Atherosclerosis Plus 50 (2022) 65–71

Contents lists available at ScienceDirect

Atherosclerosis Plus

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

Subclinical atherosclerosis determined by coronary artery calcium deposition in patients with clinical familial hypercholesterolemia

Sanna á Borg [a, *], Christian Sørensen Bork [b], Michael René Skjelbo Nielsen [c], Jan Jóanesarson [a], Tomas Zaremba [b], Ihab Bishara Yousef Lolas [d], Søren Lundbye-Christensen [e], Peter Søgaard [b], Erik Berg Schmidt [f], Albert Marni Joensen [b]

[a] Department of Medicine, National Hospital of the Faroe Islands, Faroe Islands
[b] Department of Cardiology, Aalborg University Hospital, Denmark
[c] Heart Clinic of Northern Jutland, Denmark
[d] Department of Molecular Diagnostics, Aalborg University Hospital, Denmark
[e] Unit of Clinical Biostatistics, Aalborg University Hospital, Denmark
[f] Department of Clinical Medicine, Aalborg University, Denmark

ARTICLE INFO

Article history:
Received 13 June 2022
Received in revised form
18 October 2022
Accepted 30 October 2022
Available online 11 November 2022

ABSTRACT

Background and aims: Limited knowledge exists regarding the association between coronary artery calcium (CAC) deposition in patients with clinical familial hypercholesterolemia (FH) and FH subtypes such as polygenic causes. We studied CAC score in patients with clinical FH and subtypes including polygenic causes of FH compared to healthy controls.

Methods: In a case-control study, we identified potential clinical FH cases registered with an LDL-C >6.7 mmol/l within a nationwide clinical laboratory database on the Faroe Islands and invited them for diagnostic evaluation according to clinical FH scoring systems. Controls were identified in the background population. All subjects were aged 18–75 years and without a history of cardiovascular disease. FH mutation testing and genotypes of twelve LDL-C associated single nucleotide polymorphisms were determined using conventional methods in selected individuals. CAC scores were assessed by cardiac CT. Odds ratios obtained using multivariate logistic regression were used as measures of association.

Results: A total of 120 clinical FH patients and 117 age- and sex-matched controls were recruited. We found a very low frequency of monogenic FH (3%), but a high level of polygenic FH (60%) in those genetically tested (54%). There was a statistically significant association between the CAC score and a diagnosis of clinical FH with the highest observed odds ratio of 5.59 (95% CI 1.65; 18.94, p = 0.006) in those with a CAC score ≥300 compared to those with a CAC of zero. In supplemental analyses, there was a strong association between CAC scores and clinical FH of a polygenic cause.

Conclusion: We found a statistically significant association between CAC levels and clinical FH with the highest observed risk estimates among clinical FH cases of a presumed polygenic cause.

Introduction

Familial hypercholesterolemia (FH) is a common hereditary condition occurring in 1:250–300 individuals, which is associated with lifelong exposure to low-density lipoprotein cholesterol (LDL-C) and a high risk of premature atherosclerotic cardiovascular disease (ASCVD) [1]. The most common genetic causes of FH are mutations in the low-density lipoprotein receptor (*LDLR*) gene (>90%) and the apolipoprotein B (*APOB*) gene (5–10%), while gain-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene and other causative mutations are rare (<1%) [2]. Genetic testing represents a valuable tool to diagnose FH, but the molecular genetics of FH are complex [3] and the diagnosis in daily clinic is often based on clinical information alone using clinical scoring systems such as the Dutch Lipid Clinical Network [4], the Simon Broome [5] or the Make Early Diagnosis to Prevent Early

* Corresponding author. Department of Medicine, National Hospital of the Faroe Islands, J.C. Svabosgøta 41-19, 100, Tórshavn, Faroe Islands.
E-mail address: sanbo@ls.fo (S. Borg).

https://doi.org/10.1016/j.athplu.2022.10.002

Death (MEDPED) criteria [6]. However, in a large proportion of subjects fulfilling a clinical diagnosis of FH, no monogenic mutation in the major FH-causing genes can be identified [7]. Interestingly, recent studies have suggested that combinations of inherited LDL-C raising alleles of single nucleotide polymorphisms (SNPs) gene variations may contribute to severe polygenic hypercholesterolemia [2,8]. Other important factors may include highly elevated levels of lipoprotein(a) [9] as well as lifestyle factors such as dietary habits.

While clinical FH represents a heterogeneous condition, current guidelines do not differentiate prevention strategies according to the FH subset in question although previous studies have reported that subjects with monogenic FH have a higher cardiovascular risk compared to patients with a polygenic cause of hypercholesterolemia as well as patients with no pathogenic FH mutation identified [10–13]. Novel LDL-C lowering medications such as PCSK9-inhibitors and small interfering RNA therapy are very potent, but these agents are currently very expensive and appropriate risk stratification is important to identify FH patients that may require more intensive lipid-lowering therapy and earlier intervention than others.

The amount of calcium deposited in the coronary arteries is considered a well-established surrogate of subclinical atherosclerosis, and the Agatston coronary artery calcium (CAC) scoring has been shown to be a reliable predictor of cardiovascular events and mortality risk in asymptomatic individuals both with and without FH [14–20]. Thus, the CAC score may represent a valuable tool for risk stratification to guide clinicians with regard to the intensity of lipid-lowering and lifestyle modifying therapies. However, limited knowledge exists on levels of CAC scores across subtypes of clinical FH and particularly in subjects with polygenic hypercholesterolemia.

