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Original Article

Correlates of Coronary Artery Calcification Prevalence and Severity in Patients With Heterozygous Familial Hypercholesterolemia

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ABSTRACT

Background: Determinants of coronary artery calcification (CAC) prevalence and severity in heterozygous familial hypercholesterolemia (HeFH) remain understudied. The objective of this cross-sectional study was to investigate correlates of CAC in patients with HeFH.

Methods: A CAC score was calculated by a noncontrast computed tomography scan in women (n = 68) and men (n = 78) with genetically defined HeFH. We classified CAC prevalence and severity using 3 categories: CAC score = 0 Agatston Unit (AU), CAC score = 1-100 AU, and CAC score > 100 AU. Information on potential correlates of CAC including familial and personal health history, cardiovascular risk factors, lipid-lowering medication, and lifestyle habits was collected.

RÉSUMÉ

Contexte : Les déterminants de la prévalence et de la sévérité de la calcification des artères coronaires (CAC) dans l'hypercholestérolémie familiale hétérozygote (HFHe) demeurent peu étudiés. L'objectif de cette étude transversale était d'identifier les corrélats de la CAC chez des patients atteints d'HFHe.

Méthodologie : Un score calcique coronarien (SCC) a été calculé par un examen de tomodensitométrie sans contraste chez des femmes (n = 68) et des hommes (n = 78) avec HFHe génétiquement définie. Nous avons classé la prévalence et la gravité de la CAC en trois catégories : SCC = 0 unité d'Agatston (UA), SCC = 1 à 100 UA et SCC > 100 UA. Des renseignements ont été recueillis sur des corrélats

Heterozygous (He) familial hypercholesterolemia (FH) is an inherited, autosomal dominant disease caused by genetic mutations in the low-density lipoprotein (LDL) receptor (LDLR), apolipoprotein B (apo B), or proprotein convertase subtilisin/kexin type 9.[1] By disrupting the normal clearance of LDLs from the plasma, these mutations cause a marked hypercholesterolemia across the lifespan. HeFH's main clinical feature is a 2- to 3-fold increase in plasma LDL-cholesterol (LDL-C) concentrations, typically ranging from 5.0 to 14.0 mmol/L. If untreated, individuals with HeFH face a 10- to 20-fold increased risk of coronary heart disease (CHD) compared with unaffected individuals, and CHD usually occurs prematurely before the age of 55 years. HeFH is estimated to affect 1 in 310 individuals according to most recent estimates.[2,3] Worldwide, HeFH is the most prevalent genetic disorder, causing premature coronary events and deaths.[1]

Although individuals with HeFH undisputedly face a lifelong increased risk of CHD compared with non-affected individuals,[4] they also present highly heterogeneous CHD risk profiles.[5] Age, male sex, body mass index (BMI), smoking status, blood pressure, and concentrations of LDL-C, HDL-C, and lipoprotein(a) (Lp(a)) are all associated with the prevalence and incidence of CHD in HeFH.[6,7] However, prevalence and

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Ethics Statement: The study was approved by the CHUQ Research Center ethical review committee, and informed consent was obtained from each patient.

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See page 69 for disclosure information.

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Results: A total of 95 patients had prevalent CAC. Independent correlates of CAC prevalence and severity included age (odds ratio [OR] per 10 years: 5.06, 95% confidence interval [CI]: 3.19, 7.93, $P < 0.0001$), family history of premature cardiovascular disease (OR: 3.88, 95% CI: 1.71, 8.81, $P = 0.001$), male sex (OR: 3.40, 95% CI: 1.49, 7.78, $P = 0.004$), statin use (OR: 15.5, 95% CI: 1.89, 126, $P = 0.01$), diet quality assessed with the Alternative Healthy Eating Index score (OR per 1 standard deviation: 0.59, 95% CI: 0.39, 0.90, $P = 0.01$), ever smoking (OR: 3.06, 95% CI: 1.20, 7.81, $P = 0.02$), receptor-negative genotype (OR: 3.17, 95% CI: 1.16, 8.66, $P = 0.02$), lipoprotein(a) year-score (OR per 1 standard deviation of log-transformed year-score: 1.53, 95% CI: 0.99, 2.36, $P = 0.05$).

Conclusions: In individuals with HeFH, age, family history of premature cardiovascular disease, sex, statin use, diet quality, smoking status, the *LDLR* genotype, and lipoprotein(a) concentrations were independently associated with CAC prevalence and severity.

