Familian Hypercholesterolemia in the Faroe Islands

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DOI (link to publication from Publisher):
10.54337/aau518616112

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

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BY
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DISSERTATION SUBMITTED 2022

AALBORG UNIVERSITY
DENMARK

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Dissertation submitted 2022

Dissertation submitted: August 2022

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ISSN (online): 2246-1302
ISBN (online): 978-87-7573-855-7

Published by:
Aalborg University Press
Kroghstræde 3
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Stibo Complete, 2022

ACKNOWLEDGEMENTS

I want to express my gratitude to those who have joined me on this journey to complete this thesis by educating, guiding and supporting me.

To my supervisors. I feel honored and privileged to have had a team of such competent and engaged supervisors. You have all contributed to this work in your own special way and I have learned so much from each one of you. To my main supervisor, Albert Marni Joensen. You were one of the initiators of this project and it has been a pleasure working with you. Thank you for your hard work, your advice on every aspect of this journey and your encouragement. For always taking time to talk and for understanding Faroese during times of despair. To my other supervisors, Michael René Skjelbo Nielsen, Erik Berg Schmidt, Christian Sørensen Bork and Peter Søgaard. Michael, thank you for always being constructive and honest and focusing on the clinical aspects of the results. Thank you for your great contribution with the genetic interpretations. Erik, it has been an honor working with you. Your vast experience and great knowledge within the field of dyslipidemia and your way of conveying this knowledge to others have been highly appreciated. Christian, for hour upon hour of scientific and methodological guidance and support. Your knowledge, bright mind, patience and kindness amaze me and have in many ways carried me through this process. Peter, for echocardiographic sparring. For several reasons, the echocardiographic data unfortunately were not included in this thesis, but the plan is to continue working on them in the future.

To those, who initiated this project back in the day and especially Rudi Kollslíð. Thank you for your help and support. To the National Hospital of the Faroe Islands for making this project possible. To Jan Jóanesarson for assistance with data collection and for conducting and analyzing the cardiac CTs. To Anna Maria Mortensen for assisting with the data collection. To Ása Wraae Olsen for helping with the recruitment of participants and data collection. You made hour upon hour of hard work bearable with your humor. To the staff working in the laboratory for your help and kindness and to Mogens Eriksen for data retrieval. To Guðrið Andorsdóttir for help and guidance with data management and storage. To Søren Lundbye-Christensen for good statistical guidance and humor. To Ihab Bishara Lolas and Henrik Okkels for conducting and interpreting the genetic analyses. To all the authors on the four papers included in this thesis, thank you for your contribution.

To my coworkers in Ílegusavnið, FarGen and Fólkaheilsuráðið. It has been a pleasure getting to know all of you. To my office-partners Marnar, Jacobina and Tórur. For

making this journey bearable, for sparring, laughter and encouragement. You will be missed.

A special thanks to the Research Council Faroe Islands, the National Hospital of the Faroe Islands, Amgen AB, Betri P/F and Karl G. Andersen foundation for supporting this project financially. This work could not have been done without it.

To my friends and family, my parents and my mother-in-law. To my father-in-law, who sadly passed away during this time. Thank you all for your endless support and help.

To Rógvi, my partner in life and biggest supporter in everything I do. Thank you is not enough. For endless encouragement and for always believing in me. For taking care of our family. I could not have done this without you. I owe you one.

To my beautiful children, Bjarti, Bára and Bjørk. You will always be my biggest achievement in life.

Sanna á Borg

July 2022

LIST OF PAPERS

This thesis is based on the following four papers:

Paper I

Borg SÁ, Nielsen MRS, Søgaard P, Lundbye-Christensen S, Jóanesarson J, Zaremba T, Kollslíð R, Schmidt EB, Joensen AM, Bork CS. Familial hypercholesterolemia: 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.

Paper II

Borg S, Bork CS, Nielsen MRS, Schmidt EB, Kollslíð R, Lundbye-Christensen S, Joensen AM. Lipids, lipoproteins and prevalence of familial hypercholesterolemia in the Faroe Islands – Results from a nationwide laboratory database. Atherosclerosis Plus. 2022;48:55-59.

Paper III

Borg S, Joensen AM, Nielsen MRS, Olsen ÁW, Lolas IBY, Okkels H, Lundbye-Christensen S, Schmidt EB, Bork CS. Possible explanations for the common clinical familial hypercholesterolemia phenotypes in the Faroe Islands. Manuscript attached.

Paper IV

Borg S, Bork CS, Nielsen MRS, Jóanesarson J, Zaremba T, Lolas IBY, Lundbye-Christensen S, Søgaard P, Schmidt EB, Joensen AM. Subclinical atherosclerosis determined by coronary artery calcium in patients with clinical familial hypercholesterolemia. Under review.

ABBREVIATIONS

ASCVD, Atherosclerotic cardiovascular disease

BMI, Body mass index

CAD, Coronary artery disease

CI, Confidence interval

CVD, Cardiovascular disease

DLCN, Dutch Lipid Clinic Network

FFQ, Food frequency questionnaire

FH, Familial hypercholesterolemia

LDL-C, Low-density lipoprotein cholesterol

MEDPED, Make Early Diagnosis Prevent Early Death

MUFAs, Monounsaturated fatty acids

OR, Odds ratio

PUFAs, Polyunsaturated fatty acids

SFAs, Saturated fatty acids

TFAs, Trans fatty acids

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CHAPTER 1. INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) incidence and mortality rates are declining in many European countries. However, both in Europe and globally, ASCVD including coronary artery disease (CAD) and ischemic stroke, remains a leading cause of morbidity and mortality with CAD accounting for almost 16% of deaths worldwide [1]. The pathophysiological mechanisms leading to atherosclerosis are complex, but central is an accumulation of low-density lipoprotein cholesterol (LDL-C) in a modified (including oxidized) form in the arterial intima [2–4]. The key events in the onset of atherosclerosis are the retention and accumulation of cholesterol-rich apoB-containing lipoproteins within the artery intima at sites of susceptibility for plaque formation. LDL-C and other apoB-containing lipoproteins <70 nm in diameter efficiently enter and exit the arterial intima [4]. LDL-C particles that are not catabolized in the liver may undergo alteration including oxidation and are absorbed by arterial wall macrophages inducing inflammation [2]. Arterial wall macrophages become overloaded with cholesterol and transform into foam cells that contribute to the formation of atherogenic plaques, which can expand and eventually rupture resulting in tissue ischemia [2,3].

Despite significant progress in the knowledge of these processes and the identification of multiple factors associated with disease risk, the burden of ASCVD remains high [5]. Other established risk factors for cardiovascular disease (CVD) apart from high circulating levels of blood apolipoprotein-B-containing lipoproteins (notably LDL-C) include hypertension, diabetes mellitus and lifestyle associated factors like smoking, unhealthy diets, lack of exercise and adiposity as well as family history of ASCVD and genetic factors [3,6].

This thesis aimed to assess the lipid and lipoprotein distribution in the Faroese population and to estimate the prevalence of FH here. Also, we aimed to investigate genetic and clinical characteristics and potential causes of FH in the Faroe Islands and to investigate whether coronary artery calcium (CAC) as a marker for subclinical atherosclerosis was associated with clinical FH. The included papers are based on data from lipid and lipoprotein measurements collected in the Faroe Islands over a 14-year period and a case-control study of subjects with clinical FH compared to age- and sex-matched healthy controls with focus on genetics, plasma lipoprotein(a) levels, dietary habits, the content of fatty acids in adipose tissue as a biomarker for their intake, and CAC levels.

CHAPTER 2. BACKGROUND

2.1 FAMILIAL HYPERCHOLESTEROLEMIA

Familial hypercholesterolemia is the most common monogenic autosomal dominant genetic disorder resulting in lifelong elevated plasma levels of LDL-C and a predisposition to premature ASCVD [3,4]. A key element in the diagnosis of FH is the detection of elevated plasma LDL-C levels in the absence of secondary causes of dyslipidemia such as hypothyroidism, liver or renal disease (i.e. nephrotic syndrome). Several diagnostic criteria for FH have been developed and in clinical practice, an FH diagnosis is commonly reached from clinical scores such as the Make Early Diagnosis Prevent Early Death (MEDPED) criteria [7] which is based on plasma LDL-C cutoffs stratified by age and family history. These LDL-C cutoffs can be applied to the general population (Table 1), but are lower in subjects with first-, second- and third-degree relatives with FH. Another well-known and used diagnostic tool is the Simon Broome diagnostic criteria [8] which is based on raised cholesterol levels, the presence of tendon xanthomas, genetic mutations, family history of premature myocardial infarction and a family history of hypercholesterolemia (Table 1). A definite diagnosis of FH according to the Simon Broome criteria can be made, if the subject has elevated cholesterol levels and tendon xanthoma or if an FH-causing mutation is identified during genetic testing. The most widely used criteria internationally now is the Dutch Lipid Clinic Network (DLCN) criteria [9], which is also the most comprehensive scoring system. Thus, in addition to plasma LDL-C levels it also includes information on first-degree relatives with premature CVD or elevated LDL-C, clinical history of premature CVD, FH stigmata such as tendon xanthomas or arcus cornealis and detection of pathogenic mutations in genes causing FH [9]. According to scores obtained, patients can be divided into those with definite (>8 points), probable (6-8 points), possible (3-5 points) or unlikely (<3 points) FH by the DLCN criteria (Table 1).