Therefore, we aimed to describe CAC score in clinical FH cases and healthy controls and to investigate the associations between levels of CAC and clinical FH and subtypes including polygenic causes.

Materials and methods

Study population and design

The recruitment and data collection in this case-control study have previously been described in detail elsewhere [21]. In brief, potential FH cases were identified based on a review of plasma lipids and lipoproteins collected in a nationwide clinical laboratory database (BCC-Web, CGI) in the Faroe Islands between 2006 and 2020. Subjects aged 18–75 years with a registered plasma LDL-C above 6.7 mmol/l were contacted by letter and invited to attend a clinical examination for diagnostic evaluation of FH and eligibility for the study. Prior to the clinical examination, subjects were required to have screening blood samples taken including plasma lipid- and lipoprotein levels (total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglycerides) and measurements to evaluate possible secondary causes of dyslipidemia. Subjects were considered eligible if they met the criteria of probable or definite FH according to the DLCN criteria [4], definite FH according to the Simon Broome criteria [5], and/or definite FH according to the MEDPED criteria [6]. First-degree relatives that met age- and sex-specific LDL-C cutoffs [22] were also considered eligible FH cases. Potential controls were identified in the background population through the National Register of Persons in the Faroe Islands. Subjects with a plasma LDL-C <3.5 mmol/L, DLCN score $\leq$3 and without a history of ASCVD and without the use of lipid-lowering medication were considered eligible as controls. Cases and controls were matched according to sex and age (5-year age intervals).

Subjects with a history of ASCVD were excluded from this study as we aimed to investigate CAC scores as an indicator of subclinical coronary artery disease. Also, subjects with persistent atrial fibrillation/flutter were not considered eligible, as these arrhythmias could impair the quality of the cardiac CT scans. Also, subjects with elevated creatinine levels >120 μmol/L and subjects who were pregnant were not considered eligible for the present study.

The study protocol is in accordance with the Declaration of Helsinki and was approved by the Ethics Committee in the Faroe Islands and the Faroese Data Protection Agency. All participants gave informed written consent.

Data collection

Each participant fulfilled a detailed questionnaire on medical history and lifestyle factors such as clinical history, family history of hypercholesterolemia and premature cardiovascular disease (CVD), educational level, physical activity, alcohol consumption, smoking habits and use of medications [21].

During a clinical examination a family pedigree was drawn for all clinical FH cases and family history of CVD and hypercholesterolemia was registered. Also, clinical information used in the clinical FH criteria was retrieved from the subjects' medical records during the interview.

The highest measured fasting or non-fasting plasma LDL-C without concomitant lipid-lowering medication was registered. In subjects with no available untreated plasma LDL-C measurement, the highest treated value of LDL-C was registered together with information on medication number, type, dose and frequency. In patients on lipid regulating treatment, plasma LDL-C value was estimated according to the average lipid-lowering effect of the medication [23]. Physical examination included examination for FH stigmata (tendon xanthomas, arcus cornealis, xanthelasmata), anthropometrics (height, weight, abdominal waist circumference), blood pressure and an electrocardiogram (ECG). Blood samples for genetic analyses and lipoprotein(a) were collected [21].

Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, and/or use of antihypertensive medications. Diabetes mellitus was defined as hemoglobin A1c $\geq$48 mmol/mol, and/or use of antidiabetic medications.

Genetic analyses

Genomic DNA was extracted from whole blood samples using Maxwell system (Promega). All probands with clinical FH and an untreated plasma LDL-C $\geq$ 6.7 mmol/L (n = 65) were tested for genetic monogenic mutations with an initial standard FH panel including the *LDLR*, *APOB* and *PCSK9* genes. DNA libraries were prepared using Agilent SureSelect target enrichment system and the sequencing was performed using Illumina platform. Twenty subjects with family pedigrees highly suggestive of a monogenic inheritance and without detected monogenic FH causing variants in the initial gene panel were analyzed with an extended next generation sequencing (NGS) panel. The panel included 11 FH-related genes previously described in the literature to be causally associated with FH (*LDLR, LDLRAP1, PCSK9, LIPA, LPA, ABCG5, ABCG8, APOB, APOE, ANGPTL3, STAP1*) [2]. DNA libraries were prepared using SureSelect Human All Exons v6 kit (Agilent) and were sequenced on Illumina platform (Novaseq 6000). The coverage of the gene panel was higher than 98% for variants with reading depth >30X. All variants with likely clinical significance were confirmed with Sanger sequencing. Large deletions/ duplications in the LDLR gene were tested using multiplex ligation-dependent probe amplification (MLPA, kit P062 from MRC Holland) or the CNV caller tool (VarSeq v.2.2.5).

Subjects with clinical FH without causative monogenic FH

mutations identified (n = 63) underwent genotyping for 12 LDL-C raising SNPs (rs2479409, rs629301, rs1367117, rs4299376, rs1564348, rs1800562, rs3757354, rs11220462, rs8017377, rs6511720, rs429358, rs7412). A weighted LDL-C raising polygenic risk score (PRS) was subsequently calculated based on these 12 SNPs as previously described by Futema et al. [24], Talmud et al. [25] and Olmastroni et al. [8]. Subjects with a PRS >80th percentile according to a UK reference population were considered to have a polygenic cause of FH.