Résultats : Au total, 95 patients présentaient une CAC. Les corrélats indépendants de la prévalence et de la gravité de la CAC comprenaient l'âge (rapport de cotes [RC] par tranche de 10 ans : 5,06; intervalle de confiance [IC] à 95 % : 3,19 à 7,93; $p < 0,0001$), des antécédents familiaux de maladie cardiovasculaire précoce (RC : 3,88; IC à 95 % : 1,71 à 8,81; $p = 0,001$), le sexe masculin (RC : 3,40; IC à 95 % : 1,49 à 7,78; $p = 0,004$), l'emploi de statines (RC : 15,5; IC à 95 % : 1,89 à 126; $p = 0,01$), la qualité du régime alimentaire évaluée selon le score AHEI (*Alternative Healthy Eating Index*) (RC par écart-type : 0,59; IC à 95 % : 0,39 à 0,90; $p = 0,01$), le tabagisme (RC : 3,06; IC à 95 % : 1,20 à 7,81; $p = 0,02$), le génotype récepteur-négatif (RC : 3,17; IC à 95 % : 1,16 à 8,66; $p = 0,02$) et le score lipoprotéine(a)-année (RC par écart-type du score-année transformé en logarithme : 1,53; IC à 95 % : 0,99 à 2,36; $p = 0,05$).

Conclusions : Chez les personnes atteintes d'HFHe, l'âge, les antécédents familiaux de maladie cardiovasculaire précoce, le sexe, l'emploi de statines, la qualité du régime alimentaire, le statut de tabagisme, le génotype du *LDLR* et les concentrations de lipoprotéine(a) ont été associés de façon indépendante à la prévalence et à la gravité de la CAC.

severity of coronary artery calcification (CAC) was recently identified as the most discriminant risk factor associated with incidence of cardiovascular events in patients with HeFH.[8] A CAC score of 0 Agatston Unit (AU) was associated with no event occurrence, and the risk increased proportionally with a CAC score > 0 AU over the course of up to 4 years of follow-up.[8] The superior predictive value of CAC with regard to CHD risk likely relies on the fact that CAC represents the cumulative downstream effects of any risk factors over a lifetime.[9] Thus, identifying correlates of the prevalence and severity of CAC will be informative regarding determinants of atherosclerosis development and CHD risk heterogeneity in HeFH.[10] In that regard, the cholesterol burden and concentrations of Lp(a) have been identified as correlates of CAC presence and severity in patients with HeFH.[8,11,12] However, no comprehensive assessment of correlates of CAC in this high-risk population has been conducted to date.

In the current cross-sectional study, we aimed to identify independent correlates of CAC prevalence and severity in a cohort of French-Canadian women and men with genetically defined HeFH. We investigated the associations between multiple potential correlates, including genetic, lipid, clinical, and lifestyle factors, and the prevalence and severity of CAC. We hypothesized that documented risk factors of CHD among patients with HeFH, namely age, male sex, BMI, smoking status, blood pressure, and concentrations of LDL-C, HDL-C, and Lp(a) are associated with CAC burden.[6,7]

Materials and Methods

Study participants and design

We recruited 172 patients with genetically defined HeFH via a routine visit at the Lipid Clinic of the CHUQ Research Center. To be included in the study, patients had to be a carrier of a documented FH-causing mutation and aged ≥ 18 years. Individuals with homozygous FH were ineligible. The study was approved by the CHUQ Research Center ethical review committee, and informed consent was obtained from each patient. This study was registered at clinicaltrials.gov as NCT02225340.

Data collection and clinical assessments

Recruited patients underwent a complete clinical assessment. Study staff (JPDC, AJT) measured patients' height, weight, and waist circumference. Patients' blood pressure was measured using an automatic blood pressure monitor (BP Thru, Omron, Kyoto, Japan) after they had been sitting quietly for 10 minutes. Three sequential readings were taken with 3 minutes between readings. Fasting blood samples were collected from an antecubital vein in all patients. Patients underwent the non-contrast computed tomography (CT) scan later on the same day.

Information on *LDLR* mutation, family history of premature cardiovascular disease (defined as first-degree relatives with cardiovascular disease occurrence before the age of 55 years for men or 65 years for women), smoking status (never, ever), history of hypertension, diabetes, and cardiovascular disease, as well as current cholesterol-lowering drug use (type and dose) was collected from medical records of selected patients. Study staff also collected information on total cholesterol (total-C), LDL-C, and Lp(a) concentrations available in the medical records since FH diagnosis. These data were used to calculate total-C, LDL-C, and Lp(a) year-scores.[13]

Serum lipids and lipoprotein measurements

Serum was separated from blood cells in samples collected on the morning of the clinical assessment by centrifugation at 2200 rpm (1100 g) for 10 minutes at 18 °C. Serum cholesterol and triglyceride concentrations were determined with a Roche/Hitachi MODULAR analyzer (Roche Diagnostics,

Indianapolis, IN) using proper reagents. Lp(a) concentrations were measured by nephelometry using a BN ProSpec system (Siemens Healthcare, Erlangen, Germany). Glucose levels were measured using colorimetry (Roche Diagnostics, Indianapolis, IN).