Table 1. Comparison between clinical diagnostic FH criteria

Diagnostic criteria	**MEDPED for the general population**	**Dutch Lipid Clinic Network**	**Simon Broome**
Family history			
ASCVD	NA	First-degree relative with premature coronary or vascular disease (1 point)	MI aged <50 in second-degree relative or <60 in first-degree relative (d)
Hypercholesterolemia	NA	First-degree relative with LDL-C above 95th percentile (1 point) Child with LDL-C >95th percentile (2 points)	First-degree or second-degree relative with total cholesterol above 7.5 mmol/L (e)
Clinical history			
ASCVD	NA	Premature[a] CAD (2 points) or premature cerebral or PAD (1 point)	NA
Objective FH stigmata			
Personal	NA	Tendon xanthoma (6 points) or arcus cornealis <45 years of age (4 points)	Tendon xanthoma (b)
Family	NA	Tendon xanthoma or arcus cornealis in first-degree relative (2 points)	Tendon xanthoma in first-degree relative (b)

LDL-C levels (mmol/L)			
	>6.7 (≥40 years) >6.2 (30-39 years) >5.7 (20-29 years) >5.2 (<20 years)	>8.5 (8 points) 6.5-8.4 (5 points) 5.0-6.4 (3 points) 4.0-4.9 (1 point)	>4.9 (adults) (a) >4.0 (children) (a)
Genetics			
Genetic mutations	NA	*LDLR*, *APOB* or *PCSK9* mutation (8 points)	*LDLR*, *APOB* or *PCSK9* mutation (c)
Diagnosis			
FH diagnosis	Definite (meets LDL-C cutoffs)	Definite: >8 points Probable: 6-8 points Possible: 3-5 points	Definite: a+b, or c Probable: a+d, or a+e

This table is based on references [7–10]. Abbreviations: MEDPED, Make Early Diagnosis Prevent Early Death; DLCN, Dutch Lipid Clinic Network; ASCVD, atherosclerotic cardiovascular disease, NA, not applicable; MI, myocardial infarction; LDL-C, low-density lipoprotein cholesterol; CAD, coronary artery disease; PAD, peripheral arterial disease

[a] Aged <55 years in men, aged <60 years in women

FH is caused by mutations in genes producing essential proteins involved in the LDL receptor endocytic and recycling pathways, which results in reduced cellular absorption of LDL-C, reduced production of functional LDL receptors and increased plasma LDL-C levels [11]. The use of genetic testing represents an important diagnostic tool, but genetics in FH have proven more complex than previously thought [12]. Thus, mutations in several genes may cause FH, but mutations in the LDL-receptor (*LDLR*) gene are by far the most frequent (>90%) with >1700 mutations identified and of these >1200 are believed to be expressed as a severe hypercholesterolemic phenotype [13,14]. Receptor binding defects in the

apolipoprotein B caused by mutations in the *APOB* gene account for approximately 5-10% of FH cases while gain-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene account for <1% [11]. Other rare mutations may also cause FH, like dominant mutations in *APOE* (which encodes apolipoprotein E) and *STAP1* (which encodes signal-transducing adaptor protein 1). Also, mutations can be found in the *LDLRAP1* gene, which can cause an autosomal recessive form of FH [12,14]. When a mutation is identified in a person with FH, European and American guidelines recommend cascade screening by actively testing possibly affected relatives using the family mutation for identification of FH, preferably at an early age [11,15,16]. Cascade testing has been proven to be a cost-effective strategy not only for identifying new individuals with FH, but also for initiating treatment and preventing CVD [17].

The frequency of monogenic mutations in the most prevalent genes varies significantly according to the degree of certainty of the FH diagnosis. Thus, a pathogenic variant in one of the three most common FH-causing genes can be detected in 60-80% of individuals with definite FH according to clinical diagnostic criteria [13,18,19]. However, in those with a possible clinical FH diagnosis, a pathogenic variant can only be found in approximately 20-40% [15,20–23]. A positive genetic test result may also be more common in individuals with a personal or family history of tendon xanthomas, a personal history of CVD, or imaging evidence of increased atheroma burden [24,25]. There may be several explanations for this differing confirmation rate, one being the possibility of yet unknown novel mutations. Also, high plasma lipoprotein(a) concentrations are not uncommon in patients with phenotypic FH with no detectable pathogenic mutation [26], and one study has suggested that up to 25% of subjects with clinical FH reached their diagnosis due to high plasma lipoprotein(a) concentrations [27]. A polygenic etiology may also explain the clinical FH phenotype in individuals without a pathogenic variant. Genome-wide association studies (GWAS) have led to the discovery of hundreds of single nucleotide polymorphisms (SNPs) associated with plasma lipoprotein traits [28,29]. Individually these SNPs are associated with either slightly higher or lower plasma LDL-C levels, but studies have suggested that the cumulative contribution of several inherited SNPs can raise LDL-C into the levels seen in subjects with monogenic FH [30]. Thus, a large proportion of individuals with FH without a monogenetic cause may instead have polygenic hypercholesterolemia [30–32]. This has led to the development of so-called polygenic risk scores (PRS) based on a specific number of SNPs by summing the number of raising alleles an individual carries and by weighting the carriage of each SNP by the size of its estimated effect on LDL-C levels [19]. In this group of individuals cascade testing is less cost-effective, since only approximately 30% of

relatives are likely to have LDL-C elevated above the diagnostic threshold, compared to 50% of mutation carriers seen in first-degree relatives of monogenic FH families [19]. Therefore, according to Berberich et al. [12], FH is more accurately described as a group of conditions with diverse inheritance patterns, molecular etiologies and clinical presentations, with autosomal dominant monogenic FH serving as the classic or textbook example [12].

The prevalence of heterozygote FH has previously been estimated to be 1 in 500 [14], but more recent data suggests a twofold higher prevalence of approximately 1 in 250-300 [9,33,34]. Thus, a recent large meta-analysis of 11 million individuals including 33,036 subjects with both genetic and clinical FH reported a worldwide prevalence of 1 in 313 [35]. Another large meta-analysis of 7.3 million individuals including 24,636 with FH found a prevalence of 1 in 311 [36]. Interestingly, even higher prevalence of FH can be found due to founder effects in specific populations such as French Canadians [37], South African Afrikaners [38], Finns [39], Christian Lebanese [40] and Icelanders [41]. Founder effects are characterized by reduced genetic diversity that occurs when relatively few individuals carrying FH-causing genetic mutations migrate and establish a new population while living in relative geographic and cultural isolation [12,42]. Rarely, individuals with FH may have a homozygous genotype, where both alleles have a mutation, and the prevalence of homozygote FH has also recently been revised upwards from 1 in a 1.000.000 to 1 in 160.000-300.000 individuals [12,22,43].

Assessing cardiovascular risk in individuals with FH can be challenging. Risk calculators as the European SCORE [6] are not appropriate for FH subjects, as such individuals are at a considerably higher cardiovascular risk due to lifelong elevated plasma LDL-C levels [9]. The significance of "accumulated cholesterol load" based on plasma LDL-C levels multiplied by the number of years is being increasingly recognized [14]. In FH subjects with a monogenic mutation, the artery wall has been exposed to high LDL-C levels and accelerated atherosclerosis from birth. Individuals with homozygous FH with extremely elevated LDL-C concentrations have an onset of CVD as early as first or second decade in life [4]. Additional CVD risk factors in individuals with FH include reduced HDL-C, elevated triglyceride levels, elevated lipoprotein(a) levels, diabetes mellitus, hypertension and lifestyle factors including smoking, unhealthy diet and lack of exercise [44,45]. Also, studies have shown that the presence of a causative mutation of FH is independently associated with a higher CVD risk in patients with FH compared to individuals with a clinical FH diagnosis but without a causative FH mutation identified as well as those with polygenic hypercholesterolemia [4,46–49]. Thus, Khera et al. [50] found, that the presence of a monogenic FH causing mutation was associated with a 3-fold higher risk of CAD

compared with individuals with severe hypercholesterolemia with comparable LDL-C levels who did not carry a causative FH mutation. Compared with a reference group with a plasma LDL-C <3.4 mmol/L, participants with LDL-C >4.9 mmol/L and no FH mutation had a 6-fold higher risk for CAD [50].

FH is underdiagnosed and undertreated in most parts of the world and according to Nordestgaard et al. [11] less than 1% have been diagnosed in most countries. This implicates undertreatment with lipid-lowering treatment, which may result in development of severe atherosclerosis and cardiovascular events before the lipid-lowering treatment is initiated.

2.2 THE FAROE ISLANDS

Population and genetics

The Faroe Islands are an isolated archipelago located in the middle of the North Atlantic Ocean, northwest of Scotland, between Iceland and Norway. According to the Faroese saga, the first settlements were dated to approximately 825-875 AD by Scandinavian Vikings [51]. However, other written historical sources and botanical findings indicate that the first settlers may have been Irish monks in the 6th century AD, who later deserted the islands due to the appearance of the Vikings [52]. There is extensive evidence of settlers from Scandinavia and the British Isles inhabiting the islands during the Viking era [53]. Studies have suggested an excess of Scandinavian ancestry among the male settlers of the Faroe Islands and an excess of British Isles ancestry among the female settlers [52,53].

After the initial settlements, migration to the Faroe Islands has probably been sparse probably due to the islands´ isolated geographic position [54]. The population size was as small as 4000 inhabitants in the late 1300s increasing to 9000 inhabitants in the 1800s [53] and further to the more than 53,000 people now in 2022 living in the Faroe Islands [55] (Figure 1). As a consequence of the demographic history of the Faroe Islands, levels of genetic diversity within the population have been predicted to be relatively low compared to other larger and less isolated European populations [53]. The Faroese population had a small number of founders, had slow population growth over centuries and assumed sporadic reductions in population size due to epidemics, followed by a recent population expansion. Such a demographic history makes genetic drift likely to have played a significant role in shaping the genetic diversity in Faroese, and the population of the Faroe Islands is considered the genetically most homogenous and isolated population in the North Atlantic region [52,53].