Calcium score assessed by cardiac CT

Coronary artery calcium was quantified using a non-contrast cardiac CT scan on a 320-detector row Toshiba Aquilion One scanner (Canon Medical Systems, Otawara, Japan). The scans were acquired using prospective ECG-gating with imaging trigger at 75% of the R-R interval and a slice thickness of 0.5 mm during one inspiratory breath-hold. Scan parameters: tube voltage 120 kV, tube current 40–370 mA, expected radiation dose 1–3 mSv. Calcium scores were calculated using Vitrea Cardiac software on reconstructed 3.0 mm images on a post-processing workstation (Vitrea Enterprise Suite version 6.4.3, Minnetonka, US).

The presence of coronary calcification was identified as at least three "face-connected" voxels in the course of a coronary artery as areas of hyperattenuation of at least 1 mm^2 with >130 Hounsfield units. Abnormal CAC scores were defined as an Agatston score >0.

Statistical analyses

Descriptive statistics are presented as means with standard deviation (SD) for continuous covariates and as number (percentage) for categorical variables. P-values were obtained using an unpaired *t*-test for continuous covariates and Fisher's exact test for categorical variables. Odds ratios with 95% confidence intervals (CI) obtained using multivariable logistic regression were used to investigate associations between the CAC score and clinical FH. We categorized CAC scores into dichotomized groups including zero and above zero as well as below or above the median for age, sex and ethnicity, respectively [26]. Also, we categorized CAC scores according to traditional risk categories (0, 1–299, ≥300 Hounsfield units) [27]. In model 1, we adjusted for age (continuous, years) and sex (men; women). In model 2, we additionally adjusted for selected major risk factors for ASCVD including smoking (never, former, current), hypertension (yes, no), waist circumference (continuous, cm) and lipoprotein(a) (continuous, mg/L). Continuous covariates were included in the models using restricted cubic splines with three knots placed at the 10th, 50th and 90th percentiles. In supplemental analyses, we used multivariable logistic regression to investigate associations between the CAC score and subgroups of clinical FH including PRS >80th percentile, PRS ≤80th percentile and subjects not genetically tested, respectively. Furthermore, in explorative post hoc analyses we compared those with a PRS >80th percentile with those with a PRS ≤80th using logistic regression, but the multivariable analyses were limited to age, sex, smoking status and hypertension due to the lower number of individuals in these analyses.

The statistical analyses were conducted using Stata (version 16, StataCorp). A p-value below 0.05 was considered statistically significant.

Results

General characteristics

A total of 120 clinical FH cases and 117 age- and sex-matched controls with complete exposure and covariate information were included in the present study. General characteristics of the study population are shown in Table 1. The mean age among clinical FH cases was 56.6 years and 57.5% were women with no statistically significant difference between clinical FH cases and controls. Half of the cases received lipid-lowering treatment at recruitment. Clinical FH cases had significantly larger waist circumference compared to controls (94.6 cm vs. 88.3 cm, p = 0.001) and had statistically significantly higher levels of lipoprotein(a) (p = 0.024). Also, among clinical FH cases there was a trend of more frequent former or current smokers and a larger proportion had hypertension and diabetes mellitus, but the observed differences were not statistically significantly different between cases and controls.

Within the group of clinically defined and genetically tested FH patients (n = 65), two (3.1%) subjects had a pathogenic FH mutation identified, while 39 (60.0%) were classified as having a polygenic (PRS >80th percentile) cause of FH and 24 (36.9%) had a PRS <80th percentile. A total of 55 clinical FH cases (first-degree relatives) were not genetically tested (45.8%).

Coronary artery calcium scores

Table 2 shows the number of individuals (%) by categories of CAC and age intervals among controls and clinical FH cases. A CAC score above zero was found in 49.2% (59/120) in the clinical FH group compared to 29.1% (34/117) in the control group (p = 0.002). A calcium score above the 50th percentile for sex and age was reported in 38.3% (46/120) among cases and 21.4% (25/117) in controls (p = 0.005). In the youngest age group <45 years, all cases had a CAC score of zero, but the proportions of subjects with elevated calcium levels increased with increasing age intervals in both cases and controls. The most notable difference was seen among subjects with a CAC score above 300, as 16.7% of cases compared to 4.3% of controls had calcium levels in this category.

Table 3 shows the association between categories of CAC scores and clinical FH. We found statistically significant higher odds of clinical FH with increasing levels of CAC scores compared to controls. Thus, in analyses adjusted for age- and sex, the OR of clinical FH was 2.12 (95% CI 1.11; 4.06, p = 0.024) in those with a CAC score ranging between 1 and 299 and 7.04 (95% CI 2.28; 21.76, p = 0.001) in those with a CAC score ≥300 when compared with subjects with a CAC level of zero. In analyses including adjustment for smoking, hypertension, waist circumference and levels of lipoprotein(a), we observed a similar pattern of association with an OR of clinical FH of 2.23 (95% CI 1.07; 4.64, p = 0.032) in those with a CAC score ranging between 1 and 299 and 5.59 (95% CI 1.65; 18.94; p = 0.006) in those with a CAC score ≥300. Also, we observed statistically significant associations between CAC scores >0 and CAC scores >50th percentile for age and sex and clinical FH when compared with a CAC score of zero with a multivariate adjusted OR of 2.66 (95% CI 1.32; 5.37, p = 0.006) and 2.26 (95% CI 1.13; 4.54, p = 0.022), respectively.

