg, diastolic blood pressure (PAD) $\geq$ 90 mmHg and/or the use of antihypertensive drugs. The status of active smoker was defined by having smoked at least one cigarette in the last 30 days.

Statistical analysis

Data are reported as mean $\pm$ SD for continuous parametric and median (interquartile range) for continuous non-parametric variables, and as frequency (percentage) for categorical variables. Normality of continuous variables distribution was tested using Shapiro–Wilk's test. Differences between groups were evaluated by ANOVA. Distribution of categorical variables between groups was evaluated using the χ^2 test. The association between Total Cholesterol Burden (TCB) and coronary, carotid and femoral atherosclerosis was examined in a multivariate regression analysis including sex (male $= 1$), smoking status (current smoking $= 1$), SBP, Fasting Plasma Glucose (FPG), High-density Lipoprotein-cholesterol (HDL-C), triglycerides, and BMI. According to Schmidt et al. [11] the cubic root of CAC score was used for parametrical tests. Statistical analyses were performed using SAS® software and a p-value < 0.05 was considered significant.

Results

Distribution of subclinical atherosclerosis

Table 1 shows the main characteristics of the whole sample ($n = 154$) and the population stratified according to gender. Men showed higher TC and HDL-C levels and were more treated with hypolipidemic treatment while Lp(a) was found to be higher in women. Cholesterol burden at diagnosis and total CB did not differ between men and women, who were also less treated with lipid-lowering drugs. Forty-two patients (27%) were smokers and 19 (14%) had arterial hypertension. The coronary calcium score was significantly higher in men. No differences in peripheral vascular parameters were found between men and women, except for the highest average carotid c-IMT in men. When adjusted for all the variables that differed between men and women, only CACS remained significantly different between genders.

The prevalence of CAC, carotid and femoral atherosclerosis was of 62%, 55% and 56%, respectively. No differences were found between men and women, despite a trend in a higher prevalence of coronary atherosclerosis in men and carotid atherosclerosis in women (Table 1). Approximately 17% of the entire sample showed no atherosclerosis in any district whereas 33% had all three districts explored involved (Fig. 1). When stratified according to sex, 85% of women and 82% of men presented overall subclinical atherosclerosis.

In the whole population the coronary district was the most affected, even when two vascular districts were involved (Fig. 2). When stratified according to gender, however, the coronary district was the least involved in women, who had a higher prevalence in carotid atherosclerosis. Interestingly, when two vascular districts were affected, women had an increased prevalence of both femoral and carotid atherosclerosis whereas men had a higher prevalence of coronary and femoral district involvement.

The presence of subclinical atherosclerosis in the three vascular districts was strongly correlated with age (Table 2). CB and no other measured lipid parameters identified patients with coronary, femoral or carotid atherosclerosis, who were also diagnosed with FH at a later age. Only Lp(a) was slightly higher in those with femoral vascular involvement. The presence of xanthomas was associated with a higher prevalence of carotid and coronary vascular involvement.

An increased prevalence of CAC was found in statin treated patients, whereas patients treated with ezetimibe had more atherosclerosis in all three vascular districts. Patients with hypertension also exhibited an increased prevalence of CAC.

Cholesterol burden and atherosclerotic burden

Total CB was significantly associated with CACS, femoral and carotid IMT as well as with femoral and carotid plaques (Table 3). Total CB was confirmed as an independent predictor of coronary, carotid and femoral atherosclerotic burden (after adjusting for SBP, sex, FPG, HDL-C, triglycerides, smoking status and BMI (Table 4)). Interestingly, a higher number of vascular districts affected by atherosclerosis were associated with an increased TCB (p for trend < 0.0001, Fig. 3).

Table 1 Main characteristics of whole sample.

