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Familial hypercholesterolaemia: a study protocol for identification and investigation of potential causes and markers of subclinical coronary artery disease in the Faroe Islands

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ABSTRACT

Introduction Familial hypercholesterolaemia (FH) is the most common monogenic autosomal dominant genetic disorder and is associated with a high risk of premature atherosclerotic cardiovascular disease. The prevalence of FH has been reported to be particularly high in certain founder populations. The population of the Faroe Islands is a founder population, but the prevalence of FH has never been investigated here. We aim to assess the prevalence of FH and to describe the genetic and clinical characteristics and potential causes of FH in the Faroe Islands. Furthermore, we aim to investigate whether indicators of subclinical coronary artery disease are associated with FH.

Methods and analysis The prevalence of FH will be estimated based on an electronic nationwide laboratory database that includes all measurements of plasma lipid levels in the Faroe Islands since 2006. Subsequently, we will identify and invite subjects aged between 18 and 75 years registered with a plasma low-density lipoprotein cholesterol above 6.7 mmol/L for diagnostic evaluation. Eligible FH cases will be matched to controls on age and sex. We aim to include 120 FH cases and 120 controls. Detailed information will be collected using questionnaires and interviews, and a physical examination will be undertaken. An adipose tissue biopsy and blood samples for genetic testing, detailed lipid analyses and samples for storage in a biobank for future research will be collected. Furthermore, FH cases and controls will be invited to have a transthoracic echocardiography and a cardiac CT performed.

Ethics and dissemination The project has been approved by the Ethical Committee and the Data Protection Agency of the Faroe Islands. The project is expected to provide important information, which will be published in international peer-reviewed journals.

Strengths and limitations of this study

► The prevalence of familial hypercholesterolaemia (FH) in the whole country of the Faroe Islands will be estimated using an electronic nationwide laboratory database that includes all laboratory measurements of plasma cholesterol levels in the Faroe Islands since 2006 covering approximately 60% of the population.
► Detailed information from questionnaires and interviews will be collected from subjects with clinical FH and a randomly selected control group and a comprehensive physical examination will be conducted including collection of biological material.
► Potential objective markers of subclinical coronary artery disease, which may be used for risk stratification and identification of subjects who may require additional and more aggressive lipid-lowering treatment to reduce their risk of developing atherosclerotic cardiovascular disease will be investigated.
► The prevalence of FH will be based on low-density lipoprotein cholesterol levels alone, which may underestimate the prevalence.
► Genetic testing will only be performed in a subset of the study participants.

by lifelong highly elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) resulting in a markedly increased risk of (premature) atherosclerotic cardiovascular disease (ASCVD).[1–3] Accumulating evidence supports that aggressive treatment of FH from early ages may eliminate the increased risk of coronary artery disease (CAD)[4 5] but a major challenge is that the vast majority of individuals with FH are undiagnosed and under-treated.[6] The prevalence of heterozygote FH in the general population is unknown in most countries of the world, but a recent meta-analysis of 11 million individuals including

INTRODUCTION

Familial hypercholesterolaemia (FH) is the most common human monogenic autosomal dominant genetic disorder characterised

33 036 patients with FH reported an overall prevalence of FH of 1 in 313.[7] However, the prevalence of FH is more frequent—up to 1 in 100 individuals—in certain founder populations owing to accumulation of pathogenic FH genetic variants as a result of consanguinity and genetic drift.[8]

FH can be diagnosed by identification of a known pathogenetic mutation, but molecular testing is expensive and the underlying genetics of FH is more complex than previously thought.[8] In clinical practice, an FH diagnosis is often based on clinical scores such as the Dutch Lipid Clinic Network (DLCN) criteria,[9] the Simon Broome criteria[10] or the Make Early Diagnosis Prevent Early Death (MEDPED) criteria.[11] The DLCN and the Simon Broome criteria both include information on genetic testing as an important part of making a definite diagnosis of FH, but a diagnosis is often made from a clinical evaluation alone due to cost and availability of genetic analysis. Interestingly, studies have shown that 20%–60% of patients with phenotypical FH according to clinical criteria have no detectable mutation in the most common genes causing FH.[12] Explanations for this remain uncertain, but polygenic predisposition to elevated LDL-C and gene–environment interactions as well as other molecular aetiologies are likely to be of importance for the variable inheritance patterns and clinical presentations observed in patients with FH.[8] Thus, further research on the complexity of the underlying pathophysiology of FH including molecular genetics and importance of environmental lifestyle factors is warranted. Also, limited knowledge exists on whether markers of subclinical ASCVD can be used to personalise medical treatment by identifying patients that may require additional and more aggressive lipid-lowering treatment to reduce their risk of ASCVD events.