Dietary assessment

Dietary intakes were assessed within 1 week of the clinical assessments using a self-administered, web-based food frequency questionnaire (FFQ) inquiring about patients' food intake over the preceding month.[14] The FFQ contains 136 questions split into 8 sections: dairy products, fruits, vegetables, meat and alternatives, cereals and grain products, beverages, other foods, and supplements. For each food item, patients were first asked to recall the frequency of consumption. Answer choices offered between 8 and 9 continuous responses ranging from "never" to "four or more times per day." Once a food item was reported to be consumed, participants had to detail the type of food most frequently eaten over the preceding month (for example, skimmed, reduced-fat, or full-fat milk), if applicable. Finally, respondents had to select a portion size representative of usual intake over the preceding month (clickable image). The validity and reproducibility of the FFQ has been previously demonstrated.[14]

Overall, diet quality was determined using the Alternative Healthy Eating Index (AHEI) 2010 score. The AHEI was created based on intakes of foods and nutrients that have been consistently associated with a lower risk of chronic disease.[15] The score is calculated from 11 components reflective of adherence to healthy dietary habits: higher intakes of (i) vegetables, (ii) fruits, (iii) whole grains, (iv) nuts and legumes, (v) long-chain n-3 fatty acids, and (vi) polyunsaturated fatty acids (excluding long-chain n-3 fatty acids); and lower intakes of (vii) red/processed meat, (viii) sugar-sweetened beverages and fruit juice, (ix) trans fat, and (x) sodium; and (xi) moderate alcohol consumption. Each component score ranged from 0 (least-healthy eating behavior) to 10 (healthiest eating behavior). The total AHEI score ranged from 0 to 110 (maximum adherence). The AHEI 2010 score was previously demonstrated to be negatively associated with incident CHD and mortality in the general population.[16,17] We calculated the AHEI score from FFQ data. We imputed median dietary intake values to study participants with missing or incomplete information on diet (n = 10).

Measurement of coronary artery calcification (CAC)

Multidetector CT scans without contrast were performed using a 256-slices helical scanner (Brilliance iCT, Philips, Netherlands) with a tube potential at 120 kV and a tube current-time product at 60 to 80 mAS. The region of the coronary arteries was assessed in contiguous axial slices from carina to bottom of the heart by 2.4- to 3-mm-thick transverse slices with a pitch of 0.15 to 0.25 mm during end-inspiration breath-hold. Acquisition was triggered by electrocardiography at 60% to 70% of the R-to-R-wave interval. State-of-the-art dose-reduction strategies, including adjusting tube current to chest wall morphology, prospective electrocardiogram gating, and dose modulation were used. The CT scans were performed as part of this study, not in the course of routine care.

Offline image analysis was conducted on dedicated workstations using validated software (Aquarius iNtuition from TeraRecon, Inc, San Mateo, CA). CAC scores were quantified with the Agatston scoring method.[18] All CAC data are expressed in AU. Calcification was defined as 4 adjacent pixels with a density > 130 Hounsfield units. The summation of per-slice lesion scores was performed individually for each CAC score. Operators blinded to patient data performed all scans.

Statistical analyses

For the main analyses, we used scores of 0 AU (ie, absence of CAC), 1-100 AU, and > 100 AU to classify CAC prevalence and severity in 3 ordinal categories. These thresholds were previously found to be clinically meaningful for CHD risk prediction in patients with HeFH,[8] for whom a CAC score > 100 AU is associated with the highest risk, and a CAC score of 0 AU is associated with no CHD occurrence over a 4-year period.

Comparisons of patients' characteristics between CAC categories were conducted using analyses of variance followed by Tukey's post hoc tests for multiple comparisons. We used ordinal logistic regression models to evaluate how potential correlates (age, sex, *LDLR* genotype, family history of premature cardiovascular disease, smoking status, prevalent hypertension, prevalent diabetes, statin use, BMI, LDL-C, and Lp(a) year-scores, fasting glucose and HDL-C concentrations, and diet quality [AHEI score]) were associated with CAC prevalence and severity. This approach was preferred over linear regressions because of the highly skewed distribution of CAC scores. We first used simple ordinal logistic regressions to evaluate the association between each potential correlate and CAC prevalence and severity, and to calculate the corresponding proportional odds ratios (ORs). We then used multiple ordinal logistic regression models with a backward stepwise approach with threshold for leaving the model set at $P = 0.10$ to identify independent correlates for CAC prevalence and severity, and calculate the corresponding proportional ORs. Proportional ORs reflect how much an increase in the potential risk factor is associated with the probability of higher CAC score category.

We performed different sensitivity analyses to evaluate the robustness of the main analyses. First, we repeated the stepwise multiple ordinal logistic regression by excluding the *LDLR* genotype from the original model, as this information is often unavailable to clinicians. Second, we investigated the correlates for prevalent CAC without consideration of CAC severity (ie, CAC score = 0 AU vs CAC score > 0 AU) using nominal logistic regression. Finally, we repeated the main analyses by excluding patients with a personal history of CHD (n = 17) to limit the risk for potential reverse causation associated with changes in modifiable risk factors (lifestyle habits, cholesterol-lowering drugs, and plasma lipids) following a coronary event. Statistical analyses were performed using SAS v9.4.