In supplemental analyses of the associations between categories of CAC scores and subtypes of clinical FH cases (Table 4), we found the highest odds in clinical FH cases with a PRS >80th percentile in analyses adjusted for age and sex. Thus, the OR for a CAC score above zero was 5.50 (95% CI 2.18; 13.85, p < 0.001), the OR for a CAC score >50th percentile for age and sex was 4.99 (95% CI 2.14; 11.64, p < 0.001) and the OR for a CAC score ≥300 was 20.49 (95% CI 4.54; 92.47, p < 0.001). For subjects with PRS <80th percentile and those not genetically tested, we found weaker and not statistically significant associations compared to controls. Interestingly, we found higher odds of a PRS >80th percentile when compared to those with a PRS ≤80th percentile by categories of CAC (Table 5).

Table 1
General characteristics of the study population.

	Controls (n = 117)	Clinical FH (n = 120)	p-value
Matching variables			
Age, years (SD)	55.2 (11.4)	56.6 (11.3)	0.319
Women (%)	55.6 (65)	57.5 (69)	0.794
General characteristics			
Waist circumference, cm (SD)	88.3 (13.1)	94.6 (11.4)	<0.001
Physical activity, % (n)			
<1 h/week	27.4 (32)	26.7 (32)	0.274
1–3 h/week	42.7 (50)	51.7 (62)	
>3 h/week	29.9 (35)	21.7 (26)	
Education, % (n)			
Low	19.7 (23)	25.0 (30)	0.446
Medium	52.1 (61)	44.2 (53)	
High	28.2 (33)	30.8 (37)	
Smoking, % (n)			
Never	47.0 (55)	37.5 (45)	0.323
Former	32.5 (38)	36.7 (44)	
Current	20.5 (24)	25.8 (31)	
Hypertension, % (n)	43.6 (51)	54.2 (65)	0.123
Diabetes mellitus, % (n)	1.7 (2)	4.2 (5)	0.446
Lipid-lowering treatment, % (n)	0 (0)	50.0 (60)	<0.001
Lipoprotein(a), % (n)			
<50th percentile	57.3 (67)	43.3 (52)	0.024
50–80th percentile	29.9 (35)	30.8 (37)	
80–100th percentile	12.8 (15)	25.8 (31)	
Agatston CAC-score, % (n)			
CAC >0	29.1 (34)	49.2 (59)	0.002
CAC >50th percentile	21.4 (25)	38.3 (46)	0.005
CAC = 0	70.9 (83)	50.8 (61)	0.001
CAC-score 1-299	24.8 (29)	32.5 (39)	
CAC-score ≥300	4.3 (5)	16.7 (20)	

Abbreviations: SD, standard deviation; CAC, coronary artery calcium.

Table 2
Number of individuals (%) by categories of CAC and age intervals.

Age intervals	Controls, % (n)			Clinical FH, % (n)		
	Individuals	CAC>0	CAC>50th percentile	Individuals	CAC>0	CAC>50th percentile
≤45	18.8 (22)	0 (0)	0 (0)	16.7 (20)	0 (0)	0 (0)
>45 ≤ 55	24.8 (29)	17.2 (5)	17.2 (5)	23.3 (28)	42.9 (12)	35.7 (10)
>55 ≤ 65	35.0 (41)	41.5 (17)	34.2 (14)	33.3 (40)	65.0 (26)	60.0 (24)
>65	21.4 (25)	48.0 (12)	24.0 (6)	26.7 (32)	65.6 (21)	37.5 (12)
Total	117	29.1 (34)	21.4 (25)	120	49.2 (59)	38.3 (46)

Abbreviations: CAC, coronary artery calcium.

Table 3
Association between CAC score and clinical FH.

	Age- and sex adjusted OR (95% CI)	p-value	Multivariable adjusted OR[a] (95% CI)	p-value
CAC score				
0	1 (reference)		1 (reference)	
>0	2.68 (1.44; 4.98)	0.002	2.66 (1.32; 5.37)	0.006
≤50th percentile	1 (reference)		1 (reference)	
>50th percentile	2.38 (1.29; 4.40)	0.005	2.26 (1.13; 4.54)	0.022
0	1 (reference)		1 (reference)	
1-299	2.12 (1.11; 4.06)	0.024	2.23 (1.07; 4.64)	0.032
≥300	7.04 (2.28; 21.76)	0.001	5.59 (1.65; 18.94)	0.006

Abbreviations: CAC, coronary artery calcium; FH, familial hypercholesterolemia; OR, odds ratio; CI, confidence interval.
[a] Multivariable adjustments included smoking, hypertension, waist circumference and lipoprotein(a).

Discussion

In this study, we found that CAC score was strongly associated with clinical FH compared to controls and the observed measures of association were highest in individuals with a CAC score ≥300, usually classified as a very high cardiovascular risk. Also, we found that clinical FH cases with a likely polygenic cause had high levels of CAC compared to controls as well as FH cases without a polygenic cause.

This study had limitations that should be considered. First, we primarily recruited clinical FH cases within a nationwide laboratory database covering cholesterol levels on approximately 60% of the entire Faroese population and our study population was therefore limited to individuals that had cholesterol levels measured previously as part of clinical practice. Furthermore, we initially invited subjects with a previously measured of LDL-C above 6.7 mmol/L,

Table 4
Association between CAC score and subtypes of clinical FH.