The population of the Faroe Islands has been significantly influenced by genetic drift and is considered the genetically most homogeneous and isolated population in the North Atlantic region.[13] The population of the Faroe Islands was established by few founders and the isolated geographical location limited migration and resulted in a slow growth over several hundreds of years followed by a rapid increase during recent years to the present 52 000 inhabitants.[13] Thus, a significant proportion of Faroese may have inherited gene variations from a relatively close ancestor and several genetic disorders have been shown to be more prevalent in the Faroe Islands than elsewhere.[14–17] While the prevalence of FH in the Faroe Islands is unknown, a review of previous plasma LDL-C measurements obtained in the Faroe Islands has indicated that a significant number of individuals may have markedly elevated plasma LDL-C levels suggestive of FH.

The objectives of this project are to investigate the prevalence of FH and to describe the genetic and clinical characteristics and potential causes of FH in the Faroe Islands. Furthermore, we aim to investigate whether objective markers of subclinical CAD are more common in subjects with FH, which could be important for their treatment.

METHODS AND ANALYSES
Study population(s) and study design(s)
We will describe the lipid distribution (total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C) and triglycerides) and estimate the prevalence of FH in the Faroe Islands according to the DLCN and MEDPED criteria based on registered plasma cholesterol levels measured in hospitals in the Faroe Islands between January 2006 and September 2020. In the Faroe Islands, all blood samples from both hospital and general practitioners are collected and analysed at hospitals and registered in a nationwide clinical laboratory database (BCC-Web, CGI). Subsequently, we will investigate the clinical characteristics and potential causes of FH in the Faroe Islands as well as objective markers of subclinical CAD using a case–control design. Potential FH cases will be identified based on diagnostic evaluation of subjects registered with a plasma LDL-C above 6.7 mmol/L. Subjects aged between 18 and 75 years that meet definite or probable FH according to DLCN criteria, definite FH according to the Simon Broome criteria and/or definite FH according to the MEDPED criteria will be considered eligible for inclusion as FH cases (table 1). Also, first-degree relatives to subjects with FH that meet age-specific and sex-specific LDL-C cut-offs identified during cascade screening will be considered valid FH cases.[18 19]

We will include subjects with FH until a total of 120 FH cases without persistent atrial fibrillation/flutter or a history of ASCVD have been enrolled into the substudy to have an advanced transthoracic echocardiography and cardiac CT performed. FH cases included in the substudy will be matched to cases by age and sex. Potential controls will be identified in the background population through linkage with the National Register of Persons in the Faroe Islands. Subjects without persistent atrial fibrillation/flutter and history of ASCVD and an LDL-C <3.5 mmol/L, DLCN score ≤3 and current use of lipid-lowering therapy will be considered eligible as controls. We decided to exclude subjects with persistent atrial fibrillation/flutter in the substudy because these arrhythmias may interfere with the quality of the imaging modalities. Lipid-lowering treatment is defined as current treatment with a statin in any dose, ezetimibe, proprotein-convertase subtilisin/kexin type 9 (PCSK-9) inhibitors, fibrates, red yeast rice, bile acid sequestrants or nicotinic acid. Subjects either pregnant or with elevated plasma creatinine (>120 μmol/L) will not be considered eligible for cardiac CT.

Recruitment
Potential participants aged between 18 and 75 years registered with a plasma LDL-C >6.7 mmol/L measured between January 2006 and September 2020 in the Faroe Islands will be identified from the laboratory database

Table 1 Comparison between clinical scoring systems for FH used in this study

Lipids	Dutch Lipid Clinic Network criteria[9]	Simon Broome register criteria[10]*	MEDPED criteria for the general population[11]*†
LDL-C (mmol/L)	≥8.5 (8 points) 6.5–8.4 (5 points) 5.0–6.4 (3 points) 4.0–4.9 (1 point)	>4.9 in adults (A) >4.0 in children (A)	>6.7 (≥40 years of age) >6.2 (30–39 years of age) >5.7 (20–29 years of age) >5.2 (<20 years of age)
Clinical history			
ASCVD	Premature CAD (2 points), or premature cerebral or PVD (1 point)‡§	Not applicable	Not applicable
Physical FH stigmata			
Personal	Tendinous xanthomata (6 points), or arcus cornealis before age 45 years (4 points)§	Tendinous xanthomata (B)	Not applicable
Genetics			
Functional DNA mutation in FH genes	LDLR, apoB or PCSK-9 mutation (8 points)	LDLR, apoB or PCSK-9 mutation (C)	Not applicable
Family history			
Premature ASCVD and elevated LDL-C	First-degree relative with premature coronary or vascular disease in a first-degree relative‡ or LDL-C above the 95th percentile (1 point)	MI before age 50 years in a second-degree relative or before 60 years in a first-degree relative (D)	Not applicable
Physical FH stigmata	First-degree relative with tendinous xanthomata and/ or arcus cornealis (2 points)§	Tendinous xanthomata in a first-degree relative (B)	Not applicable
Diagnostic criteria			
	Definite FH >8 points Probable FH 6–8 points Possible FH 3–5 points	Definite FH A+B or C Probable FH A+D	Definite FH includes subjects that meet the LDL-C cut-off point

*We excluded scores related to total cholesterol.
†LDL-C cut-off points are only shown for subjects without relatives with known FH. Specific cut-off points apply to first-degree, second-degree and third-degree relatives with FH.
‡Premature was defined as age before 55 years in men and age 60 years in women.
§Exclusive of each other (only the highest score counts for each group).
apoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; CAD, coronary artery disease; FH, familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MEDPED, Make Early Diagnosis Prevent Early Death; MI, myocardial infarction; PCSK-9, proprotein-convertase subtilisin/kexin type 9; PVD, peripheral vascular disease.