Results

Supplemental Figure S1 presents the flowchart of participants. Of the 172 recruited patients, one completed the

Table 1. Characteristics of the 146 patients with heterozygous familial hypercholesterolemia*

Characteristics	Mean ± SD or n (%)
Age, y	47.8 ± 14.1
Age at diagnosis,* y	34.3 ± 14.1
Male sex	78 (53)
LDLR mutation	
Del > 15 kb	90 (62)
W66G (exon 3)	30 (21)
C152W (exon 4)	6 (4)
E207K (exon 4)	4 (3)
R329X (exon 7)	1 (1)
C347R (exon 8)	2 (1)
Y468X (exon 10)	5 (3)
C646Y (exon 14)	8 (5)
Receptor-negative genotype	114 (78)
Family history of premature cardiovascular disease	81 (55)
History of coronary heart disease	17 (12)
Prevalent hypertension	25 (17)
Prevalent diabetes	6 (4)
Ever smoking	35 (24)
Current drug therapy	
Statin	142 (97)
Ezetimibe	117 (80)
PCSK9 inhibitors	40 (27)
Body mass index, kg/m^2	27.4 ± 4.9
Waist circumference, cm	91.8 ± 12.5
Systolic blood pressure, mm Hg	107 ± 12
Diastolic blood pressure, mm Hg	70 ± 9
Total-C,† mmol/L	4.58 ± 1.41
Highest recorded Total-C, mmol/L	9.13 ± 2.21
Mean lifetime Total-C, mmol/L	7.23 ± 1.48
Total-C year-score, mmol-year/L	350 ± 138
TG,† mmol/L	1.15 ± 0.61
HDL-C,† mmol/L	1.41 ± 0.37
LDL-C,† mmol/L	2.65 ± 1.30
Highest recorded LDL-C, mmol/L	7.25 ± 2.14
Mean lifetime LDL-C, mmol/L	5.41 ± 1.39
LDL-C year-score, mmol-year/L	262 ± 114
Non-HDL-C,† mmol/L	3.17 ± 1.40
Total-C/HDL-C†	3.46 ± 1.57
Apo B,† mg/L	0.95 ± 0.35
Lp(a),† nmol/L	106 ± 123
Highest recorded Lp(a), nmol/L	120 ± 131
Mean lifetime Lp(a), nmol/L	102 ± 118
Lp(a) year-score, nmol-year/L	5157 ± 6637
Glucose,† mmol/L	5.46 ± 0.90
Alternative Healthy Eating Index (AHEI) Score	56.2 ± 13.5
CAC score, AU	352 ± 679

Apo B, apolipoprotein B; AU, Agatston Unit; CAC, coronary artery calcification; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lpa, lipoprotein(a); PCSK9, proprotein convertase subtilisin/kexin type 9; SD, standard deviation; TG, triglycerides; Total-C, total cholesterol.

*Information available in 134 patients.

†Concentrations measured from the blood sample collected before the scan.

clinical assessment but did not undergo the CT scan. Additionally, the CAC score was impossible to calculate because of technical problems in 25 patients. Therefore, our main analyses include 146 patients (men, n = 78; women, n = 68). Patients in whom the CAC score was impossible to calculate (n = 25) were older and more likely to be women, compared with patients included in the main analyses (n = 146) (Supplemental Table S1). No other difference was noted between the 2 groups.

Table 1 presents characteristics of the 146 patients included in the main analyses. Mean age was 47.8 ± 14.1 years. All patients were carriers of a mutation affecting the *LDLR* gene. A total of 90 patients had the > 15-kb deletion at the 5' end of the gene; 30 had the W66G mutation in exon 3; 6 had the C152W mutation in exon 4; 4 had the E207K mutation in exon 4; 1 had the R329X mutation in exon 7; 2 had the C347R mutation in exon 8; 5 had the Y468X mutation in exon 10; and 8 had the C646Y mutation in exon 14. Overall, 114 patients (78%) were carriers of a receptor-negative mutation. At the moment of the study, 142 patients were treated with statin, mean LDL-C concentrations were 2.65 ± 1.30 mmol/L, and mean CAC scores were 352 ± 679 AU.

Figure 1 presents the distribution of the patients according to age and CAC score. A total of 95 patients (65%) had prevalent CAC (score > 0 AU). Among the patients aged 18 to < 35 years (n = 25), 3 had a CAC score between 1 and 100 AU, but none had a CAC score > 100 AU. Conversely, among patients aged 65 to 80 years (n = 13), none had a CAC score of 0 AU, and 10 had a CAC score > 100 AU.

Table 2 presents differences in age-adjusted characteristics of the study patients according to CAC score. Patients with CAC score > 100 AU were older and more likely to have a family history of premature cardiovascular disease, a personal history of CHD, and hypertension, compared with patients in lower CAC score categories. Patients with CAC score > 100 AU were also more likely to be treated with ezetimibe and proprotein convertase subtilisin/kexin type 9 inhibitors in addition to statins, and they had lower plasma concentrations of LDL-C and apolipoprotein B at the moment of the study. Finally, patients with higher CAC score also had a lower-quality diet, as demonstrated by their lower AHEI score, compared with patients in lower CAC score categories.