	Age- and sex adjusted OR (95% CI)			Multivariable adjusted OR[a] (95% CI)		
	PRS >80th percentile (n = 39)	PRS ≤80th percentile (n = 24)	Not genetically tested (n = 55)	PRS >80th percentile (n = 39)	PRS ≤80th percentile (n = 24)	Not genetically tested (n = 55)
CAC-score						
0	1 (reference)			1 (reference)		
>0	5.50 (2.18; 13.85)	1.66 (0.62; 4.44)	1.83 (0.83; 4.03)	6.29 (2.05; 19.33)	2.09 (0.60; 7.24)	1.76 (0.74; 4.22)
p-value	<0.001	0.317	0.135	0.001	0.244	0.199
<50th percentile	1 (reference)			1 (reference)		
>50th percentile	4.99 (2.14; 11.64)	1.97 (0.75; 5.19)	1.29 (0.58; 2.89)	6.22 (2.11; 18.34)	2.69 (0.76; 9.58)1	16 (0.47; 2.88)
p-value	<0.001	0.168	0.536	0.001	0.127	0.753
0	1 (reference)			1 (reference)		
1-299	4.27 (1.61; 11.29)	0.89 (0.27; 2.91)	1.74 (0.77; 3.94)	4.51 (1.38; 14.73)	1.22 (0.29; 5.07)	1.78 (0.73; 4.38)
p-value	0.003	0.847	0.187	0.013	0.782	0.207
≥300	20.49 (4.54; 92.47)	7.64 (1.73; 33.72)	2.51 (0.56; 11.22)	24.99 (4.33; 144.37)	17.81 (2.12; 149.70)	1.68 (0.34; 8.23)
p-value	<0.001	0.007	0.227	<0.001	0.008	0.522

Abbreviations: CAC, coronary artery calcium; FH, familial hypercholesterolemia; OR, odds ratio; CI, confidence interval; PRS, polygenic risk score.
[a] Multivariable adjustments included smoking, hypertension, waist circumference and lipoprotein(a).

Table 5
Association between CAC score in subjects with a PRS >80th percentile (n = 39) compared with a PRS ≤80th percentile (n = 24).

	Age- and sex adjusted OR (95% CI)	p-value	Multivariable adjusted OR[a] (95% CI)	p-value
CAC score				
0	1 (reference)		1 (reference)	
>0	4.58 (1.21; 17.35)	0.025	8.05 (1.65; 39.29)	0.010
≤50th percentile	1 (reference)		1 (reference)	
>50th percentile	3.44 (1.02; 11.53)	0.046	5.12 (1.25; 20.90)	0.023
0	1 (reference)		1 (reference)	
1-299	5.63 (1.32; 24.11)	0.020	9.66 (1.77; 52.73)	0.009
≥300	3.11 (0.60; 16.06)	0.175	5.65 (0.86; 37.33)	0.072

Abbreviations: CAC, coronary artery calcium; FH, familial hypercholesterolemia; OR, odds ratio; CI, confidence interval.
[a] Multivariable adjustments included smoking and hypertension.

which corresponds to definite FH according to the MEDPED criteria and this approach was chosen to improve the cost-benefit of the recruitment process by limiting our comprehensive examinations to those individuals most likely to fulfill a clinical diagnosis of FH. However, this may imply that our study population did not include clinical FH cases with less severe hypercholesterolemia. Also, we did not take into account potential cluster effects among relatives. We genetically tested all probands with clinical FH with LDL-C above 6.7 mmol/l. However, first-degree relatives to probands with clinical FH as well as controls were not genetically tested for pathogenic FH mutations, which is a limitation of this study. This decision was based on economic considerations as molecular screening of genetic mutations is expensive. However, we considered the presence of pathogenic FH mutations unlikely among healthy controls and clinical FH cases that were first-degree relatives to mutation negative probands with clinical FH. Also, no first-degree relatives to probands with identified pathogenic FH mutations were included in the study. We used the CAC score as a surrogate of subclinical atherosclerosis, but we did not have information on possible noncalcified plaques. We adjusted for major ASCVD risk factors including age, sex, smoking status, hypertension, waist circumference and levels of lipoprotein(a), but we cannot rule out residual confounding. Finally, supplemental analyses of subtypes of clinical FH were limited by few cases and multivariate adjusted OR for subjects with a CAC score ≥300 should in particular be interpreted with great caution due to low statistical power. However, these results were presented for the completeness of our analyses. Also, analyses comparing clinical FH cases with a high PRS with a low PRS were also limited by a low statistical power and hence wide confidence intervals and should also be interpreted with caution. Finally, our study population included Faroese men and women fulfilling a clinical FH diagnosis without history of ASCVD, which may limit the generalizability of our study findings to other populations.