(figure 1). This LDL cut-off defines definite FH according to the MEDPED criteria. Subsequently, subjects will be invited by letter to attend a clinical examination for diagnostic evaluation of FH and eligibility for the study. The invitation letter contains general information on the study and its aims. Prior to the diagnostic evaluation, subjects will be asked to have a screening blood sample taken for measurement of plasma lipids and lipoproteins (total cholesterol, LDL-C, HDL-C and triglycerides) and evaluation of possible secondary causes of dyslipidaemia (table 1) as part of routine clinical practice. Also, detailed questionnaires including medical history, social and life-style factors, use of medications and dietary habits among others will be sent along with the invitation letter. Subjects not responding to the letter invitation within 2 weeks will be contacted by telephone, but no more than three times.

Furthermore, subjects diagnosed with FH will be recommended to advise their first-degree relatives to contact their primary physician for measurement of their lipid profile. First-degree relatives that meet age-specific and sex-specific LDL-C cut-offs[18 19] in the laboratory database will be contacted and invited to attend a clinical examination (figure 1).

Data collection
Self-reported background data
All participants will be asked to report their history of ASCVD and year of possible manifestation of myocardial infarction (yes/no), percutaneous coronary intervention (yes/no), coronary artery bypass graft surgery (CABG) (yes/no), cerebral infarction (yes/no), and angina pectoris (yes/no) or peripheral artery disease (yes/no) diagnosed at a hospital. Also, information on
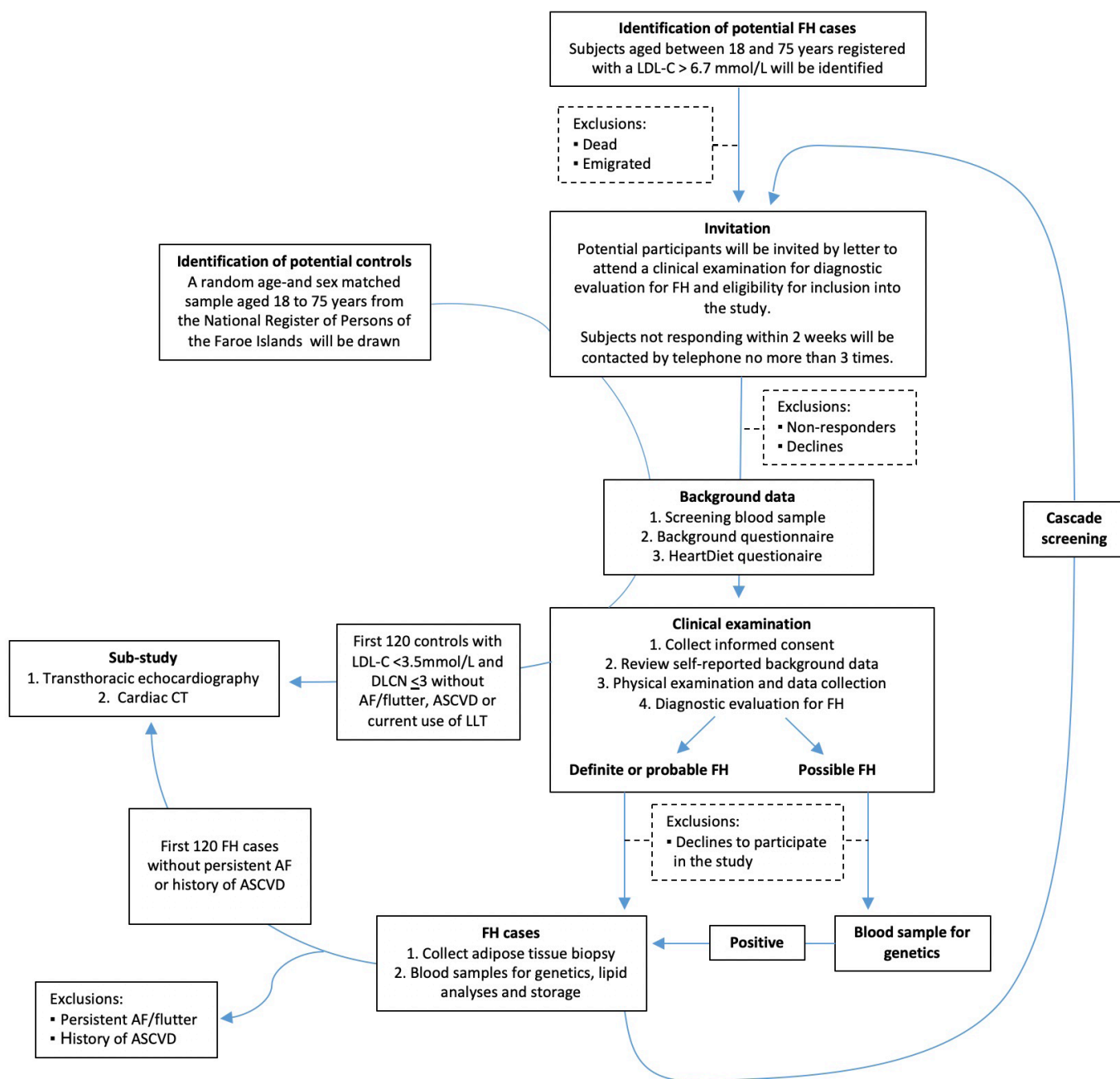
Figure 1 Flow chart showing the identification and enrolment of participants into the study. AF, atrial fibrillation; ASCVD, atherosclerotic cardiovascular disease; DLCN, Dutch Lipid Clinic Network; FH, familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering treatment.