Table 3 presents proportional ORs of potential correlates of CAC prevalence and severity. Simple ordinal regressions showed that CAC prevalence and severity were associated with age, sex, family history of premature cardiovascular disease, prevalent hypertension, LDL-C year-score, Lp(a) year-score, and fasting glucose concentrations. The *LDLR* genotype, smoking status, prevalent diabetes, statin use, BMI, HDL-C concentrations, and AHEI score were not associated with the CAC burden in simple ordinal regressions. We subsequently analyzed the independent associations between the above potential correlates and CAC burden using a multiple ordinal logistic regression model with a backward stepwise approach. The original model included all potential correlates, and the threshold for leaving the model was set at $P = 0.10$. In the final model, independent correlates of CAC burden were—from the factor the most strongly associated with CAC burden—age, family history of premature cardiovascular disease, male sex, statin use, diet quality (inverse association), ever smoking, receptor-negative genotype, and Lp(a) year-score. These 8 factors collectively explained 40.2% of the variance of the CAC. The areas under the receiver operating characteristic curves (AUROCs) of the final model discriminating between patients with CAC score 1-100 AU vs patients with CAC score of 0 AU, and between those with CAC score

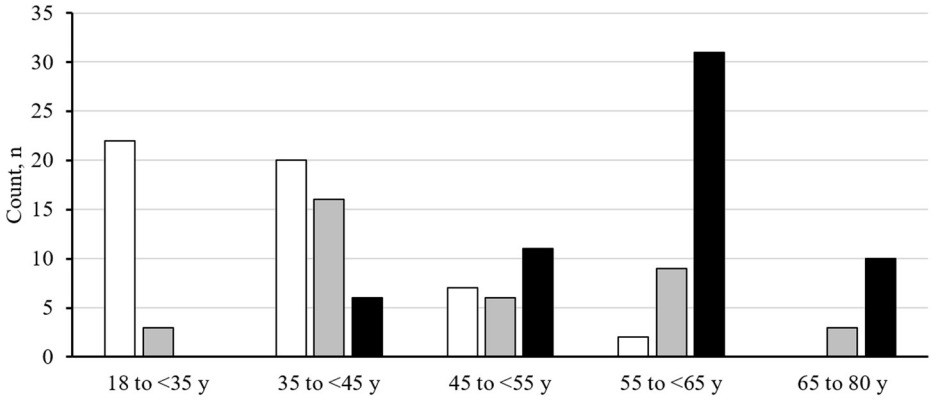

Figure 1. Distribution of the 146 patients with heterozygous familial hypercholesterolemia included in the study according to age and coronary artery calcification (CAC) scores. The **vertical bars** represent the count of patients according to CAC score: **white bars**, CAC score of 0 Agatston units (AU); **gray bars**, CAC score between 1 and 100 AU; **black bars**, CAC score > 100 AU.

> 100 vs those with score of 0 AU, were 0.90 and 0.91, respectively.

In sensitivity analyses, when we repeated the stepwise multiple ordinal logistic regression by excluding the *LDLR* genotype from the original model to take into consideration that this information is often not available for clinicians, results were mostly unchanged. Independent correlates of CAC prevalence and severity included age, family history of premature cardiovascular disease, male sex, ever smoking, statin use, AHEI score (negative association), and the Lp(a) year-score (Supplemental Table S2). This model explained 38.6% of the CAC variance. The AUROC discriminating between patients with CAC score between 1 and100 AU vs patients with CAC score of 0, and between those with CAC score >100 AU vs those with score of 0 AU, were both 0.90.

We also investigated correlates of the presence of CAC (ie, CAC score > 0 AU vs CAC score = 0 AU; Supplemental Table S3). Simple nominal logistic regressions showed age, family history of premature cardiovascular disease, smoking status, prevalent hypertension, BMI, LDL-C and Lp(a) year-scores, and fasting glucose concentrations all to be positive correlates for prevalent CAC. In the final multiple nominal logistic regression model, age, the *LDLR* genotype, smoking status, and AHEI score (negative association) were found to be independent correlates for presence of CAC. This model explained 43.6% of the CAC variance. The AUROC discriminating between patients with CAC vs those without CAC was 0.90.

Finally, we repeated the main analyses excluding patients with a history of CHD (n = 17) and restricting analyses to patients without personal history of CHD (n = 129). Among the 129 patients free of CHD, 79 patients (61%) had prevalent CAC (score > 0 AU). In the simple ordinal regressions, the CAC burden was associated with age, family history of premature cardiovascular disease, prevalent hypertension, BMI, LDL-C year-score, Lp(a) year-score, and fasting glucose concentrations (Supplemental Table S4). In the backward stepwise multiple ordinal regression approach (threshold for leaving the model, $P = 0.10$), the final model yielded results concordant with the main analysis. Age, family history of premature cardiovascular disease, male sex, statin use, AHEI score (negative association), ever smoking, receptor-negative genotype, and Lp(a) year-score were independently associated with CAC prevalence and severity. These 8 correlates collectively explained 38.2% of the CAC variance. The AUROCs discriminating between patients with CAC score of 1-100 AU vs patients with CAC score of 0, and between those with CAC score > 100 vs those with score of 0 were 0.91 and 0.90, respectively.