CAC is considered a solid surrogate marker of subclinical atherosclerosis, and the absence of CAC deposition has been identified as a favorable prognostic marker also among individuals at high cardiovascular risk [19,20,28]. Interestingly, in our study, we found that in 50% of cases with clinical FH and a history of severely elevated plasma LDL-C levels had a CAC score of zero. However, several other studies have reported, that in patients with highly elevated LDL-C levels, absence of calcified plaques may be frequent and in particular among subjects below 45 years of age [4,29]. Thus, a systematic review by Mszar et al. [29] including nine studies representing 1176 asymptomatic heterozygote FH patients with a mean age of 47 years, found a pooled prevalence of a CAC score of zero to be 45%. In a recent study by Mortensen et al. [30], 46.2% of 948 subjects (median age 57, 57.2% women) with LDL-C >4.9 mmol and with presumptive symptoms of CVD had absence of calcified plaques. This might suggest that some individuals might be less prone to development of early ASCVD despite a genetic susceptibility and lifelong exposure to significantly high LDL-C levels. Miname and Gallo [18,20] found a very low risk of ASCVD events in asymptomatic individuals with proven genetic diagnosis of FH and CAC scores of 0 after a median of 3.7 and 2.7 years of follow-up, respectively. These studies suggest the role of the CAC score in risk stratification in subjects with FH. Sandesara [19] followed 246 individuals (mean age 63 years, 58% women) with LDL-C levels ≥4.9 mmol/L from The Multi-Ethnic Study of Atherosclerosis (MESA) for a median of 13.2 years, and those with a CAC score of

0 had a risk of cardiovascular events of 4.7 per 1000 person-years, while the ASCVD risk was 5-fold higher in those with CAC scores greater than 0.

In our study, we observed that subjects with clinical FH had higher levels of CAC compared to controls, which is supported by few previous studies [31,32]. However, it has been observed over the last few years that the risk of ASCVD varies more among persons with FH than previously recognized. In fact, several studies have shown that subjects with monogenic FH have a higher risk of ASCVD compared to severe hypercholesterolemia due to other causes [10–12]. Khera et al. found, that the presence of a monogenic FH causing mutation implicates a 3-fold greater ASCVD risk compared with individuals with severe hypercholesterolemia with comparable LDL-C levels who did not carry an FH-variant [10]. Similarly, previous studies have shown heterogeneity in CAC levels among individuals with FH and that subjects with a monogenic cause of FH had higher severity of preclinical atherosclerosis than those with a polygenic cause [11,33]. Also, a previous study found that among those referred to specialized lipid clinics with suspected FH, up to 70% were mutation negative [34] and depending on the definitions of a high polygenic score, approximately 20–40% of mutation negative individuals have increased polygenic risk [34]. These individuals are considered to have an elevated ASCVD risk compared to the general population [13,28], but one study found no statistically significant difference in CAC levels in 40 polygenic/undetermined FH cases compared to healthy controls [33].

LDL-C is a causal factor for development of ASCVD [35] and subjects with severely elevated LDL-C levels are by guidelines uniformly considered to be at high risk independently of the presence of other cardiovascular risk factors. According to current ESC/EAS dyslipidemia guidelines [4], subjects with FH without other major risk factors and subjects with markedly elevated single risk factors, e.g. LDL >4.9 mmol/L (>190 mg/dL), are considered at high risk and the recommended intervention strategies are lifestyle interventions and concomitant lipid-lowering treatment. In these subjects, an LDL-C reduction of ≥50% from baseline and an LDL-C goal of <1.8 mmol/L is recommended. However, residual elevations in LDL-C persist in many individuals despite dual therapy with statins and ezetimibe [36]. These patients could be eligible for more intensive treatment with PCSK9-inhibitors or other potent LDL-C lowering medications. Interestingly, current guidelines do not differentiate treatment goals between individuals with monogenic FH and those without monogenic mutations identified that fulfill a clinical diagnosis of FH.

We conducted advanced genetic analyses in subjects with clinical FH for whom family pedigrees suggested monogenic inheritance. Surprisingly, we found only two patients (3.1%) with monogenic FH among those genetically tested (n = 65). We found strong associations between categories of Agatston CAC score and clinical FH with the highest observed measures of associations found in those with a CAC score ≥300, which can be classified as very high cardiovascular risk. In supplemental analyses we found, that subjects with polygenic FH (PRS >80th percentile) had higher levels of CAC compared to those with PRS ≤80th percentile which again had higher levels of CAC than those, who were not genetically tested. This could be expected, as those individuals who were not genetically tested also would be those with the lowest LDL-C levels.

In conclusion, we found that CAC levels were higher in the older age groups in both clinical FH cases and controls. In this study population with a very low frequency of monogenic FH mutations identified we found a strong positive association between CAC levels and clinical FH, in particular in those with a likely polygenic cause. Further studies investigating risk stratification in individuals fulfilling a clinical diagnosis of FH including polygenic causes are warranted.

Financial support

The study was funded by the Research Council Faroe Islands (grant number: 0334), the National Hospital of the Faroe Islands, Amgen AB and Betri P/F. The funding sources had no impact on the study design, data analysis, report writing or on the decision to submit the paper for publication.

Author contributions

All authors contributed to the conception of this work. SB drafted the first draft of the manuscript, performed the statistical analyses and created the tables. CSB, MSN, JJ, TZ, IBL, SLC, PS, EBS and AMJ assisted in the planning of the statistical analyses and data interpretation. CSB and SLC supervised the conduct of the statistical analyses. All authors have contributed to the content and have approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2022.10.002.