family history of premature (before 55 years of age in men and 60 years of age in women) ASCVD (yes/no) and hypercholesterolaemia (yes/no) in first-degree relatives will be collected. Furthermore, information on social and lifestyle factors including educational level, physical activity, smoking habits and alcohol consumption will also be obtained. Education level will be defined as the highest completed level of education according to the International Standard Classification of Education categorised into low (primary and lower secondary education), medium (general upper secondary education, vocational education and training and short-cycle tertiary education) and high level of education (bachelor and master degrees or equivalent levels and doctoral or equivalent level).[20] Information on physical activity will be collected in terms of hours per week spent on strenuous walking, running, bicycling and swimming categorised into <1, 1–3 and 3 hours and above per week. Information on alcohol consumption will be collected as number of alcohol units per week categorised into <7, 8–14 and 15 units and above per week. One unit of alcohol is defined as 12.5 cL of wine, 33 cL of beer or 4 cL of spirits. Information on smoking status will be categorised into never (smoked less than 100 cigarettes or equivalent during

lifetime), former (smoked more than 100 cigarettes or equivalent during lifetime but not within the last 28 days) and current smokers (smoked more than 100 cigarettes or equivalent during lifetime and has smoked within the last 28 days). For former and current smokers, the average daily use of cigarettes or equivalent and years of smoking will be obtained. We will consider one pipe bowl or cheroot equivalent to three cigarettes, while one cigar will be equivalent to four cigarettes. Furthermore, information on current use of lipid-lowering therapy including number, type, dose and frequency of medications used will be obtained as well as year of initiation of lipid-lowering treatment. Furthermore, the participants will be asked to report use of antihypertensive (yes/no), diuretics (yes/no), antidiabetic and anticoagulant/anti-platelet medication(s) (yes/no) as well as use of fish oil supplements (yes/no). All participants will be asked to report muscle symptoms including muscle pain and/or tenderness (yes/no), muscle tiredness and/or weakness (yes/no) and muscle cramps (yes/no), and whether muscle complaints are symmetrical (yes/no) as well as their severity graded from zero (no complain) to 10 (worst imaginable complain). This is done because statin-associated muscle symptoms are a major reason for statin non-adherence.[21]

The habitual diet of the participants will be assessed using the Danish HeartDiet Questionnaire, which was developed as a clinical tool to evaluate the diet of healthy subjects and patients with dyslipidaemia and/or coronary heart disease.[22 23] The HeartDiet Questionnaire includes 19 items on quality and quantity regarding intake of dairy products, bread, cereals, potato/rice/pasta, fats, meat, fish, vegetables/legumes, fruits, nuts, sweets and different kinds of snacks and fast food. The quantity of the most frequently consumed foods will be assessed in terms of everyday portion sizes. Each item ranges from one of three to five possible answers with scores empirically assigned ranging from 0 to 18 points, which can be used to calculate a fat-score and a fish-fruit-vegetable score. These two scores range from 0 to 100 points. A heart-healthy diet with respect to intake of saturated fat and a combination of fruit, vegetable, fish and wholegrain intake is defined as a diet fulfilling both a fat-score and fish-fruit-vegetable score of at least 75 points.[22]

Clinical examination

At the clinical examination, all subjects will be assigned a study ID, and date and year of birth and date of examination will be registered. The following data will be collected at the clinical examination (table 2):