	Total	Women	Men
n	154	84	70
Age, years	48.3 ± 13.8	49.8 ± 13.7	46.5 ± 12.8
BMI, kg/m^2	24.8 ± 5.0	24.1 ± 4.9	25.6 ± 5.1
Cardiovascular risk factors			
Smoke, *n* (%)	42 (27)	19 (23)	23 (33)
Obesity, *n* (%)	17 (11)	11(13)	6 (9)
Arterial hypertension, *n* (%)	19 (12)	10 (12)	9 (13)
SBP, mmHg	113.7 ± 11.9	110.7 ± 12.9	117.2 ± 9.5‡
DBP, mmHg	65.9 ± 10.2	64.5 ± 11.3	67.6 ± 8.6
Lipid profile			
TC, mmol/L	6.52 ± 1.75	6.09 ± 1.55	6.88 ± 1.84†
TG, mmol/L	1.18 ± 0.71	1.25 ± 0.60	1.13 ± 0.80
HDL-C, mmol/L	1.46 ± 0.52	1.26 ± 0.37	1.62 ± 0.57‡
LDL-C, mmol/L	4.55 ± 1.67	4.26 ± 1.47	4.78 ± 1.80
Lp(a), mg/dl	27 (10–54)	33 (14–60)	21 (10–44)*
Lipid-lowering treatment			
Overall, *n* (%)	123 (80)	58 (69)	65 (93)‡
Statins, *n* (%)	120 (78)	56 (67)	64 (91)‡
Ezetimibe, *n* (%)	52 (34)	20 (24)	32 (46)†
Familial hypercholesterolemia			
Age of diagnosis, years	21.8 ± 12.5	22.9 ± 12.5	20.6 ± 12.5
Xanthomas, *n* (%)	36 (23)	17 (20)	19 (27)
Cholesterol burden			
CB at diagnosis, mmol-years/L	209.4 ± 126.4	213.9 ± 114.2	204.0 ± 140.1
CB post-diagnosis, mmol-years/L	191.2 ± 89.3	199.2 ± 92.8	181.6 ± 84.6
Total CB, mmol-years/L	400.6 ± 137.2	413.2 ± 128.2	385.5 ± 146.8
Coronary atherosclerosis			
CAC score	16 (0–163)	7.5 (0–70)	54.4 (0–348)‡$
CACS = 0, *n* (%)	59 (38)	38 (45)	31 (30)
CACS ≥ 1, *n* (%)	95 (62)	46 (55)	49 (70)
Carotid atherosclerosis			
Mean c-IMT, mm	0.62 ± 0.16	0.59 ± 0.02	0.65 ± 0.02*
No plaque, *n* (%)	69 (45)	33 (39)	36 (51)
Presence of plaque, *n* (%)	85 (55)	51 (61)	34 (49)
Maximum stenosis, %	5 (0–15)	10 (0–15)	0 (0–11)
Femoral atherosclerosis			
Mean f-IMT, mm	0.54 (0.22)	0.56 ± 0.20	0.61 ± 0.23
No plaque, *n* (%)	67 (44)	37 (44)	30 (43)
Presence of plaque, *n* (%)	87 (56)	47 (56)	40 (57)
Maximum stenosis, %	10 (0–20)	10 (0–20)	10 (0–20)

BMI, body mass index; CAC, coronary artery calcium; CACS, coronary artery calcium score; c-IMT, carotid intima-media thickness; f-IMT, femoral intima-media thickness; CB, cholesterol burden; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Lp(a), Lipoprotein(a). *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$ women vs. men. $$p$ 0.0011 women vs. men adjusted for SBP, TC, HDL-C and lipid-lowering treatment.

Discussion

In this study, we explored subclinical atherosclerosis in three different vascular districts in a sample of patients with clinically and genetically determined familial hypercholesterolemia using non-invasive methods. The vast majority of patients (83%) had at least one territory involved. These results confirm the severity of atherosclerotic disease in asymptomatic HeFH patients. Independently from the number of vascular districts involved, the coronary district was the most affected in the whole population. However, only 11% of patients had coronary involvement without any carotid or femoral atherosclerotic lesion.

The CAC has been shown to correlate with the overall extent of coronary heart disease identified by the presence of coronary artery stenosis on autopsied subjects [12]. Asymptomatic individuals with a higher CAC have a higher risk of myocardial infarction and sudden cardiac death than subjects without CAC [13,14]. In a study by He et al. [15], 411 out of 3895 patients underwent both CAC score (CACS) and stress myocardial perfusion tomography (SPECT): CACS predicted an abnormal SPECT in an age and sex-independent fashion. Interestingly, none of the patients without CAC had an abnormal SPECT. This work, together with other prospective studies on the incidence of cardiovascular events in asymptomatic patients [16,17], allowed to establish the role of CACS in better stratifying CV risk. Coronary calcium is strongly associated with the incidence of cardiovascular events [13]. In the CARDYA study (Coronary Artery Risk Development in Young Adults) the incidence of CACS > 0 in young adults (mean age of 40 years), was 9.9% [18]. We previously reported an increased prevalence of CAC in HeFH patients, this being particularly