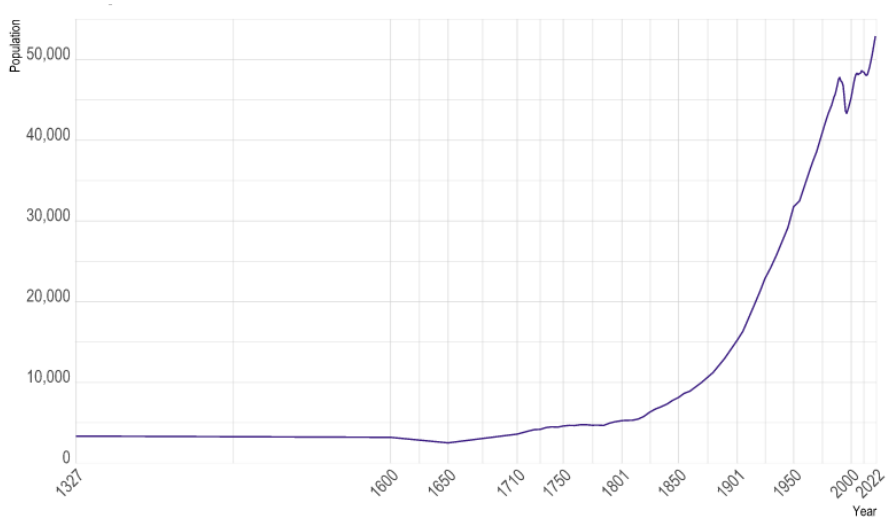

Figure 1. The Faroese population from 1327 to 2022. Data are based on population counts and figures from the national statistics office.

The presence of a few ancestors carrying pathogenetic variants, both of Scandinavian and British Isles origin, may have contributed in combination with possible inbreeding

to the current high prevalence of several genetic diseases in the Faroe Islands. Thus, inflammatory bowel disease [56], carnitine transporter deficiency [57], glycogen storage disease type IIIA [58], and amyotrophic lateral sclerosis [59] have been shown to be more prevalent in the Faroe Islands than elsewhere.

Traditional Faroese diet

Traditional Faroese diet is dominated by animal products and a high intake of saturated fat with little or no consumption of fruit and vegetables. Important parts of traditional Faroese food are fish and seafood, lamb, seabirds, whale meat and blubber. Potatoes and a few root vegetables have been grown on the islands [60,61]. However, during the past few decades, the Faroese diet has undergone significant changes and has shifted to a more western-style diet. A study from 1985 by Vestergaard and Zachariassen [62] explored the Faroese diet from 1981 to 1982 compared with the dietary habits in Denmark and other industrialized countries. They observed a lower consumption of dairy products, meat and vegetables, while the intake of fish was higher in the Faroe Islands. The Faroese also consumed whale meat and blubber, which contain high amounts of organic pollutants [62,63]. Since then, two studies have followed, the first from 2000-01 [64] and the second from 2013-16 [61], which have allowed for an assessment of how the dietary habits of the Faroese have changed. These studies suggest a decrease in the intake of fish, seafood and whale meat and blubber, while the intake of meat and vegetables has increased.

Population characteristics

In many aspects, the Faroese population is similar to the populations in the other Nordic countries with high income and a universal welfare state with free access to the healthcare system. Interestingly, according to Statistic Faroe Islands, the Faroe Islands had the highest life expectancy of the Nordic countries in 2021 [65] at 83.5 years compared to 81.4 years in Denmark and 83.2 years in Iceland. However, a report from 2017 from the Nordic Medico-Statistical Committee [66] found that the Faroese had the highest proportion of daily smokers among the Nordic countries. Smoking has decreased in the course of the last many years, and a survey from 2019 [67] showed that 19% of adults in the Faroe Islands smoked daily. Further, a survey from 2019 [68] found that 62% of the Faroese adult population could be classified as overweight (BMI >25kg/m^2) which is higher than in other Nordic countries with the exception of Iceland [69]. Further, according to the survey a total of 21% could be classified as

obese (BMI >30kg/m^2) and 30% reported that they did not meet the level of physical activity recommended by the WHO [68].

The cardiovascular mortality rate has declined in the Faroe Islands over the last two decades and has been replaced by cancer as the leading cause of death since 2016 [70]. In 2020, the mortality rate of CVD was 140 per 100.000 for both men and women [70]. Due to the geographic distance to the nearest percutaneous coronary intervention (PCI) facility, patients with ST-elevation myocardial infarction are initially treated with fibrinolysis. All patients diagnosed with acute coronary syndrome are transported to Denmark for coronary angiography and, if necessary, PCI or coronary artery bypass grafting (CABG). No regularly reported data exist on the incidence of CVD in the Faroe Islands. However, data from an unpublished study by Kristiansen et al. reports the incidence of CABG and PCI for the years 2015-2019. The incidence rate for CABG/PCI was 182 per 100.000 individuals, which seems to be a lower incidence rate compared to Denmark [71].

The prevalence of FH in the Faroe Islands is unknown. To our knowledge, this is the first FH research conducted in the Faroe Islands. Based on evaluation of lipid and lipoprotein measurements from a large part of the population, many Faroese, however, have severely elevated plasma LDL-C levels. Possible explanations for these findings have previously not been investigated.

CHAPTER 3. AIMS AND HYPOTHESES

The overall aim of this thesis was to describe the distribution of lipids and lipoproteins in the Faroe Islands and to investigate the prevalence of FH in the Faroe Islands. Furthermore, we aimed to examine the genetic and clinical characteristics and potential causes of FH in the Faroe Islands and to investigate whether coronary artery calcium deposition as a marker of subclinical atherosclerosis was more prevalent in subjects with FH compared to healthy controls. We described the methodological considerations and data collection in a protocol paper and studied our aims and hypothesis in three papers as specified below:

Protocol paper

The aim of this paper was to describe the methods and methodological considerations of a large FH project in the Faroe Islands of which this thesis is a part.

Study I:

The aim of this study was to describe the lipid and lipoprotein distribution in the Faroe Islands and to estimate the prevalence of FH in the Faroe Islands. We hypothesized that the prevalence of FH would be high in the Faroe Islands and that the lipid distribution would differ according to sex and age.

Study II:

The aim of this study was to describe potential genetic causes of FH in the Faroe Islands and to investigate whether plasma levels of lipoprotein(a) and dietary habits were associated with clinical FH in the Faroe Islands. Given the genetic history of the Faroe Islands, we expected to identify a high prevalence of monogenic autosomal dominant FH in the study population. Also, we hypothesized that subjects with FH would have a diet high in saturated fat and low in fish, fruit and vegetables.

Study III:

The aim of this study was to describe CAC scores in clinical FH cases and healthy controls and to investigate the associations between levels of CAC and clinical FH and FH subtypes including polygenic causes. We hypothesized that subjects with clinical FH would have higher levels of CAC as a biomarker for subclinical atherosclerosis compared to controls.

CHAPTER 4. METHODS

In this thesis, the distribution of lipids and lipoproteins and the prevalence of clinical FH in the Faroe Islands were based on data from a nationwide laboratory database, while investigation of possible causes of FH in the Faroe Islands as well as the association between CAC score and clinical FH were based on a case-control study as described below. The recruitment of participants, as well as the data collection and methodological considerations have previously been described in detail [10].

4.1 STUDY POPULATION

Potential FH cases were identified based on a review of results of blood samples taken over a 14-year period (2006-2020) in a nationwide laboratory database. Subjects between 18 and 75 years and with a plasma LDL-C above 6.7 mmol/L (cutoff for definite FH according to the MEDPED criteria for the general population) were invited by letter for diagnostic evaluation for FH. Subjects not responding within two weeks were contacted by telephone up to three times. Prior to a clinical examination, subjects were required to have a non-fasting screening blood sample taken including plasma lipids and lipoproteins (total cholesterol, LDL-C, HDL-C and triglycerides) as well as indicators for secondary causes of dyslipidemia. Subjects without secondary dyslipidemia who met the criteria of definite or probable FH according to the DLCN criteria, definite FH according to the Simon Broome criteria and/ or definite FH according to the MEDPED criteria were considered eligible for inclusion in the study as FH cases. First-degree relatives who met age- and sex-specific LDL-C cutoffs were also considered eligible FH cases [72].

Control subjects were identified from the background population through the National Register of Persons in the Faroe Islands. Subjects without persistent atrial fibrillation/ flutter and a history of ASCVD and an LDL-C <3.5 mmol/L, DLCN score ≤3 and current use of lipid-lowering therapy were considered eligible as controls. Controls and cases were matched according to sex and age (5-year age intervals) [10].

For the substudy on subclinical atherosclerosis assessed by cardiac CT, subjects with a history of ASCVD were excluded. Also, subjects with persistent atrial fibrillation/ flutter were excluded, as these arrhythmias may interfere with the quality of the cardiac CT imaging. Subjects with elevated creatinine levels >120 μmol/L and pregnant women were not eligible for inclusion into the substudy.

The study was approved by the Ethics Committee and the Data Protection Agency in the Faroe Islands (registration number: 17/00208-4). All participants gave written informed consent at inclusion.

4.2 LIPID MEASUREMENTS

In the Faroe Islands, all blood samples from general practitioners and the three hospitals are collected and analyzed in hospital laboratories and then entered into a nationwide clinical laboratory database (BCC-Web, GCI). We examined all lipid and lipoprotein measurements (total cholesterol, LDL-C, HDL-C, and triglycerides) collected in the Faroe Islands between 2006 and 2020. Individuals lacking a complete lipid profile and those with a foreign civil registration number were not included. The Friedewald formula [73,74] was used to determine plasma LDL-C levels in mmol/L in this database, and according to local practice we excluded individuals with LDL-C values that were calculated despite triglyceride levels exceeding 4 mmol/L, as the Friedewald equation is not valid for such values.