Highest measured LDL-C

The highest measured fasting or non-fasting LDL-C (mmol/L) registered in the nationwide clinical laboratory database of the Faroe Islands (BCC-Web, CGI) will be noted. In subjects with no available untreated LDL-C measurement, the highest registered value of LDL-C (mmol/L) on lipid-lowering therapy including number,

type, dose and the frequency of medications used will be registered. Then plasma LDL-C value will be estimated according to average treatment effect of the reported lipid-lowering treatment.[24]

Clinical and family history of ASCVD and hypercholesterolaemia

A clinical history of premature CAD and cerebral and peripheral vascular disease used in the DLCN criteria as well as a history of myocardial infarction used in the Simon Broome criteria will be based on review of the subjects' medical records (table 1). A family history of highly elevated plasma LDL-C, premature coronary vascular disease, premature myocardial infarction, tendinous xanthomata and arcus cornealis in first-degree relatives will be obtained based on interview, physical examination and medical records. Information on clinical and family history of ASCVD and hypercholesterolaemia will be collected by categorisation (yes/no/unknown). Highly elevated plasma LDL-C levels in first-degree relatives will be defined according to the 95th percentile of LDL-C based on Danish data used in Danish Lipid Clinics.[25] We define premature coronary vascular disease as acute myocardial infarction, procedural revascularisation, coronary artery bypass surgery and/or objective evidence of CAD (exercise stress test, stress echocardiography, myocardial perfusion scintigraphy, stress cardiac magnetic resonance, coronary angiography, cardiac CT). Cerebral disease will be defined as a prior ischaemic stroke or a medically (aspirin, clopidogrel, persantine) treated transitory ischaemic attack. Peripheral vascular disease will be defined as relevant symptoms and an ankle-brachial index below 0.9 or procedural revascularisation. Premature cardiovascular disease is defined as age below 55 years in men and below 60 years in women.

Family data

A family tree will be drawn for each participant based on interview, and information on history of cardiovascular disease and hypercholesterolaemia will be registered when available. Also, each subject with included first-degree relatives will be linked by ID numbers.

Physical examination for clinical signs of FH

The physical examination for FH stigmata will include evaluation of arcus cornealis (white or grey opaque full or partial ring in the corneal margin) and tendinous xanthomata (lipid deposits) in the finger extensors and/or the Achilles tendon(s). Also, xanthelasmata (lipid deposits underneath the skin on or around the eyelids) will be searched for (yes/no). Finally, all subjects will be classified according to the DLCN criteria, Simon Broome criteria and MEDPED criteria. The Simon Broome criteria and the MEDPED criteria traditionally include information on elevated total cholesterol in the patient or their relatives as differentiation into lipoprotein particles was not uniformly available. However, total cholesterol may be elevated due to several other causes than FH and therefore we decided to exclude scores based on

Table 2	Overview of data collection
Screening blood analyses	Total cholesterol, LDL-C, HDL-C, triglycerides, haemoglobin, thrombocytes, glucose, haemoglobin A1c, creatinine and eGFR, creatine kinase, ALT, bilirubin, alkaline phosphatase, TSH, albumin and electrolytes (sodium and potassium)
General information	Patient ID, date of birth, date of enrolment and informed consent
Self-reported data	
Personal history of ASCVD	History and calendar year of manifestation of myocardial infarction (yes/no), PCI (yes/no), CABG (yes/no), cerebral infarction (yes/no), and peripheral artery disease (yes/no) and angina pectoris (yes/no) diagnosed at a hospital
Familial history of premature ASCVD and hypercholesterolaemia	First-degree relative with premature* ASCVD (yes/no) and hypercholesterolaemia (yes/no)
Social and lifestyle factors	Educational level (low, medium or high), physical activity (<1, 1–3 or $\geq$3 hours/week), alcohol consumption (<7, 8–14 and >15 units/week), smoking status (never, former and current smokers) and years of smoking (continuous)
Use of lipid-lowering treatment	Type, dose and frequency of lipid-lowering† treatment and year of initiation (calendar year)
Use of other medications	Antihypertensive (yes/no), diuretic (yes/no), antidiabetic, anticoagulant medication(s) (yes/no) as well as use of fish oil supplements (yes/no)
Muscle complaints	Muscle pain and/or tenderness (yes/no), muscle tiredness and/or weakness (yes/no) and muscle cramps (yes/no) and whether muscle complaint is symmetrical (yes/no) and graded severity of muscle complaints (0–10)
Dietary habits	HeartDiet Questionnaire including 19 items on quality and quantity regarding intake of selected foods
Physical examination	
Medical interview and review of medical record	Highest measured or estimated LDL-C, clinical history of premature ASCVD and family history of premature ASCVD, hypercholesterolaemia or clinical signs of FH according to the DLCN criteria and Simon Broome criteria (table 1)
Family tree	A family tree will be drawn and history of ASCVD and hypercholesterolaemia will be registered
History of statin intolerance	Inability to tolerate at least two statins at any dose due to muscle-related symptoms (yes/no), inability to tolerate at least two statins at any dose due to symptoms other than muscle-related symptoms (yes/no) and statin naïve (yes/no)
Anthropometrics and blood pressure	Abdominal waist circumference (cm), height (cm), body weight (kilograms) and blood pressure (mm Hg)
Physical examination for clinical signs of FH	Arcus cornealis (yes/no), tendinous xanthomas (yes/no) and xanthelasmata (yes/no)
Diagnostic criteria for FH	Classification according to the DLCN criteria, Simon Broome criteria and the MEDPED criteria (table 1)
ECG	12-lead electronic ECG
Hand-grip strength test	Average hand muscle strength (kilograms)
Biological material	Adipose tissue sample, venous blood samples for genetic testing for FH, detailed lipid analyses and storage in biobank
Substudy	Eligibility for inclusion into substudy (yes/no). Invitation for transthoracic echocardiography and calcium score measurement by cardiac CT