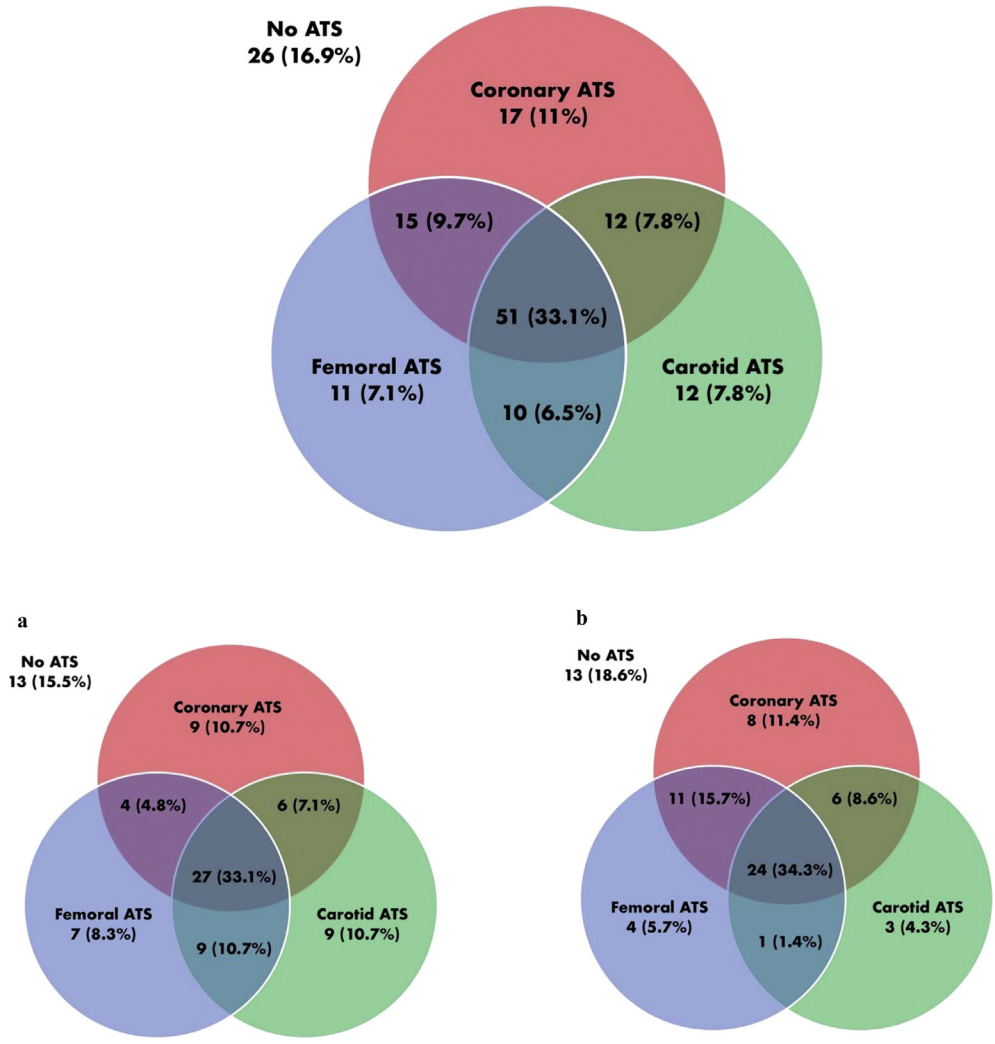

Figure 1 Distribution of atherosclerosis in study population. The red round represents the coronary territory, the purple and the green rounds represent the femoral and carotid vascular districts, respectively. The presence of 2 or 3 overlapping rounds suggests the involvement of 2 or 3 vascular districts. a. Distribution of subclinical atherosclerosis in women ($n = 84$). b. Distribution of subclinical atherosclerosis in men ($n = 70$). ATS = atherosclerosis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

increased in young subjects aged from 20 to 45 years old [8]. The early appearance of calcified plaques in FH patients can be partially explained by the potential role of LDL-receptor (LDL-R) mutation in directly determining a higher arterial inflammation [19] and a greater calcium deposition in the vascular wall of young subjects [20]. On the other hand, the association found between CACS and CB suggests the main role played by the early and lasting exposure to high plasmatic levels of cholesterol.