We described the lipid distribution of the major lipids and lipoproteins (total cholesterol, LDL-C, HDL-C, triglycerides) in the Faroese population by providing age- and sex-specific lipid values based on each individual's first available complete lipid profile measurement. We classified participants with a complete lipid profile based on their highest recorded LDL-C level according to the MEDPED diagnostic criteria and the LDL-C cutoffs included in the DLCN diagnostic criteria for FH. The MEDPED criteria are based on age-specific cholesterol cutoffs (total-C or LDL-C), and we classified the subjects as having FH if their highest measured cholesterol level exceeded one of these cutoffs. Based on the LDL-C cutoffs provided in the DLCN criteria, probable FH was defined as LDL-C $\geq$8.5 mmol/L and possible FH was defined as LDL-C $\geq$5 mmol/L.

4.3 SELF-REPORTED PATIENT DATA

All participants in the case-control study were asked to fill in a detailed questionnaire before the physical examination including information on their previous history of ASCVD, information on family history of premature ASCVD (before 55 years of age in men and before 60 years of age in women) and known hypercholesterolemia in first-degree relatives. Also, information on social and lifestyle factors including educational level (low (primary and lower secondary education), medium (general

upper secondary education, vocational education and training and short-cycle tertiary education) and high (bachelor and master degrees or equivalent levels and doctoral or equivalent level)), physical activity (strenuous walking, running, bicycling, swimming categorized into <1, 1-3 and 3 hours and above per week), alcohol consumption (<7, 8-14 and 15 alcohol units and above per week), smoking (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), current smoker (smoked more than 100 cigarettes or equivalent during lifetime and has smoked within the last 28 days) and use of medications (lipid-lowering therapy, antihypertensive, diuretic, antidiabetic or anticoagulant/ antiplatelet medications, fish-oil supplements) was collected.

4.4 ASSESSMENT OF HABITUAL DIETARY HABITS

All participants in the case-control study received the Danish HeartDiet Questionnaire by letter and were asked to report their habitual intake of selected foods prior to the physical examination. The HeartDiet Questionnaire was based on Danish national dietary recommendations for consumption of fruits and vegetables, fish, whole-grain and saturated fatty acids (SFAs) and was designed to evaluate the habitual diet of individuals with dyslipidemia and/ or CAD [75]. The questionnaire contains 19 questions and covers the intake of different food groups: dairy products, bread, cereals, potato/rice/pasta, fats, meat, fish, vegetables/legumes, fruits, nuts, sweets and different kinds of snacks and fast-food. Each question may be answered with one of three to five options, equivalent to 0-18 possible points. The points can be summarized into two scores: a fat score (sum of questions 1-9) and a fish, fruit and vegetable score (sum of questions 10-19). The fat-score and the fish-fruit-vegetable-score range from 0 to 100 points. A score of 75 or more has been arbitrarily defined as indicative of a heart healthy diet in terms of intake of SFAs and a combination of fruit, vegetable, fish and whole-grains. If both scores were at least 75, the overall diet was considered heart healthy [75].

4.5 ASSESSMENT OF ADIPOSE TISSUE CONTENT OF FATTY ACIDS

To have a biomarker and objective indicator of exposure to fatty acids, all participants in the case-control study were asked to have a subcutaneous adipose tissue biopsy taken from the buttocks during the clinical examination. The content of fatty acids in

adipose tissue represents the intake and metabolism during the preceding 1-2 years of polyunsaturated fatty acids (PUFAs) while other fatty acids are to some extent made endogenously in the body [76]. The content of fatty acids in adipose tissue are indicators of the underlying dietary pattern.

The adipose tissue biopsies were obtained with a Luer-Lock system consisting of a needle, a venoject multi-sample Luer adapter and an evacuated blood tube according to the method of Beyen and Katan [77]. Samples were subsequently flushed with nitrogen and stored at minus 80°C until further analyses. The fatty acid analyses have previously been described in detail [78]. In brief, the fatty acid composition of adipose tissue was determined using gas chromatography. The biopsies were defrozen and 1–2 mg of tissue was extracted and preheated at 50°C for 10 min before analysis. The fat was subsequently dissolved in heptane at 50°C, and the fatty acids were trans-esterified by 2 mol/L potassium hydroxide in methanol at 50°C for 2 min, in accordance with the Union of Pure and Applied Chemistry Standard Methods techniques [79]. Using a Varian 3900 gas chromatograph with a CP-8400 auto-sampler (Varian, Middleburg, The Netherlands) and a flame ionization detector, the fatty acid composition was examined. Split injecting mode, a CP-sil 88 60 m × 0.25 mm capillary column and temperature programming (90–210 °C) and constant flow were used. Helium was employed as the carrier gas. Using commercially available standards, the peak retention durations and area fractions of fatty acid methyl esters were determined (Nu-check-Prep, Inc., US). The content of the separated 34 individual fatty acids was expressed as relative percentage of total fatty acids when analyzed.

4.6 GENETIC ANALYSES

All subjects with clinical FH and an untreated plasma LDL-C ≥6.7 mmol/L (n = 81) were tested for genetic monogenic mutations with an initial standard FH panel including the *LDLR*, *APOB* and *PCSK9* genes. Genomic DNA was extracted from whole blood samples using Maxwell system (Promega). DNA libraries were prepared using Agilent SureSelect target enrichment system and the sequencing was performed using Illumina platform.

Twenty individuals with pedigrees strongly suggestive of a monogenic inheritance but without detectable monogenic FH-causing variants in the initial gene panel were analyzed with an extended next generation sequencing (NGS) panel. The panel consisted of 11 FH-related genes previously described in the literature as being causally associated with FH (*LDLR, LDLRAP1, PCSK9, LIPA, LPA, ABCG5,*

ABCG8, APOB, APOE, ANGPTL3, STAP1) [12]. 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 and an untreated plasma LDL-C ≥ 6.7 mmol/L without causative monogenic FH mutations identified 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. [32] and Talmud et al. [31]. Subjects without monogenic genetic cause of FH identified and a PRS >80th percentile according to a UK reference population were considered to have a polygenic cause of FH [19].

4.7 DETERMINATION OF CORONARY ARTERY CALCIUM

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

Calcium scores were assessed on reconstructed 3.0 mm images using Vitrea Cardiac software on a post-processing workstation (Vitrea Enterprise Suite V.6.4.3, Minnetonka, United States). Coronary calcification was determined as the presence of at least three 'face-connected' voxels with >130 Hounsfield units per 1 mm2 along the course of a coronary artery. Abnormal calcium scores were defined as Agatston values greater than zero.

4.8 STATISTICAL ANALYSES

In study I, we used quantile regression to report the different percentiles (1th, 5th, 10th, 25th, 50th, 75th, 90th, 95th and 99th percentiles) for plasma total cholesterol, LDL-C, HDL-C and triglycerides based on first registered lipid profile measurement in each

individual. The prevalence of FH was estimated with corresponding 95% confidence intervals (CI) using a generalized linear model with robust variance estimation based on highest registered lipid level in each individual.

In study II, we compared the characteristics among cases and controls using Fischer´s exact test for categorical variables and an unpaired t-test for continuous covariates. The associations between exposures of interest (standardized fat-score and fish-fruit-vegetable-score, standardized content of major fatty acids in adipose tissue and categorized levels of plasma lipoprotein(a) and clinical FH were investigated using multivariable logistic regression. In model 1, we adjusted for matching factors including age (continuous, years) and sex (men; women). In model 2, we further adjusted for alcohol intake (0-7, ≥8 units/ week) and educational level (low, medium, high). In supplemental analysis, we in addition adjusted for potential intermediates including physical activity (<1, 1-3, >3 hours/ week) and waist circumference (continuous, cm). In post hoc analysis, we investigated the association between waist circumference (continuous, cm) and clinical FH. The continuous exposures were standardized by dividing each exposure with the standard deviation to allow for comparison across exposures of interest. All continuous covariates were included in the statistical models using restricted cubic splines with three knots placed at the 10^{th}, 50^{th} and 90^{th} percentiles.

In study III, we used multivariable logistic regression to investigate associations between CAC score and clinical FH. We categorized CAC scores into dichotomized groups including zero and above zero Hounsfield units as well as below or above the median CAC score for age, sex and ethnicity [80]. We also categorized CAC scores according to traditional risk categories (0, 1-299, ≥300 Hounsfield units) [81]. In model 1, we adjusted for matching factors including age (continuous, years) and sex (men; women). In model 2, we additionally adjusted for major risk factors for ASCVD including smoking (never, former, current), hypertension (yes, no), waist circumference (continuous, cm) and levels of plasma lipoprotein(a) (continuous, mg/L). Continuous covariates were included in the statistical models using restricted cubic splines with three knots placed at the 10^{th}, 50^{th} and 90^{th} percentiles. In supplemental analyses, we investigated the associations between CAC score and subtypes of clinical FH, including PRS >80^{th} percentile, PRS <80^{th} percentile and those not genetically tested, respectively.

All statistical analyses were conducted using Stata statistical software (version 16, StataCorp) and a p-value below 0.05 was considered statistically significant.

CHAPTER 5. STUDIES

The data collection and recruitment of participants and the methodological considerations in the studies included in this thesis were described in a protocol paper entitled “Familial hypercholesterolemia: a study protocol for identification and investigation of potential causes and markers of subclinical coronary artery disease in the Faroe Islands” [10].