*Defined as ASCVD before age 55 years in men and age 60 years in women.
†Lipid-lowering treatment was defined as use of atorvastatin, simvastatin, rosuvastatin, pravastatin, fluvastatin, lovastatin, ezetimibe and proprotein-convertase subtilisin/kexin type 9 inhibitors.
ALT, alanine transaminase; ASCVD, atherosclerotic cardiovascular disease; CABG, coronary artery bypass grafting; DLCN, Dutch Lipid Clinical Network; eGFR, estimated glomerular filtration rate; FH, familial hypercholesterolaemia; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MEDPED, Make Early Diagnosis Prevent Early Death; PCI, percutaneous coronary intervention; TSH, thyroid-stimulating hormone.

total cholesterol in the Simon Broome criteria and the MEDPED criteria.

History of statin intolerance

Statin therapy together with lifestyle modification represent the cornerstone in prevention and treatment of ASCVD. Side-effects such as muscle complaints are unfortunately frequent reasons for discontinuation of statin therapy.[21] Information on statin intolerance in this study will be based on self-reported information by the patients and review of their medical records. History of statin intolerance will be defined as inability to tolerate at least two statins at any dose due to muscle-related symptoms that started or increased markedly during statin therapy and stopped after discontinuation (yes/no). We decided not to include information on statin rechallenge or increases in creatine kinase levels on statin therapy in our definition because this information would be impossible to reliably obtain. In addition, we will also obtain information on whether the participants were statin intolerant due to other symptoms than muscle-related symptoms (yes/no). Furthermore, we will identify subjects considered statin naïve defined as less than 2 months of statin therapy (yes/no). Prior statin treatment will be limited to 2 months in order not to exclude subjects that initiated statin treatment but were unable to tolerate continued treatment.

Anthropometrics

Abdominal waist circumference (centimetres) will be measured in standing position using a flexible tape placed on a horizontal plane around the abdomen at the level of the iliac crest. The measurement will be undertaken at the end of a normal expiration. The height (centimetres) and total body weight (kilograms with one decimal) will be obtained in standing position with indoor clothing without wearing shoes.

Blood pressure

The brachial blood pressure will be measured after 5–10 min of rest using an automated blood pressure cuff (Omron Automatic upper arm blood pressure monitor, M3 Comfort, Japan). The lowest of two measurements will be registered.

Electrocardiogram

An electronic 12-lead ECG will be obtained according to international recommendations[26] using a GE Healthcare MAC 2000 resting ECG system.

Hand-grip dynamometer strength test

The maximum achieved muscle strength of the subjects will be investigated (CAMRY Electronic Hand Dynamometer model EH101, USA). The average muscle strength of two measurements (right hand, left hand, right hand and left hand) according to the manufacturer instructions will be registered in kilograms with one decimal for each hand.

Blood samples

Blood samples for genetic analyses, detailed laboratory analyses and samples for storage in a biobank for future research will be collected at the physical examination. A blood sample for genetic analysis will be collected in a 3 mL EDTA vacuum blood tubes and subsequently refrigerated at 5°C. Detailed laboratory analyses including apolipoprotein B (apoB), high sensitive C reactive protein and lipoprotein(a) will be collected in a 6 mL blood tube with a coagulant activator added. The blood sample will be centrifuged at 3220 g for 10 min within 6 hours of collection followed by pipetting into two cryotubes, which subsequently will be stored at −80°C. A 6 mL EDTA vacuum blood tube will be drawn and centrifuged at 3220 g for 10 min within 6 hours of sampling. Plasma, buffy coat and erythrocytes will be transferred to three separate cryotubes and stored at −80°C in a biobank.

Adipose tissue

A subcutaneous adipose tissue biopsy will be taken from the buttock from FH cases and matched controls included in the substudy using a luer-lock system consisting of a needle, a venoject multisample luer adapter and an evacuated blood tube according to the method of Beynen and Katan.[27] The adipose tissue biopsy will be transferred to a cryotube and flushed with nitrogen immediately after collection to limit oxidation. The samples will be stored at −80°C until analysis. The content of 34 individual fatty acids will be expressed as area percentage of total fatty acids when analysed.