If overall subclinical atherosclerosis did not differ between men and women, sex-related differences were found in the distribution of atherosclerosis: when atherosclerosis was present simultaneously in two vascular districts, men showed a higher prevalence of coronary involvement than women. Although the distribution of atherosclerosis has never been investigated according to the number of districts explored, these results are in line with the data already published in the literature [21]. Interestingly, women had an increased involvement of the carotid district. Few studies have explored the role of sex in determining the risk of carotid atherosclerosis. A study conducted on 154 HeFH patients (40.9% men) found that, among women, HDL-c and Apo-A1 levels were associated with a decreased prevalence of carotid plaque [22] whereas in men ApoB levels were directly related to carotid atherosclerosis. Interestingly in our cohort, HDL-C levels were higher in men than in women; despite a role of hypolipidemic treatment cannot be excluded, this might in part explain the higher prevalence of carotid plaque in women than in men.

Multiple factors are involved in determining sex-specific vascular atherosclerosis in HeFH. The protective role of estrogens is considered the main cause of lower incidence of CV diseases in premenopausal women [23,24]. Indeed, estrogens have been shown to induce an increased activity of LDL receptor which corresponds to a reduction of TC and LDL-C levels [25,26]. However, apart from the role of estrogens, already in childhood HeFH girls have increased TC

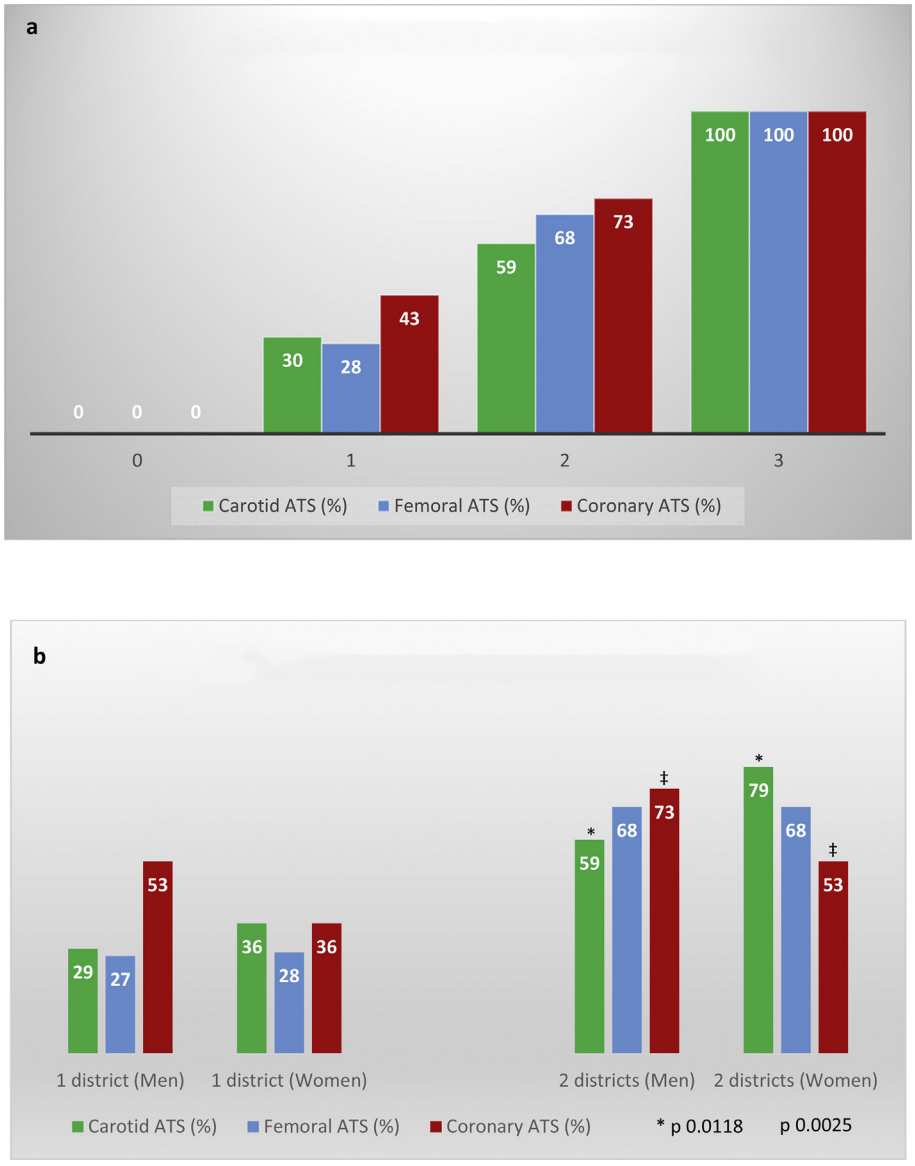

Figure 2 a. Subclinical atherosclerosis distribution depending on the number of involved districts. b. Subclinical atherosclerosis distribution: differences between men and women when 1 or 2 districts are considered. ATS = atherosclerosis.

levels compared to boys, independently from age or LDL-R mutation [27]. Also, the electronegativity of LDL particles may play a role in determining a more prevalent involvement of coronary arteries in men [28].