The scientific rationale for this paper was to provide detailed information on the data collection and recruitment of participants and the methodological considerations underlying the studies included in this thesis. The objective was to collect data for future studies with FH patients, focusing on possible explanations for lipid disorders such as genetics, dietary habits and biological materials including detailed lipid measurements, markers of inflammation and analysis of adipose tissue content of fatty acids. Biological material was stored in a biobank for possible later studies. Also, the aim was to collect data on potential markers for subclinical atherosclerosis such as electrocardiogram, advanced echocardiographic investigations and cardiac CT.

The recruitment of potential FH cases and controls was described in detail. Data collection including self-reported data on personal and familial ASCVD, educational level, physical activity, alcohol consumption, smoking and assessment of diet by the Danish HeartDiet Questionnaire were obtained. The clinical examination and measurements obtained from the participants at inclusion were thoroughly explained. Also, substudies with advanced echocardiography and coronary calcium determinations by CT scans were described.

5.1 STUDY I

Aim

The aim of this study was to describe the lipid and lipoprotein distribution in the Faroese population by providing sex- and age-specific lipid and lipoprotein levels including total cholesterol, LDL-C, HDL-C and triglycerides. Further, we aimed to investigate the prevalence of FH in the Faroe Islands according to the MEDPED diagnostic criteria and according to the LDL-C cutoff values included in the DLCN diagnostic criteria.

Key methods

We used an electronic nationwide laboratory database that included all lipid and lipoprotein measurements obtained in the Faroe Islands between 2006 and 2020. We used quantile regression to estimate the percentiles for plasma total cholesterol, LDL-C, HDL-C and triglycerides based on first registered lipid level in each individual. Subjects were categorized according to the MEDPED diagnostic criteria for FH and the plasma LDL-C cutoff values in the DLCN criteria including possible (LDL-C $\geq$5 mmol/L) and probable (LDL-C $\geq$8.5 mmol/L) FH based on highest registered lipid level in each individual. Subsequently, we estimated the prevalence of FH with 95% confidence intervals using generalized linear models with robust variance estimation.

Main results

We identified a total of 30,711 Faroese men and women registered with a complete lipid profile. Overall, we found major differences in lipid levels according to sex and age (Table 2). In both men and women, lipid levels were higher in the older age groups. The highest median plasma LDL-C across age categories among men was observed in those aged 45-50 years (median 3.6, 95th percentile 5.3), while the highest median LDL-C in women was seen later in life at age 60-65 years (median 3.7, 95th percentile 5.4). In men aged above 50 years, we observed a 0.22 mmol lower LDL-C for every ten-year age interval and a 0.18 mmol/L lower LDL-C in women above 60 years of age for every ten-year age interval, respectively.

Table 2. Medians with 5th and 95th percentiles of plasma total-cholesterol, LDL-C, HDL-C and triglycerides for sex and age groups

Age (yrs)	**Men**				
	n	**Total-C**	**HDL-C**	**TRG**	**n**	**LDL-C**
0-5	18	4.0 (2.5;6.9)	1.1 (0.6;1.9)	1.1 (0.5;3.4)	18	2.2 (1.4;5.1)
5-10	36	4.1 (3.0;5.4)	1.5 (0.9;2.1)	0.7 (0.3;2.5)	36	2.4 (1.2;3.2)
10-15	100	4.2 (2.9;5.7)	1.3 (0.8;2.1)	1.0 (0.5;2.4)	98	2.4 (1.3;3.8)
15-20	442	3.9 (2.9;5.3)	1.2 (0.8;1.7)	0.9 (0.5;2.3)	437	2.2 (1.4;3.4)
20-25	643	4.3 (3.2;5.9)	1.2 (0.8;1.7)	1.0 (0.5;2.7)	640	2.5 (1.5;4.0)
25-30	763	4.7 (3.3;6.6)	1.2 (0.8;1.7)	1.1 (0.5;3.1)	749	2.9 (1.7;4.4)
30-35	978	5.0 (3.6;6.8)	1.2 (0.8;1.8)	1.2 (0.6;3.5)	961	3.1 (1.9;4.8)
35-40	1324	5.3 (3.8;7.2)	1.2 (0.8;1.8)	1.3 (0.6;3.8)	1315	3.3 (2.0;5.0)
40-45	1670	5.5 (4.0;7.4)	1.2 (0.8;1.9)	1.4 (0.6;3.8)	1649	3.5 (2.1;5.1)
45-50	1752	5.6 (4.1;7.6)	1.2 (0.8;1.9)	1.4 (0.6;3.9)	1732	3.6 (2.1;5.3)
50-55	1718	5.6 (3.9;7.4)	1.2 (0.8;1.9)	1.4 (0.6;3.6)	1709	3.5 (2.0;5.2)
55-60	1687	5.5 (3.8;7.4)	1.3 (0.8;2.0)	1.3 (0.6;3.3)	1693	3.5 (1.9;5.0)
60-65	1557	5.4 (3.6;7.3)	1.3 (0.8;2.1)	1.3 (0.6;3.5)	1553	3.4 (1.7;5.0)
65-70	1163	5.3 (3.4;7.1)	1.3 (0.9;2.1)	1.2 (0.6;2.8)	1164	3.4 (1.6;4.8)
70-75	833	5.1 (3.4;7.0)	1.3 (0.8;2.1)	1.2 (0.6;2.8)	833	3.2 (1.6;4.8)
75-80	597	4.9 (3.2;6.9)	1.3 (0.8;2.0)	1.2 (0.6;2.8)	595	2.9 (1.4;4.8)
80-85	306	4.7 (3.1;6.8)	1.2 (0.7;2.1)	1.1 (0.6;2.5)	309	2.8 (1.4;4.7)
85-90	168	4.6 (3.0;6.9)	1.2 (0.8;1.9)	1.2 (0.6;3.2)	167	2.8 (1.4;4.7)
90-95	61	4.5 (2.8;6.7)	1.2 (0.6;1.9)	1.2 (0.6;3.3)	61	2.7 (1.1;4.4)
95-100	11	4.4 (2.5;7.1)	1.3 (0.8;2.0)	1.1 (0.4;2.0)	11	2.7 (0.9;5.5)
	15827				15730	

Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TRG, triglycerides

Table 2. Medians with 5th and 95th percentiles of plasma total-cholesterol, LDL-C, HDL-C and triglycerides for sex and age groups

		Women				
Age (yrs)	**n**	**Total-C**	**HDL-C**	**TRG**	**n**	**LDL-C**
0-5	13	4.5 (2.9;6.9)	1.2 (0.6;1.8)	0.9 (0.5;3.0)	13	3.2 (1.4;4.5)
5-10	24	4.4 (3.4;5.1)	1.4 (0.9;1.8)	0.8 (0.5;2.2)	24	2.4 (1.6;3.3)
10-15	133	4.0 (3.1;5.2)	1.3 (0.8;2.0)	0.9 (0.4;2.7)	132	2.2 (1.4;3.2)
15-20	536	4.3 (3.2;6.1)	1.4 (0.9;2.0)	0.9 (0.5;2.1)	537	2.4 (1.5;3.9)
20-25	622	4.5 (3.2;6.3)	1.4 (0.9;2.0)	1.0 (0.5;2.5)	618	2.6 (1.5;4.1)
25-30	655	4.6 (3.4;6.2)	1.4 (0.9;2.1)	1.0 (0.5;2.5)	655	2.7 (1.7:4.2)
30-35	944	4.7 (3.4;6.4)	1.4 (0.9;2.1)	0.9 (0.5;2.3)	944	2.7 (1.7:4.2)
35-40	1267	4.8 (3.7;6.5)	1.4 (0.9;2.1)	0.9 (0.5;2.2)	1265	2.9 (1.8;4.3)
40-45	1512	5.0 (3.8;6.5)	1.5 (1.0;2.2)	1.0 (0.5;2.5)	1513	3.0 (1.9;4.6)
45-50	1637	5.4 (4.0;7.2)	1.5 (1.0;2.3)	1.0 (0.5;2.6)	1632	3.2 (2.0;5.0)
50-55	1572	5.7 (4.1;7.5)	1.6 (1.0;2.3)	1.1 (0.6;2.8)	1568	3.5 (2.1;5.1)
55-60	1415	5.8 (4.2;7.8)	1.6 (1.0;2.4)	1.1 (0.6;2.8)	1408	3.6 (2.1;5.4)
60-65	1311	5.9 (4.3;7.9)	1.6 (1.0;2.3)	1.2 (0.6;2.9)	1309	3.7 (2.2;5.4)
65-70	1021	5.8 (4.0;7.8)	1.6 (1.0;2.4)	1.2 (0.6;2.7)	1019	3.5 (1.9;5.3)
70-75	822	5.7 (3.8;7.8)	1.6 (1.0;2.4)	1.3 (0.7;2.9)	822	3.4 (1.8;5.3)
75-80	674	5.6 (3.7;7.4)	1.5 (0.9;2.4)	1.3 (0.7;2.8)	675	3.3 (1.7;5.1)
80-85	458	5.6 (3.5;7.5)	1.5 (0.9;2.4)	1.3 (0.6;2.8)	460	3.3 (1.6;5.2)
85-90	275	5.3 (3.5;7.7)	1.4 (0.9;2.2)	1.3 (0.7;2.7)	275	3.2 (1.7;5.2)
90-95	96	5.4 (3.3;7.3)	1.4 (0.7;2.4)	1.2 (0.7;3.2)	95	3.2 (1.6;4.9)
95-100	17	4.2 (2.9;7.8)	1.1 (0.5;1.7)	1.1 (0.6;3.8)	17	2.4 (1.4;5.7)
	15004				14981	

Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TRG, triglycerides

According to the MEDPED age-specific cutoffs for LDL-C, we found a prevalence of definite FH of 1 in 142 or 0.70% (95% CI: 0.62;0.80%). According to the LDL-C cutoffs included in the DLCN criteria, we found a prevalence of possible FH of 1 in 8 or 12.4% (95% CI: 12.08;12.82%) and a prevalence of probable FH of 1 in 3,071 or 0.03% (95% CI: 0.02;0.06%).