Discussion

In a sample of women and men with genetically defined HeFH, age, family history of cardiovascular disease, sex, statin use, diet quality, smoking status, the *LDLR* genotype, and Lp(a) year-score were all independently associated with CAC prevalence and severity. The ability of these correlates to collectively discriminate between patients with CAC score of 0 AU and patients with prevalent CAC was very high. Our sensitivity analyses further demonstrated that these findings were consistent across different analytical approaches or patients' characteristics, including personal history of CHD. These data provide novel information on potential determinants of atherosclerosis in HeFH.

In terms of nonmodifiable correlates, our study suggests that age is the strongest determinant of CAC prevalence and severity. Male sex, *LDLR* genotype, family history of cardiovascular disease, and Lp(a) concentrations were also nonmodifiable correlates of CAC. The relationship between age and CAC prevalence and severity observed is consistent with most studies on CAC development and progression in the general population[19,20] and in individuals with HeFH.[8,12] Increasing age undisputedly drives the cholesterol burden of patients with HeFH.[12] On the other hand, the relationship between age and CAC prevalence and severity needs to be interpreted in the context of older patients having lived a greater proportion of their lives in the pre-statin era, compared with younger patients. Considering that statin-therapy slows atherosclerosis development,[21,22] the presence of this confounding may overestimate the strength of the relationship between age and CAC in our sample. Notwithstanding the above, the strong association between age and CAC prevalence and severity among patients with HeFH that we and others have observed supports the importance of

Table 2. Differences in age-adjusted characteristics of the 146 patients with heterozygous familial hypercholesterolemia according to the coronary artery calcium score

Characteristic	Coronary calcium score = 0 AU (n = 51)	Coronary calcium score = 1-100 AU (n = 37)	Coronary calcium score >100 AU (n = 58)	*P*
CAC score,* AU	0	29 ± 25	868 ± 850	< 0.0001
CAC score range,* min-max	0-0	1-96	103-3132	
Male sex*	23 (45)	18 (49)	37 (64)	0.12
Receptor-negative genotype*	38 (75)	31 (84)	45 (78)	0.57
Age,* y	35.8 ± 10.5[a]	48.0 ± 11.4[b]	58.2 ± 9.4[c]	< 0.0001
Family history of premature cardiovascular disease*	18 (35)	18 (49)	45 (78)	< 0.0001
History of coronary heart disease*	1 (2)	0 (0)	16 (28)	< 0.0001
Ever smoking*	7 (14)	11 (30)	17 (29)	0.09
Prevalent hypertension*	2 (4)	6 (16)	17 (29)	0.001
Prevalent diabetes*	1 (2)	3 (8)	2 (3)	0.37
Current drug therapy*				
Statin	50 (98)	35 (95)	57 (98)	0.56
Ezetimibe	34 (67)	31 (84)	52 (90)	0.01
PCSK9 inhibitors	11 (22)	3 (8)	26 (45)	0.0001
Body mass index, kg/m^2	26.1 ± 5.9	28.3 ± 4.9	28.1 ± 5.8	0.13
Waist circumference, cm	88.7 ± 14.9	93.0 ± 12.2	93.8 ± 14.6	0.23
Blood pressure, mm Hg				
Systolic	106 ± 14	107 ± 12	107 ± 14	0.81
Diastolic	67 ± 11	71 ± 9	71 ± 11	0.21
Total-C,† mmol/L	4.66 ± 1.70	4.93 ± 1.40	4.29 ± 1.66	0.13
Highest recorded	8.99 ± 2.68	9.03 ± 2.20	9.30 ± 2.62	0.83
Mean lifetime	7.15 ± 1.77	6.84 ± 1.45	7.56 ± 1.73	0.10
Year-score	346 ± 91	330 ± 74	366 ± 89	0.11
TG, † mmol/L	1.01 ± 0.74	1.20 ± 0.61	1.25 ± 0.73	0.29
HDL-C, † mmol/L	1.45 ± 0.46	1.34 ± 0.38	1.42 ± 0.45	0.42
LDL-C, † mmol/L	2.75 ± 1.55[ab]	3.04 ± 1.27[a]	2.31 ± 1.51[b]	0.04
Highest recorded	7.11 ± 2.59	7.28 ± 2.13	7.35 ± 2.53	0.91
Mean lifetime	5.36 ± 1.67	5.12 ± 1.37	5.64 ± 1.63	0.24
Year-score	261 ± 85	246 ± 70	274 ± 83	0.21
Non-HDL-C,† mmol/L	3.21 ± 1.68	3.59 ± 1.38	2.87 ± 1.64	0.07
Total-C/HDL-C†	3.43 ± 1.88	3.97 ± 1.54	3.15 ± 1.84	0.05
Apo B,† mg/L	0.97 ± 0.42[ab]	1.08 ± 0.34[a]	0.85 ± 0.41[b]	0.02
Lp(a),† nmol/L	87 ± 148	110 ± 121	120 ± 145	0.57
Highest recorded	97 ± 158	123 ± 130	140 ± 154	0.45
Mean lifetime	73 ± 141	111 ± 116	123 ± 138	0.23
Year-score	3990 ± 7568	5161 ± 6222	6182 ± 7403	0.41
Glucose,† mmol/L	5.40 ± 0.99	5.72 ± 0.81	5.34 ± 0.97	0.07
Alternative Healthy Eating Index (AHEI) Score	60.9 ± 15.9[a]	56.4 ± 13.1[ab]	52.1 ± 15.6[b]	0.04

Values are means ± standard deviation or counts and percentages. Superscript letters denote significant differences.