The assessment of other vascular districts, such as the femoral arteries, to improve the predictive capacity of traditional risk factors is still not much studied. In a post-mortem study in the Netherlands, the femoral arteries were most frequently affected by atherosclerosis among 5 peripheral vascular sites, including common carotid artery [29]. Recently the PESA (Progression of Early Subclinical Atherosclerosis) study showed a higher prevalence of femoral plaques compared to carotid plaques in an asymptomatic cohort of middle-aged men and women [30]. Furthermore, the Aragon Workers's Health Study (AWHS) showed a greater association between coronary calcium and the presence of femoral plaques in 1423 asymptomatic men of 40 and 59 years old [31].

The high total CB and CB at diagnosis correlated significantly with the presence of subclinical atherosclerosis in any studied district, with an increasing trend depending on the number of districts affected. Total CB was positively associated with CACS and both carotid and femoral atherosclerosis. These results reinforce the concept of the association between an early and lasting exposure to high levels of cholesterol and premature atherosclerosis in FH patients. Furthermore, the diagnosis CB is an easily calculable index and may be more useful than the single data on total cholesterol in the identification of high-risk FH patients in whom an

Table 2 Vascular distribution of subclinical atherosclerosis in the whole sample.

	No carotid ATS	Presence of carotid ATS	*p* Value	No femoral ATS	Presence of femoral ATS	*p* Value	No coronary calcium	Presence of coronary calcium	*p* Value
n	69	85	–	67	87	–	59	95	–
Sex, *n* women/*n* men	33/36	51/34	0.1313	37/30	47/40	0.8820	38/21	49/46	0.528
Age, years	41.6 ± 12.7	53.7 ± 11	<0.001	40.7 ± 1.4	54.1 ± 11	<0.001	41.3 ± 1.6	52.6 ± 11.4	<0.001
BMI, kg/m^2	24.4 ± 5.8	25 ± 4.4	0.4465	24.7 ± 5.8	24.8 ± 4.4	0.8746	24.2 ± 4.5	25.1 ± 5.3	0.2716
Cholesterol burden									
CB at diagnosis, mmol-years/L	157.6 ± 104.6	251.4 ± 127.4	<0.001	170.2 ± 116.0	239.6 ± 126.4	<0.001	157.4 ± 112.8	241.7 ± 124.1	<0.001
CB post-diagnosis, mmol-years/L	176.8 ± 90.5	202.9 ± 87.1	0.0715	159.3 ± 86.4	215.8 ± 84.0	<0.001	159.1 ± 81.0	211.1 ± 88.8	<0.001
Total CB, mmol-years/L	334.4 ± 121.4	454.3 ± 125.8	<0.001	329.5 ± 122.1	455.4 ± 122.7	<0.001	316.4 ± 117.7	452.9 ± 122.0	<0.001
Familial hypercholesterolemia									
Age of diagnosis, years	17.3 ± 11.5	25.5 ± 12.1	<0.001	18.6 ± 12	24.3 ± 12.4	0.004	18.4 ± 13.4	23.9 ± 11.5	0.007
Xanthomas, *n* (%)	11 (15.9)	25 (29.4)	0.0495	11 (16.4)	25 (28.7)	0.0734	7 (11.9)	29 (30.5)	0.0078
Cardiovascular risk factors									
Smokers, *n* (%)	21 (30.4)	21 (24.7)	0.4273	14 (20.9)	28 (32.2)	0.049	15 (25.4)	27 (28.4)	0.6847
Arterial hypertension, *n* (%)	5 (8.2)	14 (17.8)	0.1028	5 (8.1)	14 (18)	0.089	2 (3.9)	17 (19.1)	0.0116
Lipid profile									
TC, mmol/L	6.49 ± 1.84	6.54 ± 1.71	0.0559	6.49 ± 1.81	6.57 ± 1.73	0.8757	6.18 ± 1.53	6.72 ± 1.86	0.0631
TG, mmol/L	1.16 ± 0.86	1.20 ± 0.57	0.7299	1.23 ± 0.92	1.14 ± 0.51	0.4464	1.07 ± 0.63	1.25 ± 0.75	0.1247
HDL-C, mmol/L	1.40 ± 0.59	1.50 ± 0.47	0.2718	1.42 ± 0.49	1.53 ± 0.52	0.0862	1.40 ± 0.54	1.50 ± 0.49	0.2544
LDL-C, mmol/L	4.60 ± 1.71	4.50 ± 1.66	0.7227	4.55 ± 1.66	4.55 ± 1.71	0.9832	4.32 ± 1.40	4.71 ± 1.81	0.165
Lp(a), mg/dl	23 (10–46)	31 (10–61)	0.0832	20 (10–48)	33 (14–61)	0.0187	25 (12–74)	27 (10–59)	0.4287
Lipid-lowering treatment									
Overall, *n* (%)	54 (78)	69 (81)	0.6536	49 (73)	74 (85)	0.0673	42 (71)	81 (85)	0.0342
Statins, *n* (%)	53 (77)	67 (79)	0.7647	49 (73)	71 (82)	0.2087	41 (70)	79 (83)	0.0468
Ezetimibe, *n* (%)	16 (24)	36 (42)	0.02	16 (24)	36 (41)	0.02	10 (17)	42 (45)	<0.001