References

[1] Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. Nat Rev Dis Prim 2017;3:17093. https://doi.org/10.1038/nrdp.2017.93.
[2] Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol 2019;16:9–20. https://doi.org/10.1038/s41569-018-0052-6.
[3] Di Costanzo A, Minicocci I, D'Erasmo L, Commodari D, Covino S, Bini S, et al. Refinement of pathogenicity classification of variants associated with familial hypercholesterolemia: implications for clinical diagnosis. J Clin Lipidol 2021;15:822–31. https://doi.org/10.1016/j.jacl.2021.10.001.
[4] Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Eur Heart J 2019:111. https://doi.org/10.1093/eurheartj/ehz455. –88.
[5] Betteridge DJ, Broome K, Durrington PN, Mann JI, Miller JP, Neil HAW, et al. Risk of fatal coronary heart disease in familial hypercholesterolaemia. Br Med J 1991;303:893–6.
[6] Williams RR, Hunt SC, Schumacher MC, Hegele RA, Leppert MF, Ludwig EH, et al. Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. Am J Cardiol 1993;72:171–6. https://doi.org/10.1016/0002-9149(93)90155-6.
[7] Sturm AC, Knowles JW, Gidding SS, Ahmad ZS, Ahmed CD, Ballantyne CM, et al. Clinical genetic testing for familial hypercholesterolemia: JACC scientific expert panel. J Am Coll Cardiol 2018;72:662–80. https://doi.org/10.1016/j.jacc.2018.05.044.
[8] Olmastroni E, Gazzotti M, Arca M, Averna M, Pirillo A, Catapano AL, et al. Twelve variants polygenic score for low-density lipoprotein cholesterol distribution in a large cohort of patients with clinically diagnosed familial hypercholesterolemia with or without causative mutations. J Am Heart Assoc 2022;11:23668. https://doi.org/10.1161/JAHA.121.023668.
[9] Langsted A, Kamstrup PR, Benn M, Tybjærg-Hansen A, Nordestgaard BG. High lipoprotein(a) as a possible cause of clinical familial hypercholesterolaemia: a prospective cohort study. Lancet Diabetes Endocrinol 2016;4:577–87. https://doi.org/10.1016/S2213-8587(16)30042-0.
[10] Khera AV, Won HH, Peloso GM, Lawson KS, Bartz TM, Deng X, et al. Diagnostic yield and clinical utility of sequencing familial hypercholesterolemia genes in patients with severe hypercholesterolemia. J Am Coll Cardiol 2016;67:2578–89. https://doi.org/10.1016/j.jacc.2016.03.520.
[11] Sharifi M, Higginson E, Bos S, Gallivan A, Harvey D, Li KW, et al. Greater preclinical atherosclerosis in treated monogenic familial hypercholesterolemia vs. polygenic hypercholesterolemia. Atherosclerosis 2017;263:405–11. https://doi.org/10.1016/j.atherosclerosis.2017.05.015.
[12] Trinder M, Li X, DeCastro ML, Cermakova L, Sadananda S, Jackson LM, et al. Risk of premature atherosclerotic disease in patients with monogenic versus