Substudy

The primary objective of the substudy is to investigate whether subjects with FH have a higher prevalence of indicators of subclinical CAD as investigated by ECG, echocardiography and/or cardiac CT compared with subjects without FH. Therefore, the substudy is limited to subjects without a history of ASCVD defined as myocardial infarction, procedural revascularisation, CABG and/or objective evidence if ischaemia (exercise stress test, stress echocardiography, myocardial perfusion scintigraphy, stress cardiac magnetic resonance, coronary angiography, cardiac CT), angina pectoris, ischaemic stroke or a medically (aspirin, clopidogrel, persantin) treated transitory ischaemic attack, and symptomatic peripheral vascular disease with ankle-brachial index below 0.9 or procedural revascularisation.

Echocardiography

Patients will be scanned using Vivid E9 and E95 ultrasound scanners (GE Vingmed, Norway). Two-dimensional (2D) echocardiographic measures including interventricular septum, left ventricular (LV) posterior wall thickness, LV end-diastolic dimensions and left atrial (LA) dimensions (end-systole) will be obtained from the parasternal long-axis view. The 2D greyscale and tissue Doppler imaging (TDI) images will be saved for each of the standard apical (two/three/four-chamber) views. LV ejection fraction

will be calculated by a modified Simpson biplane method, from which, also the LV systolic and diastolic volumes will be calculated. LA volumes (end-systolic) will be calculated from apical two-chamber and four-chamber views. Mitral E-wave and A-wave velocity will be measured and the E/A ratio calculated. Pulsed-wave TDI tracings will be done at the septal and lateral mitral annulus in the apical four-chamber view to obtain e' velocity, E/e' and e'/a' ratio will be calculated.

Strain measurements will be based on 2D greyscale images for each of three standard apical views obtained at a frame rate between 55 and 80 fps. An investigator who is blinded for laboratory and cardiac CT results will conduct the strain analysis by using EchoPAC software (GE Vingmed, Norway). The LV endocardial border will be traced by software tracking algorithm throughout the cardiac cycle automatically and adjusted manually in case of poor tracking. Subjects with three or more segments without tracking information will be excluded in analyses of global longitudinal strain (GLS).

Segmental peak systolic longitudinal strains will be quantified as will GLS representing an average of all 17 myocardial segments. Finally, the number of segments displaying post-systolic shortening will be assessed. The operators will be blinded to case status.

Calcium score assessed by cardiac CT

Participants will undergo a non-contrast cardiac CT scan on a 320-detector row Toshiba Aquilion One scanner (Canon Medical Systems, Otawara, Japan). The scans will be performed with an inspiratory breath-hold using prospective ECG-gating with imaging trigger at 75% of the R–R interval and a slice thickness of 0.5 mm. Scan parameters: tube voltage 120 kV, tube current 40–370 mA, expected radiation dose 1–3 mSv.

Calcium scores will be measured on reconstructed 3.0 mm images on a post-processing workstation (Vitrea Enterprise Suite V.6.4.3, Minnetonka, USA) using Vitrea Cardiac software. Coronary calcification will be 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.[28] Abnormal calcium scores will be defined as an Agatston score >0. The scans will also be evaluated for clinically significant incidental extracardiac findings.

Database

All data will be collected and stored using REDCap electronic data capture tool hosted at Aalborg University, Denmark.[29]

Planned analyses

In the first part of this project, we will describe lipid distribution in the Faroese population. We will estimate the prevalence of FH in the Faroe Islands according to the MEDPED and DLCN criteria based on registered cholesterol levels in a nationwide clinical laboratory database. Following this, we will study the genetic and clinical

characteristics and potential causes of FH in the Faroe Islands by recruiting subjects with FH as shown in figure 1. Also, we will also investigate differences in dietary habits and the content of fatty acids in adipose tissue between subjects with clinical FH and controls using a case–control design. Lipoprotein(a) will be measured in all FH cases and matched controls and the association with clinical FH will be investigated. Furthermore, we will investigate whether objective markers of subclinical CAD obtained using transthoracic echocardiography and cardiac CT are associated with clinical FH. For objective markers of subclinical CAD, we will initially focus on the emerging technique GLS, which can be used to quantify subtle disturbances in LV systolic function using echocardiography that may reflect CAD.

Power and sample size

Due to the novelty of our study objectives, the current literature did not contain information on the necessary prerequisites for detailed power calculations during the planning of this project. The sample size calculations of this project were therefore based on the substudy because investigation of objective markers of subclinical CAD in this study population was considered of major importance. We decided to estimate the sample size of the project based on echocardiographic measurements allowing for subsequent stratified analyses. We assumed an average GLS of −16% in subjects with FH and a GLS of −19% in healthy controls and an SD of 3.5. A Pearson's X^2 test suggested that a total of 23 FH cases and 23 controls would be required to detect the above differences in GLS between the groups with a level of significance of 0.05 and a power of 0.80. However, to allow for detailed subgroups analyses (eg, subjects naíve to statins), we aimed at including 120 FH cases and 120 controls in the substudy as we hypothesised that approximately 20%–30% would be naíve to statins.