Apo B, apolipoprotein B; AU, Agatston Unit; CAC, coronary artery calcification; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lpa, lipoprotein(a); PCSK9, proprotein convertase subtilisin/kexin type 9; SD, standard deviation; TG, triglycerides; Total-C, total cholesterol.

*Values are not age-adjusted.

†Concentrations measured from the blood sample collected before the scan.

implementing effective cascade screening programs in order to diagnose and treat HeFH from an early age.[23,24] With regard to *LDLR* genotype, the fact that the negative-receptor genotype was associated with higher odds of CAC compared with the defective-receptor genotype is consistent with multiple studies that have demonstrated the inverse relationship between the residual activity of the *LDLR* gene and circulating risk factors,[25,26] CAC burden,[27] and CHD risk.[28] Our finding underscores the importance of screening for *LDLR* mutation in HeFH management. Still, FH can be diagnosed without molecular diagnosis,[24] and the information on the *LDLR* genotype is not always available to the clinicians. We therefore took this element into consideration in our analyses. When we withdrew information on the *LDLR* genotype from our models, the ability of the other independent correlates of CAC burden (ie, age, sex, family history of premature cardiovascular disease, statin use, diet quality, smoking status, and Lp(a) year-score) to collectively distinguish patients with prevalent CAC from those without CAC remained very high. Moreover, considering the autosomal dominant transmission of the disease, the relationship reported between family history of cardiovascular disease and odds of higher CAC is reflective of the impact of the *LDLR* genotype on HeFH phenotype. As per age, our findings on the associations between the *LDLR* genotype, family history of premature cardiovascular disease, and CAC prevalence and severity support the implementation of effective cascade screening programs to address the genetic risk by treating from an early age. Finally, we observed that Lp(a) concentrations, assessed using the year-score, were the sole independent lipid risk factors for CAC burden. Given that Lp(a) concentrations are genetically defined,[29] they are considered to be nonmodifiable risk factors. The relationship observed between Lp(a) and CAC score is consistent with previous studies that investigated the relationship between

Table 3. Correlates of coronary artery calcification (CAC) burden in 146 patients with heterozygous familial hypercholesterolemia

	Simple ordinal logistic regression		Multiple ordinal logistic regression*	
Correlate	Proportional odds ratio (95% CI)	*P*	Proportional odds ratio (95% CI)	*P*
Age (per 10 y)	3.77 (2.67-5.37)	< 0.0001	5.06 (3.19, 7.93)	< 0.0001
Sex (male vs female)	1.85 (1.01, 3.39)	0.05	3.40 (1.49, 7.78)	0.004
LDLR genotype (receptor-negative vs receptor-defective)	1.14 (0.55, 2.35)	0.73	3.17 (1.16, 8.66)	0.02
Family history of premature cardiovascular disease (yes vs no)	4.22 (2.23, 8.01)	< 0.0001	3.88 (1.71, 8.81)	0.001
Smoking status (ever vs never)	1.92 (0.93, 3.93)	0.08	3.06 (1.20, 7.81)	0.02
Prevalent hypertension (yes vs no)	2.91 (1.52, 5.60)	0.001		
Prevalent diabetes (yes vs no)	1.23 (0.27, 5.61)	0.79		
Statin use (yes vs no)	1.11 (0.18, 6.97)	0.91	15.5 (1.89, 126)	0.01
BMI (per 1 SD)	1.34 (0.98, 1.83)	0.07		
LDL-C year-score (per 1 SD log-transformed unit)	4.82 (3.07, 7.57)	< 0.0001		
Lp(a) year-score (per 1 SD log-transformed unit)	1.94 (1.39, 2.69)	< 0.0001	1.53 (0.99, 2.36)	0.05
HDL-C concentration (per 1 SD)	1.02 (0.75, 1.38)	0.91		
Fasting glucose concentration (per 1 SD)	1.67 (1.13, 2.45)	0.009		
AHEI score (per 1 SD)	0.95 (0.70, 1.29)	0.74	0.59 (0.39, 0.90)	0.01

Odds ratios were calculated using a backward stepwise approach with threshold for leaving the model sets as $P = 0.10$. All variables listed in the table were included in the original model. Only odds ratios of variables retained in the final model (ie, $P < 0.10$) are presented.

AHEI, Alternative Healthy Eating Index; BMI, body mass index; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; Lp(a), lipoprotein a; SD, standard deviation.