Table 3. Prevalence of FH in the Faroe Islands according to the MEDPED and the DLCN diagnostic criteria among 30,711 individuals

	Individuals meeting the criteria (n)	**Prevalence**	
MEDPED criteria			
Definite FH LDL-C cutoffs: >5.2 (<20 years) >5.7 (20-29 years) >6.2 (30-39 years) >6.7 (≥40 years)	216	0.70% (95%CI 0.62;0.80)	**1:142**
DLCN criteria			
Possible FH (LDL-C ≥5 mmol/L)	3,823	12.45% (95%CI 12.08;12.82%)	**1:8**
Probable FH (LDL-C ≥8.5 mmol/L)	10	0.03% (95%CI 0.02;0.06%)	**1:3071**

Abbreviations: MEDPED, Make Early Diagnosis Prevent Early Death; LDL-C, low-density lipoprotein cholesterol; DLCN, Dutch Lipid Clinic Network

Conclusion

In conclusion, we found major differences in lipid levels according to sex and age groups. In both men and women lipid levels were higher with higher age groups, however maximum levels were seen later in women compared to men. We found a very high prevalence of definite FH of 1 in 142 (MEDPED criteria), while the prevalence of possible FH was as high as 1 in 8 (DLCN criteria), indicating that the Faroe Islands might represent an FH founder population.

5.2 STUDY II

Aim

The aim of this study was to describe potential genetic causes of FH in the Faroe Islands and to investigate, whether levels of plasma lipoprotein(a), dietary habits and the composition of major fatty acids in adipose tissue were associated with clinical FH in the Faroe Islands.

Key methods

In this case-control study possible cases between 18 and 75 years were identified by previously measured LDL-C levels above 6.7 mmol/L in a nationwide laboratory database and invited by letter to attend a clinical examination and diagnostic evaluation for FH (Figure 2). Subjects without secondary dyslipidemia who met the criteria of definite/ probable FH according to the MEDPED, Simon Broome or DLCN criteria were considered eligible FH cases. Controls were recruited from the background population and subjects with a plasma LDL-C <3.5 mmol/L, DLCN score ≤3 and without a history of ASCVD or the use of lipid-lowering medication were considered eligible as controls.

Subjects fulfilled detailed background questionnaires on medical history and social factors, and dietary habits were assessed. Physical examination included anthropometrics and examination for FH stigmata including tendon xanthoma and arcus cornealis. Blood samples were collected for genetic analysis and lipoprotein(a) together with an adipose tissue biopsy.

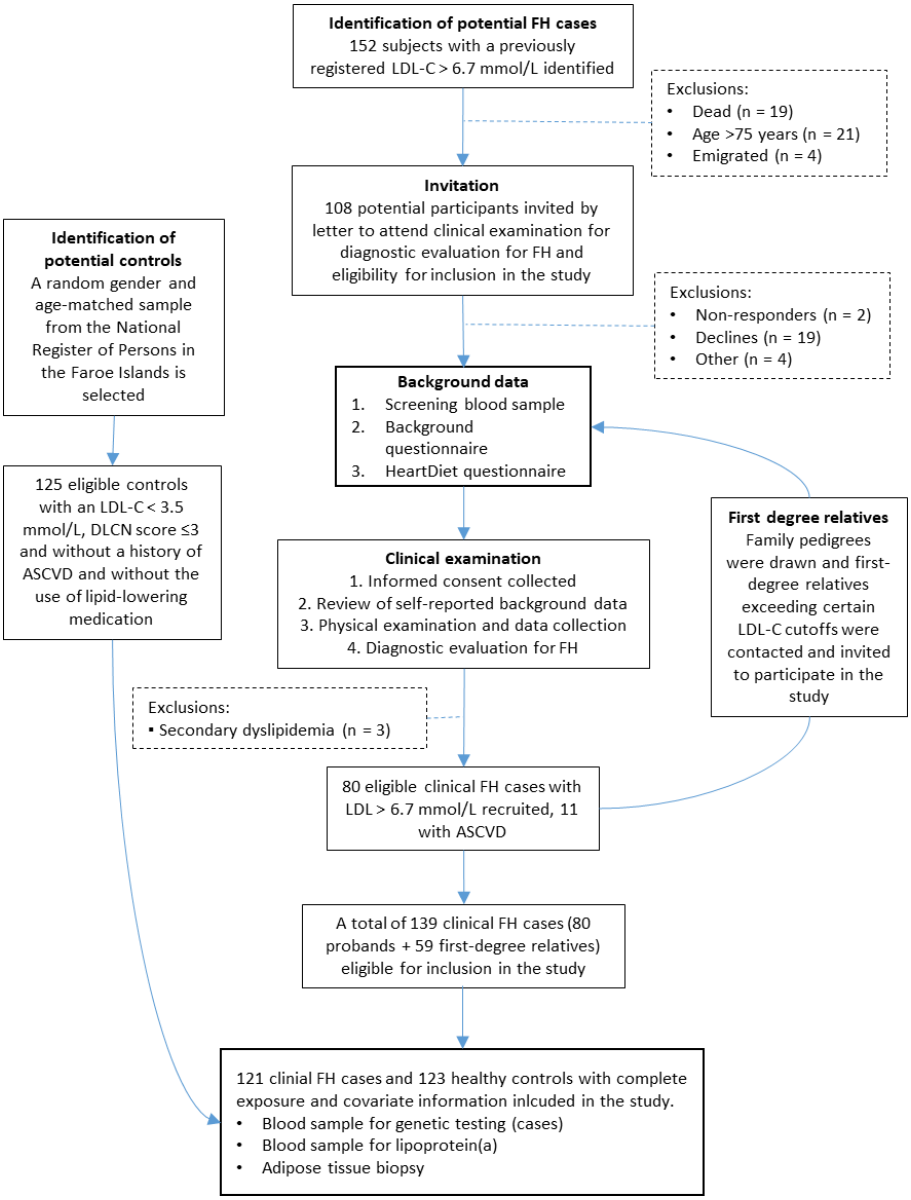

Figure 2. Flowchart for the recruitment of cases and controls in the study.

Main results

We recruited at total of 139 clinical FH cases of whom 11 had a history of ASCVD. A total of 81 probands with clinical FH were investigated by genetic testing for FH. Within this group we identified two subjects (2.5%) with a known pathogenic FH mutation, both mutations were in the *LDLR* gene. In post hoc analyses, we found that 63% of subjects with clinical FH had a PRS >80th percentile indicating a polygenic cause of hypercholesterolemia.

A total of 121 clinical FH cases without a history of ASCVD and atrial fibrillation/flutter and 123 age- and sex-matched controls with complete information and exposures were included in the study for analyses of exposures of interest (Table 4).

Clinical FH cases had significantly larger waist circumference compared to controls, but we found no statistically significant difference between cases and controls with regards to physical activity, length of education, smoking, alcohol consumption and occurrence of hypertension or diabetes mellitus (Table 4). Clinical FH cases had statistically significant higher mean fat-score. Also, we found significantly lower content of SFAs in adipose tissue and significantly higher content of marine n-3 PUFAs in adipose tissue in cases compared to controls.

In multivariable adjusted analysis, we found statistically significant associations between dietary fat-score and clinical FH with an OR of 1.75 (95% CI 1.31;2.33, p <0.001) per standard deviation increase. The content of SFAs in adipose tissue was inversely associated with an OR of 0.66 (95% CI 0.49;0.88, p = 0.006) per standard deviation increase. We found highly positive and statistically significant associations between the content of marine n-3 PUFAs in adipose tissue comparing those with clinical FH to controls with a multivariate adjusted OR of 3.17 (95% CI 2.09;4.80, p <0.001). Furthermore, we found strong and significant associations between plasma lipoprotein(a) in the 80-100th percentile and clinical FH compared to those with levels of plasma lipoprotein(a) below the 50th percentile with a multivariate adjusted OR of 2.79 (95% CI 1.35;5.78, p = 0.006).

Table 4. Baseline characteristics of the study population

	Controls (n = 123)	**Clinical FH** (n = 121)	**p-value**
Age at enrollment, years (SD)	55.1 (11.3)	56.6 (11.2)	0.301
Women (%)	54.5 (67)	57.9 (70)	0.608
Education, % (n)			
Low	20.3 (25)	24.8 (30)	0.552
Medium	51.2 (63)	44.6 (54)	
High	28.5 (35)	30.6 (37)	
Smoking, % (n)			
Never	46.3 (57)	38.0 (46)	0.425
Former	31.7 (39)	36.4 (44)	
Current	22.0 (27)	25.6 (31)	
Alcohol consumption, % (n)			
0-7 units/week	92.7 (114)	85.1 (103)	0.068
≥8 units/week	7.3 (9)	14.9 (18)	
Physical activity, % (n)			
<1 hour/week	28.5 (35)	27.3 (33)	0.289
1-3 hours/week	42.3 (52)	51.2 (62)	
>3 hours/week	29.3 (36)	21.5 (36)	
Waist cirumference, cm (SD)	89.0 (13.5)	94.6 (11.4)	<0.001
Hypertension, % (n)	44.7 (55)	54.6 (66)	0.159
Diabetes mellitus, % (n)	2.4 (3)	5.0 (6)	0.332
Lipoprotein(a), % (n)			
<50th percentile	57.7 (71)	42.2 (51)	0.009
50-80th percentile	30.1 (37)	31.4 (38)	
80-100th percentile	12.1 (15)	26.5 (32)	
Dietary habits (SD)			
Fat score	48.7 (18.1)	58.0 (19.3)	<0.001
Fish, fruit and vegetable score	46.2 (13.5)	48.8 (14.5)	0.161
Fatty acids in adipose tissue, % (SD)			
Saturated fatty acids	6.1 (1.19)	5.7 (1.20)	0.007
Monounsaturated fatty acids	58.6 (2.68)	58.6 (2.82)	0.937
N-6 PUFAs	9.4 (1.21)	9.4 (1.34)	0.785
Marine n-3 PUFAs	0.3 (0.14)	0.4 (0.19)	<0.001
Plant n-3 PUFA	0.9 (0.18)	0.9 (0.17)	0.196
Trans fatty acids	0.3 (0.06)	0.3 (0.06)	0.407