polygenic familial hypercholesterolemia. J Am Coll Cardiol 2019;74:512–22. https://doi.org/10.1016/j.jacc.2019.05.043.
[13] Trinder M, Francis GA, Brunham LR. Association of monogenic vs polygenic hypercholesterolemia with risk of atherosclerotic cardiovascular disease. JAMA Cardiol 2020;5:390–9. https://doi.org/10.1001/jamacardio.2019.5954.
[14] Paixao ARM, Ayers CR, El Sabbagh A, Sanghavi M, Berry JD, Rohatgi A, et al. Coronary artery calcium improves risk classification in younger populations. JACC Cardiovasc Imaging 2015;8:1285–93. https://doi.org/10.1016/j.jcmg.2015.06.015.
[15] Blaha MJ, Cainzos-Achirica M, Greenland P, McEvoy JW, Blankstein R, Budoff MJ, et al. Role of coronary artery calcium score of zero and other negative risk markers for cardiovascular disease : the multi-ethnic study of atherosclerosis (MESA). Circulation 2016;133:849–58. https://doi.org/10.1161/CIRCULATIONAHA.115.018524.
[16] Ferencik M, Pencina KM, Liu T, Ghemigian K, Baltrusaitis K, Massaro JM, et al. Coronary artery calcium distribution is an independent predictor of incident major coronary heart disease events: results from the framingham heart study. Circ Cardiovasc Imag 2017. https://doi.org/10.1161/CIRCIMAGING.117.006592.
[17] Bittencourt MS, Blankstein R, Blaha MJ, Sandfort V, Agatston AS, Budoff MJ, et al. Implications of coronary artery calcium testing on risk stratification for lipid-lowering therapy according to the 2016 European Society of Cardiology recommendations: the MESA study. Eur J Prev Cardiol 2018;25:1887–98. https://doi.org/10.1177/2047487318788930.
[18] Miname MH, Bittencourt MS, Moraes SR, Alves RIM, Silva PRS, Jannes CE, et al. Coronary artery calcium and cardiovascular events in patients with familial hypercholesterolemia receiving standard lipid-lowering therapy. JACC Cardiovasc Imaging 2019;12:1797–804. https://doi.org/10.1016/j.jcmg.2018.09.019.
[19] Sandesara PB, Mehta A, O'Neal WT, Kelli HM, Sathiyakumar V, Martin SS, et al. Clinical significance of zero coronary artery calcium in individuals with LDL cholesterol ≥190 mg/dL: the Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2020;292:224–9. https://doi.org/10.1016/j.atherosclerosis.2019.09.014.
[20] Gallo A, Pérez de Isla L, Charrière S, Vimont A, Alonso R, Muñiz-Grijalvo O, et al. The added value of coronary calcium score in predicting cardiovascular events in familial hypercholesterolemia. JACC Cardiovasc Imaging 2021;14:2414–24. https://doi.org/10.1016/j.jcmg.2021.06.011.
[21] Borg SÁ, Nielsen MRS, Søgaard P, Lundbye-Christensen S, Jóanesarson J, Zaremba T, et al. Familial hypercholesterolaemia: a study protocol for identification and investigation of potential causes and markers of subclinical coronary artery disease in the Faroe Islands. BMJ Open 2022;12:e050857. https://doi.org/10.1136/bmjopen-2021-050857.
[22] Starr B, Hadfield SG, Hutten BA, Lansberg PJ, Leren TP, Damgaard D, et al. Development of sensitive and specific age- and gender-specific low-density lipoprotein cholesterol cutoffs for diagnosis of first-degree relatives with familial hypercholesterolaemia in cascade testing. Clin Chem Lab Med 2008;46:791–803. https://doi.org/10.1515/CCLM.2008.135.
[23] Besseling J, Kindt I, Hof M, Kastelein JJP, Hutten BA, Hovingh GK. Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14,000 mutation carriers. Atherosclerosis 2014;233:219–23. https://doi.org/10.1016/j.atherosclerosis.2013.12.020.
[24] Futema M, Shah S, Cooper JA, Li K, Whittall RA, Sharifi M, et al. Refinement of variant selection for the LDL cholesterol genetic risk score in the diagnosis of the polygenic form of clinical familial hypercholesterolemia and replication in samples from 6 countries. Clin Chem 2015;61:231–8. https://doi.org/10.1373/clinchem.2014.231365.
[25] Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. Lancet 2013;381:1293–301. https://doi.org/10.1016/S0140-6736(12)62127-8.
[26] 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;113:30–7. https://doi.org/10.1161/CIRCULATIONAHA.105.580696.
[27] Hecht HS, Blaha MJ, Kazerooni EA, Cury RC, Budoff M, Leipsic J, et al. CAC-DRS: coronary artery calcium data and reporting system. An expert consensus document of the society of cardiovascular computed tomography (SCCT). J Cardiovasc Comput Tomogr 2018. https://doi.org/10.1016/j.jcct.2018.03.008.
[28] Miname MH, Santos RD. Reducing cardiovascular risk in patients with familial hypercholesterolemia: risk prediction and lipid management. Prog Cardiovasc Dis 2019;62:414–22. https://doi.org/10.1016/j.pcad.2019.10.003.
[29] Mszar R, Grandhi GR, Valero-Elizondo J, Virani SS, Blankstein R, Blaha M, et al. Absence of coronary artery calcification in middle-aged familial hypercholesterolemia patients without atherosclerotic cardiovascular disease. JACC Cardiovasc Imaging 2020. https://doi.org/10.1016/j.jcmg.2019.11.001.
[30] Mortensen MB, Caínzos-Achirica M, Steffensen FH, Bøtker HE, Jensen JM, Sand NPR, et al. Association of coronary plaque with low-density lipoprotein cholesterol levels and rates of cardiovascular disease events among symptomatic adults. JAMA Netw Open 2022;5:e2148139. https://doi.org/10.1001/jamanetworkopen.2021.48139.
[31] Martinez LRC, Miname MH, Bortolotto LA, Chacra APM, Rochitte CE, Sposito AC, et al. No correlation and low agreement of imaging and inflammatory atherosclerosis' markers in familial hypercholesterolemia. Atherosclerosis 2008;200:83–8. https://doi.org/10.1016/j.atherosclerosis.2007.12.014.
[32] Miname MH, Ribeiro MS, Filho JP, Avila LF, Bortolotto LA, Martinez LRC, et al. Evaluation of subclinical atherosclerosis by computed tomography coronary angiography and its association with risk factors in familial hypercholesterolemia. Atherosclerosis 2010;213:486–91. https://doi.org/10.1016/j.atherosclerosis.2010.10.001.
[33] D'erasmo L, Minicocci I, Di Costanzo A, Pigna G, Commodari D, Ceci F, et al. Clinical implications of monogenic versus polygenic hypercholesterolemia: long-term response to treatment, coronary atherosclerosis burden, and cardiovascular events. J Am Heart Assoc 2021;10:18932. https://doi.org/10.1161/JAHA.120.018932.
[34] Jacob E, Hegele RA. Monogenic versus polygenic forms of hypercholesterolemia and cardiovascular risk: are there any differences? Curr Atherosclerosis Rep 2022;1:1–8. https://doi.org/10.1007/s11883-022-01018-6.
[35] Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement fromthe European Atherosclerosis Society Consensus Panel. Eur Heart J 2017;38:2459–72. https://doi.org/10.1093/eurheartj/ehx144.
[36] Perez De Isla L, Alonso R, Watts GF, Mata N, Saltijeral Cerezo A, Muñiz O, et al. Attainment of LDL-cholesterol treatment goals in patients with familial hypercholesterolemia: 5-year SAFEHEART registry follow-up. J Am Coll Cardiol 2016;67:1278–85. https://doi.org/10.1016/j.jacc.2016.01.008.