ATS, atherosclerosis; BMI, body mass index; CB, cholesterol burden; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Lp(a), Lipoprotein(a).

Table 3 Correlation between total cholesterol burden and coronary, carotid and/or femoral atherosclerotic burden.

	Total CB					
	Total		Men		Women	
	r	*p*	*r*	*p*	*r*	*p*
CACS	0.52	*<0.0001*	0.66	*<0.0001*	0.44	*<0.0001*
Mean carotid IMT, mm	0.50	*<0.0001*	0.46	*<0.0001*	0.60	*<0.0001*
Mean femoral IMT, mm	0.25	*0.0226*	0.28	0.0772	0.25	0.0926
Carotid maximum percentage of stenosis, %	0.52	*<0.0001*	0.57	*<0.0001*	0.49	*<0.0001*
Femoral maximum percentage of stenosis, %	0.54	*<0.0001*	0.61	*<0.0001*	0.47	*<0.0001*
Carotid or femoral maximum percentage of stenosis, %	0.61	*<0.0001*	0.68	*<0.0001*	0.56	*<0.0001*

CB, cholesterol burden; CACS, coronary artery calcium score; IMT, intima-media thickness.

earlier beginning of a lipid-lowering treatment becomes necessary. Interestingly, while in men the strongest correlation was found between CB and CACs, in women c-IMT had the strongest correlation with CB, confirming a possible sex-specific impact of vascular district involvement in HeFH.

This study shows some limitations. It is a cross-sectional study of a numerically limited sample. However, accurate atherosclerotic phenotyping and calculation of cholesterol burden were performed. Coronary angiographic CT scan that could have detected non-calcified hypodense plaques was not performed. This procedure requires the injection of contrast medium and a higher radiation exposure. Furthermore, we did not compare HeFH patients with a control population. However, we used the age and sex adjusted reference values from the MESA study showing that a CACs greater than zero, exceeding the 75th percentile in younger patients, indicates a high cardiovascular risk [21].

FH is still underdiagnosed. Starting a drug treatment at the age of 8–10 may postpone the presentation of a cardiovascular event of about 10 years [32]. Although current

Table 4 Association between total cholesterol burden and coronary, carotid and femoral atherosclerosis examined in a multivariate regression analysis including other cardiovascular risk factors.

	Coronary calcium			Carotid ATS			Femoral ATS		
	R^2	β	p	R^2	β	p	R^2	β	p
	0.29			0.18			0.25		
Total CB, mmol-years/L		0.0130	*<0.0001*		0.008	*<0.0001*		0.0116	*<0.0001*
Sex, 1 = male		1.6130	*0.0023*		0.6448	0.1408		0.6744	0.1562
Smoking habits, 1 = yes		0.3539	0.4478		0.1234	0.7640		0.8345	0.0583
SBP, mmHg		0.0052	0.8097		0.008	0.6532		0.0327	0.0965
FPG, mmol/L		0.8755	*0.0496*		0.6561	0.1056		0.4753	0.2483
HDL, mmol/L		0.4203	0.4083		0.4611	0.2968		0.1538	0.7536
TG, mmol/L		0.8755	0.0496		0.2874	0.2901		0.6137	0.0858
BMI, kg/m^2		0.0224	0.6508		0.0286	0.5059		0.0170	0.7057

ATS, atherosclerosis; CB, cholesterol burden; SBP, systolic blood pressure; FPG, fasting plasma glucose; HDL, high density lipoprotein; TG, triglycerides; BMI, body mass index.