Abbreviations: SD, standard deviation; PUFAs, polyunsaturated fatty acids

Conclusion

In conclusion, in this study population of Faroese subjects with highly elevated LDL-C levels fulfilling a clinical diagnosis of FH, we found a very low proportion of monogenic FH and a high proportion of individuals with a likely polygenic cause of FH. Subjects with clinical FH had higher plasma levels of lipoprotein(a) compared to controls. Surprisingly, subjects with clinical FH had lower intake of saturated fat and lower content of SFAs and higher content of marine n-3 PUFAs in adipose tissue compared to controls. Thus, we conclude that the very high levels of LDL-C observed in the Faroese population, are mainly due to polygenic causes and to a lesser extent other genetic factors including lipoprotein(a) levels. Differences in lifestyle did not explain the elevated LDL-C levels, if anything, dietary measures seemed to be more healthy among clinical FH cases.

5.3 STUDY III

Aim

The aim of this study was to describe CAC scores in clinical FH cases compared to matched healthy controls and to investigate the associations between CAC levels and clinical FH and subtypes including polygenic causes of FH.

Key methods

In this case-control study possible cases between 18 and 75 years were identified by previously measured LDL-C levels above 6.7 mmol/L in a nationwide laboratory database and invited by letter to attend a clinical examination and diagnostic evaluation for FH. Subjects without secondary dyslipidemia who met the criteria of definite/ probable FH according to the MEDPED, Simon Broome or DLCN criteria were considered eligible FH cases. Controls were recruited in the background population and subjects with a plasma LDL-C < 3.5 mmol/L, DLCN score ≤3 and without a history of ASCVD and without the use of lipid-lowering medication were considered eligible as controls. Subjects with a history of ASCVD were excluded from this study as we aimed to investigate CAC score as an indicator of subclinical atherosclerosis. Also, subjects with persistent atrial fibrillation/ flutter were not considered eligible, as these arrhythmias could impair the quality of the cardiac CT scans. Further, subjects with elevated creatinine levels >120 μmol/L and subjects who were pregnant were not considered eligible for the present study.

Coronary artery calcium score was assessed by cardiac CT. We used multivariable logistic regression to investigate associations between CAC score and clinical FH.

Main results

A total of 120 clinical FH cases and 117 age- and sex-matched controls were included. A CAC score of zero was found in nearly 50 percent of the clinical FH cases. We found strong and statistically significant associations between levels of CAC score and clinical FH, and the observed association was strongest in individuals with a CAC score ≥300 Houndsfield units, commonly classified as very high cardiovascular risk. The multivariate adjusted OR for the association between CAC score ≥300 Houndsfield units and clinical FH was 5.59 (95% CI 1.65;18.94, p = 0.006) compared

with subjects with a CAC score of zero (Table 5). In supplemental analyses of the associations between categories of CAC scores and subtypes of clinical FH cases, we found the highest odds in clinical FH cases with a PRS >80th percentile suggesting a likely polygenic cause.

Table 4. 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)	
	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

[a] Multivariable adjustments included smoking, hypertension, waist circumference and lipoprotein(a)

Conclusion

In conclusion, 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 of hypercholesterolemia.

CHAPTER 6. DISCUSSION

6.1 STRENGTHS AND LIMITATIONS OF THE STUDIES

The strengths and limitations of the studies included in this thesis have been discussed in detail in the individual papers, but some important topics will also be addressed here. In study I, we examined the lipid and lipoprotein distribution in the Faroe Islands and estimated the prevalence of FH based on blood samples registered in a nationwide laboratory database. The strengths of this study were the large proportion of the entire Faroese population included in the analyses (approximately 60%), which allowed us to describe the lipid and lipoprotein distribution among individuals aged 0 to 100 years of age. However, because our data was cross-sectional, we were unable to draw any conclusions about true changes in lipids over time. Also, the blood samples were limited to individuals who had their cholesterol levels measured previously as part of clinical practice. Further, in this study, the prevalence of FH was based on lipid measurements alone, as we did not have information on other items included in diagnostic criteria for FH, such as clinical and family history of CVD, FH stigmata or genetics. This may have underestimated the true prevalence of FH. Neither did we have information on possible use of lipid-lowering treatment or secondary causes of dyslipidemia, and this may have overestimated or underestimated the true prevalence of FH. Further, the estimation of the prevalence of FH was based on the highest registered plasma LDL-C measurement in each individual, which could lead to an overestimation of the prevalence, as it could be affected by measurement errors, periodically extreme diets, secondary dyslipidemia etc.

In studies II and III, we used a case-control design to examine the associations between our exposures of interest and clinical FH. The advantages of case-control designs include the possibility of conducting cost-effective analyses of risk factors associated with rare outcomes. However, case-control studies may be prone to selection bias and information or recall bias, and therefore careful methodological considerations are needed to limit these sources of bias. Furthermore, the retrospective and observational nature of case-control designs may be used to provide information on associations between multiple exposures of interest and the outcome in question, but cannot be used to establish causation [82].

Selection bias

Selection bias may arise from systematic errors during the identification and recruitment of cases and controls into the study and from factors that can influence the participation in a study. Such errors may occur if the association between exposure and outcome is different for participants and non-participants [83]. In our study, we recruited FH cases within a nationwide laboratory database covering approximately 60% of the entire population. These individuals had their cholesterol levels measured as part of clinical practice, but due to the large sample covering a significant part of the population, these lipid and lipoprotein measurements are believed to be representative of the Faroese population. The participation of eligible clinical FH cases invited to participate in the study was over 80%, and even though this does not eliminate potential selection bias, the high participation rate among cases makes the data less prone to selection bias. Of those participating in the study, both cases and controls, we had complete exposure and covariate information on > 90%.

Controls were identified at random from the background population and those with a plasma LDL-C <3.5 mmol/L, a DLCN score ≤3 and without a history of ASCVD and without the use of lipid-lowering medication were considered eligible as controls. The cutoff LDL-C value of 3.5 mmol/L was chosen based on a review of 26,500 individuals from the Faroe Islands that had an LDL-C measurement performed between 2006 and 2018. The median of the first measured LDL-C was 3.3 mmol/L, whereas the median of the highest measured LDL-C was 3.7 mmol/L. The cutoff of 3.5 mmol/L was chosen to be representative of the cholesterol levels of the Faroese population. The participation rate among controls was approximately 70%, which was lower than among the clinical FH cases. However, selection bias was not considered a major concern in our study.

Information bias

Information bias may arise from inaccurate assessment of the exposures and/ or misclassification of the outcomes of interest [83]. Several types of information bias can occur during data collection. Information on dietary intake in study II was assessed by a self-reported FFQ which are prone to measurement errors. However, the FFQ used in this study (HeartDiet) has previously been carefully developed to assess the habitual diet of subjects with dyslipidemia in clinical practice and has been validated against a semi-quantitative 198-item FFQ and biomarkers for dietary intake and was found suitable to assess a heart-healthy diet [75]. However, HeartDiet was not designed to include traditional Faroese foods and we could not capture variations

in food intake over time. Furthermore, the participants may have reported a more advantageous portrayal of their diet depending on what is perceived as beneficial such as a low consumption of foods rich in saturated fat as we observed. However, we also found that the content of SFAs in adipose tissue, an objective measure of fatty acid exposure, was lower in clinical FH cases than controls and this may suggest that those with clinical FH had lowered their intake of foods rich in SFAs due to their elevated cholesterol levels.

All of the information included in the background questionnaire by both cases and controls is prone to recall bias. However, information on medical history, medications etc. was reviewed by going through the participants medical journals during the clinical examination visit. Nevertheless, information bias due to inaccurate self-reported information on covariates cannot be ruled out.

Confounding

A confounder can be defined as a variable which influences or is associated with both the exposure variable and the outcome variable, causing spurious association [82]. In studies II and III to minimize the effect of confounding, we conducted multivariable adjustment for potential risk factors defined a priori to data analysis. However, residual confounding due to inaccurate self-reported information on covariates, inadequate adjustment, or unaccounted risk factors cannot be ruled out in our research.

We applied adjustments for risk factors in different models. In study II, the observed associations for each outcome in the minimally adjusted models taking into account age and sex (Model 1) in general showed similar overall patterns of associations after additional adjustment for established risk factors for dyslipidemia, including educational level and alcohol intake (Model 2), indicating no or minimal confounding from these factors. In supplemental analysis, we included additional adjustments for potential intermediates, including physical activity and waist circumference, which also showed overall similar patterns of associations.

In study III, the observed associations in Model 1 (age- and sex-adjusted) were slightly weakened after additional adjustment for established risk factors for ASCVD, including smoking, hypertension, waist circumference, and lipoprotein(a) (Model 2). Supplemental analyses of subtypes of clinical FH were limited by few cases, and multivariate adjusted OR for subjects with a CAC ≥300 should particularly be interpreted with caution due to the risk of overfitting.