Lp(a) concentrations, CAC, and CHD risk in patients with HeFH.[7,11,30] Accordingly, our study suggests that patients with HeFH presenting high Lp(a) concentrations appear to be good candidates for high-intensity pharmacologic treatments. This finding is also of interest as therapies to lower Lp(a) levels are under development.[31]

With regard to modifiable correlates of CAC, we found that statin use, smoking status, and diet quality were independently associated with CAC prevalence and severity. Although statins reduce overall atherosclerosis development, evidence also suggests that they favor plaque calcification concomitantly.[32,33] This mechanism would be reflective of plaque repair and would not negatively affect the cardioprotective effects of statins.[33] Our results appear to be in line with these data, as statin use was associated with higher odds of CAC prevalence and severity. Still, this observation should be interpreted with caution as the number of patients included in our study who were not treated with statins was very small (n = 4), and reverse causation cannot be ruled out. Besides, it is well recognized that smoking exacerbates CHD risk among individuals with HeFH,[7,34] and our results are consistent with the literature. On the other hand, although diet undisputedly plays a major role in CHD prevention in the general population,[35] our understanding of how diet influences cardiovascular health in patients with HeFH remains highly inadequate to date, thereby limiting our appreciation of its potential in CHD prevention in this high-risk population. The literature on diet management in FH is minimal and mostly limited to dietary supplement interventions without any consideration of overall diet quality.[36,37] Because of the lack of conclusive data, diet has traditionally been considered a secondary therapy with limited potential for the management of HeFH.[4,24,36] Still, in our sample of patients with genetically defined HeFH, we observed that diet quality, assessed by the AHEI score, was inversely associated with higher CAC burden. This inverse association was independent of other common risk factors of CAC and CHD in patients with HeFH, such as age, male sex, *LDLR* genotype, and smoking status. Even though this association relies on a single assessment of diet, mostly representative of dietary intakes in the month preceding data collection, it constitutes the first evidence linking higher diet quality to lower coronary atherosclerosis in patients with HeFH. This finding supports the importance of dietary counseling in HeFH management.

Of note, LDL-C concentrations, expressed using the year-score, were associated with the CAC burden in simple logistic regressions, but not in multiple logistic regression models in the present study. One potential explanation for the lack of an independent association in the multiple logistic regression models may be related to the mediating effect of age on the relationship between the LDL-C year-score and CAC prevalence and severity: LDL-C year-score is strongly correlated with age and the CAC score. The relationship may also have been confounded by reverse causation, as individuals with higher CAC burden were more likely to receive high-intensity drug therapy. Previous studies have shown that CHD risk attributable to LDL-C among patients with HeFH is highly attenuated when patients receive cholesterol-lowering therapies.[21,22,38] For instance, a 20-years follow-up study in children with HeFH demonstrated that initiation of statin therapy during childhood slowed the progression of carotid intima−media thickness and reduced the risk of cardiovascular disease in adulthood at a level that was not higher than that in non-HeFH children.[38] Overall, our study suggests that CAC heterogeneity among patients with HeFH is independent of LDL-C.

In the current study, CAC was detected in 65% of our sample of patients with genetically defined HeFH, including

37% of patients aged < 45 years. Among patients free of CHD, CAC prevalence was 61%. These numbers are consistent with data from a meta-analysis of 9 studies in which the overall prevalence of CAC score > 0 AU was estimated to be 55% (95% CI: 45%-66%) among patients with HeFH free of CHD.[39] Considering that CAC is one of the most potent predictors of CHD,[8,19] the fact that about half of individuals with HeFH present no CAC demonstrates the heterogeneity in CHD risk profiles in this population,[5,40-43] and underscores the importance of documenting the determinants of atherosclerosis in this population. In that regard, our results support currently recommended management approaches including effective cascade screening strategies, early treatment onset, and lifestyle management education comprising counseling on diet and smoking cessation, independent of the prevalence and severity of CAC.[24]

This study has several strengths and limitations. The comprehensive data collection allowed us to identify correlates of CAC prevalence and severity that have never been documented previously among patients with HeFH, such as diet quality. On the other hand, it is very likely that other factors not documented in the current study (eg, total homocysteine, plasma metabolites, physical activity) also influence CAC in HeFH. In addition, the cross-sectional design exposes our analyses to reverse causation. To offset this limitation, we used year-scores for circulating risk factors when possible (ie, for LDL-C and Lp(a)). We also conducted sensitivity analyses by restricting the study sample to patients free of CHD, which yielded virtually unchanged results. The high number of failed scans likely limited our power to detect additional significant correlates of CAC. Still, all study subjects had genetically defined HeFH, which allowed us to explore the role of the *LDLR* genotype in CAC prevalence and severity. Similar studies among patients carrying other common *LDLR* mutations remain warranted.

Conclusions

We found that age, family history of premature cardiovascular disease, sex, statin use, diet quality, smoking status, the *LDLR* genotype, and Lp(a) year-score were independently associated with CAC prevalence and severity in patients with HeFH. The inverse association observed between diet quality and CAC burden has never been reported previously and constitutes the first evidence linking diet quality to coronary atherosclerosis in patients with HeFH. Further studies are required to ascertain whether our findings imply causality. Still, this study provides novel information on potential determinants of atherosclerosis in HeFH.

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Supplementary Material

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