Figure 3 Cholesterol burden (mmol-years/L) as a function of the number of vascular districts affected by atherosclerosis.

guidelines do not recommend the evaluation of CAC in young patients, our data suggest that this evaluation may be necessary for the identification of coronary atherosclerosis in FH patients and consequently a fundamental support for adopting appropriate therapeutic choices. Data from the SAFEHEART support this evidence: 2752 FH patients from the Spanish registry showed a greater prevalence of coronary artery disease than cerebrovascular events and peripheral arterial disease (11.8%, 1.8%, 1.4% respectively) [33].

Conclusions

Heterozygous familial hypercholesterolemia is associated with early atherosclerotic disease in the absence of any clinical signs of coronary or peripheral vascular disease. Our study showed a simultaneous involvement of coronary, femoral and carotid vascular districts in one third of the study population, with the highest prevalence of coronary atherosclerotic burden.

This suggests that the coronary CT without contrast medium for the evaluation of coronary calcium in patients with familial hypercholesterolemia may be useful in the identification of subject at higher CV risk and consequently an important support in therapeutic choices. A different involvement in carotid and coronary district was found according to gender. Mechanisms behind sex-specific phenotypic and biochemical presentation of HeFH should be further explored.

Cholesterol burden at diagnosis is an easy calculable index and may be more useful for the identification of high-risk FH patients in whom an earlier beginning of lipid-lowering treatment becomes necessary.

Acknowledgment

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Hovingh GK, Davidson MH, Kastelein JJP, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 2013;34(13):962–71.
[2] Benn M, Watts GF, Tybjærg-Hansen A, Nordestgaard BG. Mutations causative of familial hypercholesterolaemia: screening of 98 098 individuals from the Copenhagen General Population Study estimated a prevalence of 1 in 217. Eur Heart J 2016;37(17):1384–94.
[3] Conroy RM, Pyorala K, Fitzgerald AP. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 2003;24.
[4] Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97(18):1837–47.
[5] Tonstad S, Joakimsen O, Stensland-Bugge E, Ose L, Bønaa KH, Leren TP. Carotid intima-media thickness and plaque in patients with familial hypercholesterolaemia mutations and control subjects. Eur J Clin Investig 1998;28(12):971–9.
[6] Raal FJ, Pilcher GJ, Waisberg R, Buthelezi EP, Veller MG, Joffe BI. Bulk is the pivotal determinant of hypercholesterolemia. Am J Cardiol 1999;83:1330–1333.
[7] Cheng HM, Ye ZX, Chiou KR, Lin SJ, Charng MJ. Vascular stiffness in familial hypercholesterolaemia is associated with C-reactive protein and cholesterol burden. Eur J Clin Investig 2007;37(3):197–206.
[8] Gallo A, Giral P, Carrié A, Carreau V, Béliard S, Bittar R, et al. Early coronary calcifications are related to cholesterol burden in heterozygous familial hypercholesterolemia. J Clin Lipidol. June 2017;11(3):704–711.e2.
[9] Stein JH, Korcarz CE, Hurst RT. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of

Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascula. J Am Soc Echocardiogr 2008; 21.
[10] Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15(4): 827–32.
[11] Schmidt HHJ, Hill S, Makariou EV, Feuerstein IM, Dugi KA, Hoeg JM. Relation of cholesterol-year score to severity of calcific atherosclerosis and tissue deposition in homozygous familial hypercholesterolemia. Am J Cardiol 1996;77(8):575–80.
[12] Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, et al. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol 1998;31(1):126–33.
[13] Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 2008;358(13):1336–45.
[14] Becker A, Leber A, Becker C, Knez A. Predictive value of coronary calcifications for future cardiac events in asymptomatic individuals. Am Heart J 2008;155(1):154–60.
[15] He ZX, Hedrick TD, Pratt CM, Verani MS, Aquino V, Roberts R, et al. Severity of coronary artery calcification by electron beam computed tomography predicts silent myocardial ischemia. Circulation 2000;101(3):244–51.
[16] Raggi P, Callister TQ, Cooil B, He ZX, Lippolis NJ, Russo DJ, et al. Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. Circulation 2000;101(8):850–5.
[17] Hecht HS. Coronary artery calcium scanning: past, present, and future. J Am Coll Cardiol: Cardiovasc Imaging 2015;8(5):579–96.
[18] Okwuosa TM, Greenland P, Ning H, Liu K, Lloyd-Jones DM. Yield of screening for coronary artery calcium in early middle-age adults based on the 10-year Framingham Risk Score: the CARDIA Study. J Am Coll Cardiol: Cardiovasc Imaging 2012;5(9):923–30.
[19] Mattina A, Rosenbaum D, Bittar R, Bonnefont-Rousselot D, Noto D, Averna M, et al. Lipoprotein-associated phospholipase A_2 activity is increased in patients with definite familial hypercholesterolemia compared with other forms of hypercholesterolemia. Nutr Metab Cardiovasc Dis 2018;28:517–23.
[20] Pugliese G, Iacobini C, Fantauzzi CB, Menini S. The dark and bright side of atherosclerotic calcification. Atherosclerosis 2015;238(2): 220–30.
[21] McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). Circulation 2006;113(1):30–7.
[22] Waluś-Miarka M, Czarnecka D, Kloch-Badełek M, Wojciechowska W, Kapusta M, Malecki MT. Carotid artery plaques – are risk factors the same in men and women with familial hypercholesterolemia? Int J Cardiol 2017;244:290–5.
[23] Bairey Merz CN, Shaw LJ, Reis SE, Bittner V, Kelsey SF, Olson M, et al. Insights from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. Part II: gender differences in presentation, diagnosis, and outcome with regard to gender-based pathophysiology of atherosclerosis and macrovascular and microvascular cor. J Am Coll Cardiol 2006;47(3 suppl.).
[24] Polk DM, Naqvi TZ. Cardiovascular disease in women: sex differences in presentation, risk factors, and evaluation. Curr Cardiol Rep 2005;7(3):166–72.
[25] Ajit R, Srivastava K, Baumann D, Schonfeld G. In vivo regulation of low-density lipoprotein receptors by estrogen differs at the post-transcriptional level in rat and mouse. Eur J Biochem 1993.
[26] Inukai T, Takanashi K, Takebayashi K, Tayama K, Aso Y, Takiguchi Y, et al. Estrogen markedly increases LDL-receptor activity in hypercholesterolemic patients. J Med 2000;31(5–6):247–61.
[27] Holven KB, Narverud I, van Lennep JR, Versmissen J, Øyri LKL, Galema-Boers A, et al. Sex differences in cholesterol levels from birth to 19 years of age may lead to increased cholesterol burden in females with FH. J Clin Lipidol 2018;12(3):748–755.e2.
[28] Lee A-S, Chen W-Y, Chan H-C, Hsu J-F, Shen M-Y, Chang C-M, et al. Gender disparity in LDL-induced cardiovascular damage and the protective role of estrogens against electronegative LDL. Cardiovasc Diabetol 2014;13(1):64.
[29] Pasterkamp G, Schoneveld AH, Hillen B, Banga JD, Haudenschild CC, Borst C. Is plaque formation in the common carotid artery representative for plaque formation and luminal stenosis in other atherosclerotic peripheral arteries? A post mortem study. Atherosclerosis 1998;137(1):205–10.
[30] Fernández-Friera L, Peñalvo JL, Fernández-Ortiz A, Ibañez B, López-Melgar B, Laclaustra M, et al. Prevalence, vascular distribution, and multiterritorial extent of subclinical atherosclerosis in a middle-aged cohort. Circulation 2015;131(24):2104–13.
[31] Laclaustra M, Casasnovas JA, Fernández-Ortiz A, Fuster V, León-Latre M, Jiménez-Borreguero LJ, et al. Femoral and carotid subclinical atherosclerosis association with risk factors and coronary calcium: the AWHS study. J Am Coll Cardiol 2016;67(11): 1263–74.
[32] Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease. Eur Heart J 2013;34(45):3478–90.
[33] Pérez de Isla L, Alonso R, Mata N, Saltijeral A, Muñiz O, Rubio-Marin P, et al. Coronary heart disease, peripheral arterial disease, and stroke in familial hypercholesterolaemia: insights from the SAFEHEART registry (Spanish familial hypercholesterolaemia cohort study). Arterioscler Thromb Vasc Biol 2016;36(9): 2004–10.