Patient and public involvement

Information material was prepared in consultation with two subjects with a medical history of cardiovascular disease. Patient or public involvement was not considered possible in the design, conduct or dissemination of this research project.

Current status

This project is ongoing, and as of March 2022, a total of 127 cases and 125 controls has been recruited into the substudy. We await genetic results and no data analyses has begun at this stage.

DISCUSSION

The main purpose of this project is to describe the genetic and clinical characteristics and potential causes of FH in the Faroe Islands, which might represent a founder population with a high prevalence of FH. Several founder

populations among French Canadians,[30] South African Afrikaners[31] and Lebanese,[32] Finns[33] and Icelanders[34] have reported a very high frequency of embedded pathogenic mutations in FH genes. Most founder populations are characterised by the presence of few pathogenic FH variations that predominate. Identification of founder mutations confers key advantages including early screening and initiation of lipid-lowering therapy. Although FH is considered a genetic disease characterised by highly elevated LDL-C levels, the diagnosis is often based on clinical data alone (clinical FH) due to cost and availability of genetic analysis. Also, genetic testing may not be definite due to the complexity and large number of variants in the LDL receptor gene, apoB and PCSK-9 coding genes of which a significant proportion may be variants of unknown significance.[8] Furthermore, a recent study reported that FH mutations identified by genetic sequencing were found among less than 2% in subjects with severe hypercholesterolaemia (LDL-C above 4.9 mmol/L).[35] Interestingly, results from the Copenhagen General Population Study have suggested that nearly 25% of those diagnosed with clinical FH may be due to high levels of lipoprotein(a),[36] which is a lipoprotein that contains about 30% of cholesterol that are not subtracted in the calculation of total plasma LDL-C when using the Friedewald formula used by most laboratories.[37] Other important causes of clinical FH might include polygenic predisposition to elevated LDL-C, diet and other lifestyle factors, but further knowledge on potential causes of clinical FH is needed. In this project, we also aim to investigate whether potential objective markers of subclinical CAD measured by advanced echocardiography, cardiac CT and ECG differ between subjects with FH and controls, which may be used to determine whether these markers can identify subjects that require more intensive lipid-lowering treatment to lower their risk of developing manifest CAD.

Some strengths and limitations of this project should be mentioned. We will estimate the prevalence of FH in the Faroe Islands according to the MEDPED and DLCN criteria based on registered cholesterol levels in a large electronic laboratory database that contains all laboratory measurements of cholesterol levels in the Faroe Islands since 2006. However, other valid clinical data used in the DLCN criteria are not available through registries in the Faroe Islands and therefore the prevalence of FH according to the DLCN criteria may be underestimated. Also, valid information on use of lipid-lowering therapy at the time of measurement of lipid levels is not available, which is another important limitation. Major strengths of this project include collection on detailed clinical data on eligible subjects with FH in a possible founder population with a presumed high prevalence of FH. A major advantage of this project is the use of adipose tissue, which may represent an objective biomarker of intake and metabolism of fatty acids for extended periods.[38] Another major strength of this project includes collection of advanced echocardiographic measurements and

cardiac CT. Blood samples for genetic testing will be obtained from all subjects diagnosed with clinical FH, but genetic analyses will initially only be conducted among subjects with likely autosomal dominant inheritance of FH due to high cost. Also, FH cases included in the substudy will be limited to those with a history of severe hypercholesterolaemia and our findings may therefore not be generalised to individuals with clinical FH with less severe hypercholesterolaemia.

This project is expected to contribute with knowledge on the genetic and clinical characteristics and potential causes of FH in a founder population with an unknown prevalence of FH. Finally, this project is expected to promote awareness and public health relevance of identifying subjects with FH in the Faroe Islands.

ETHICS AND DISSEMINATION

All necessary permissions have been obtained prior to initiation of inclusion including approval from the Ethical Committee of the Faroe Islands and the Data Protection Agency of the Faroe Islands (registration number: 17/00208-4). All potential participants will be informed in detail about the project and will be informed of the right to refuse to participate or to withdraw their consent to participate at any time without reprisal according to the Declaration of Helsinki. All biological samples will be collected and handled according to best practice guidelines. Cardiac CT scans were not included in primary ethical approval. Therefore, an additional protocol was forwarded, which has been approved by the Ethical Committee of the Faroe Islands.

This project is expected to yield multiple publications in internationally peer-reviewed journals. Furthermore, we aim to disseminate and promote the awareness and public health relevance of identifying subjects with FH in the Faroe Islands.

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