Generalizability

The generalizability of the results in this thesis may be limited according to the eligibility criteria for being invited to participate in the study and the exclusion criteria applied. Initially, we invited individuals with a previously measured LDL-C >6.7 mmol/L, which is the cutoff that corresponds to definite FH according to the MEDPED criteria. This strategy was chosen to improve the cost-benefit of the recruitment procedure by limiting our extensive examinations to those individuals most likely to meet the clinical diagnostic criteria for FH. However, this may imply that we did not include FH cases with less severe hypercholesterolemia, and our results may not be generalizable to these subjects. But the first-degree relatives were included based on specific LDL-C cutoffs and hence had lower LDL-C levels. The Faroese population is considered isolated and homogenous, and the results obtained in this thesis may not be generalizable to other populations with different genetic backgrounds.

6.2 MAIN FINDINGS OF THE STUDIES

The lipid and lipoprotein distribution in the Faroe Islands and the prevalence of FH in the Faroe Islands have to our knowledge not previously been assessed. Further, no studies have examined possible explanations for the highly elevated cholesterol levels in this genetically homogenous population. Neither have the clinical characteristics and levels of subclinical atherosclerosis in patients with FH been investigated. In this chapter, we will discuss the main findings of the studies included in this thesis.

Lipid and lipoprotein distribution

In study I investigating the lipid and lipoprotein distribution according to age and sex, we found specific differences in lipid levels in men compared to women. In men, the highest median plasma LDL-C level across age groups was seen at 45-50 years of age, while the highest median plasma LDL-C in women was seen later in life at age 60-65 years of age and decreased onwards in both men and women. Plasma HDL-C levels were relatively stable in men in all age groups, while women had slightly higher levels, especially in age groups above the age of 50 years. Triglyceride levels in women were relatively stable in all age groups with moderately higher levels in women older than 60 years of age. In men, however, triglyceride levels were highest

in the age groups from 40 to 55 years of age and then slightly lower in older age groups.

Several studies and cohorts have previously collected distribution values for the major lipoproteins and lipids in different populations [84–89]. Balder et al. [88] thus provided complete contemporary percentile based reference values based on 133,450 individuals from the Netherlands participating in a cross-sectional population-based cohort study. Our cross-sectional data are comparable to the cross-sectional data from this Dutch study, even though their distributions were based on fasting blood samples. Also, subjects with CVD and subjects receiving lipid-lowering treatment were excluded in the study by Balder et al. However, their study also showed prominent sex- and age-related differences in main plasma lipids and lipoproteins. In men, LDL-C increased markedly from adolescence and peaked in age group 45-49 years of age. At higher age groups LDL-C levels gradually decreased. In women, by contrast, LDL-C was stable until their mid-30s, after which LDL-C increased to a maximum for the age group 55-59 years. Women also had higher maximum LDL-C levels compared to men and no clear decline at higher ages was observed as in men [88].

We found similar patterns and levels of especially plasma LDL-C in both men and women. Balder et al. [88] found a peak median plasma LDL-C among men aged 45-49 years (median 3.6 mmol/L) compared to in women aged 55-59 years (median 3.7 mmol/L). Comparable patterns and levels of plasma HDL-C were also seen in both men and women. However, although we did see similar patterns of triglyceride levels across the different age groups in men and women, triglyceride levels were higher in our study compared to Balder et al [88]. Also, we observed similar patterns of LDL-C according to sex and age as a recent large Danish cohort including 559,889 individuals [89].

Our study shows, that LDL-C levels were highest in women aged 55 to 65 years of age, and the lower LDL-C levels seen in men in older age groups, were not seen as distinctively in women. Several studies report that the menopause transition is associated with changes in lipid levels [90,91]. Postmenopausal women thus have higher levels of LDL-C than premenopausal women, and the mechanism may partially be explained by the complex hormonal effects of estrogens on lipid metabolism [92]. Also, weight-gain and lower levels of physical activity could affect cholesterol levels in postmenopausal women.

The distinct age- and sex-specific differences in lipid and lipoprotein levels seen in our study emphasize the importance of accounting for age and sex when evaluating lipid profiles.

Prevalence of FH

FH is severely underdiagnosed in most countries of the world. According to Nordestgaard et. al [11], the Netherlands have the highest estimated percent of individuals diagnosed with FH with 71% diagnosed. Of the Nordic countries, 43% are diagnosed in Norway and 19% in Iceland. However, this is theoretically predicted based on a prevalence of 1 in 500 in the general population, so the true percentages may instead be 26% and 9.5%. Based on data from an older study from the Eastern part of Norway, the prevalence of FH in Norway was found to be 1 in 300 [93]. In Denmark, a survey of approximately 69,000 individuals using the DLCN criteria (>5 points) found a frequency of FH to be 1 in 223 [34]. A Finnish study found an estimated prevalence of FH to be 1 in 600 [94]. However, these prevalence calculations were based entirely on *LDLR* mutation data. A recent study in Iceland found a prevalence of monogenic FH of 1 in 836 in a large sample of 166,281 genotyped individuals, representing a large fraction of the Icelandic population [95]. According to a modified version of the DLCN criteria (defined as probable or definite FH), they found a prevalence of 2.2% among 79.000 adults.

Limited data exists on FH prevalence based on laboratory databases only. One Italian study used electronic data in 162,864 individuals to estimate the FH prevalence [96]. The prevalence of definite FH according to the MEDPED criteria was 1 in 540 in statin-treated and 1 in 1,380 in untreated subjects. Thus, the prevalence according to diagnostic criteria based on laboratory databases was markedly higher in our study.

Due to founder effects, the prevalence of FH and especially FH causing mutations is much higher in countries with populations isolated by cultural or geographical boundaries like French-Canadians (1 in 270) [97], Christian Lebanese (1 in 90) [98] or Afrikaners in South Africa (1 in 76) [99,100]. We found a prevalence of possible FH according to the LDL-C cutoffs included in the DLCN criteria of 1 in 8 and a prevalence of definite FH according to the MEDPED criteria for plasma LDL-C of 1 in 142. A prevalence that seems to be comparable to known FH founder populations together with the genetic history of the Faroese population led us to believe, that we could possibly have discovered a new FH founder population.

Genetics

Given the very high prevalence of possible and definite FH based on cholesterol levels in the Faroese population and the genetic history of the Faroe Islands, we expected to possibly identify a “Faroese founder mutation”. However unexpectedly, we found a

surprisingly low proportion of only 2.5% of pathogenic monogenic FH causing mutations in subjects with clinical FH included in the case-control study. Founder populations are often characterized by a few pathogenic mutations dominating within the population. In Finland, three founder mutations have been found to account for approximately 80% of the known *LDLR* mutation spectrum and approximately 70% of all FH cases [94]. A few FH mutations also dominate within the population of Iceland [41,95].

The genetics of FH are complex, and studies have shown, that the probability of a positive genetic test result depends on the probability of FH as determined by diagnostic criteria. Thus, a variant in the three most common FH causing genes can be found in a larger proportion of those with definite or probable FH compared to in those with possible or unlikely FH [13,23]. In our study, we had a high proportion of individuals with probable and definite FH by diagnostic clinical criteria and in these subjects an FH mutation is typically found in approximately 60-80% [13]. This is far from our finding of only 2.5% with a monogenic FH mutation.

Interestingly, a total of 8 subjects (9.6%) had the same rare variant in the *LDLR* gene (Ser421Arg). This variant is classified as a variant of unknown significance (VUS) in the ClinVar database. In order to explore this variant further, we found the prevalence of this mutation to be 0.6% in the Faroese population based on a sample from 206 individuals without CVD (data from unpublished Faroese studies). In the gnomAD (European non-Finnish) database the minor allele frequency (MAF) is reported to be 0.00004. Further, we analyzed ten first-degree relatives for this specific variant. Eight of them had the same variant and they all had plasma LDL-C levels above the age- and sex-specific 75th percentile of LDL-C compared to data from subjects included in the Copenhagen General Population study. Two relatives did not have this specific variant and of these two, one had plasma LDL-C corresponding to the 50th percentile and the other had LDL-C above the 95th percentile. Although all mutation carriers had high LDL-C values, these results are somewhat contradictory; nonetheless, further information on non-carrier relatives is necessary. However, the combined data, including the fact that this variant is 15 times more prevalent in our cases compared to our reference Faroese population and 2,500 times more prevalent than in a non-Finnish European population, indicate that this variant might be causative of FH for some individuals in the Faroe Islands.

Due to the very few pathogenic variants found in the initial standard FH panel, we expanded the genetic analyses to include an extended next generation sequencing of FH-related genes described in the literature to be causally associated with FH in 20 selected clinical FH cases with the highest LDL-levels and a family history suggestive

of monogenic inheritance of FH. However, this did not yield more pathogenic monogenic variants. Also, in subjects with FH and an untreated plasma LDL-C ≥ 6.7 mmol/L (n=83) we conducted genotyping for 12 LDL-C raising SNPs and calculated PRS and found a very high frequency of PRS >80th percentile indicating that polygenic hypercholesterolemia may be an important explanation for the observed high prevalence of FH in the Faroe Islands. Studies have estimated, that in those without a monogenic cause of FH, up to 20-30% can be explained by polygenic causes [31,46,101]. In our study, 63% of those genetically tested had PRS over 80th percentile suggestive of polygenic hypercholesterolemia. Also, these individuals seem to have a lower risk of CVD and respond better to lipid-lowering treatment compared to subjects with monogenic FH [47,48,102,103], so the findings may have clinical implications.

Lipoprotein(a)

Since the genetic results showed a very low frequency of monogenic FH in our study population, we started investigating other possible explanations for the elevated cholesterol levels in the Faroese population, including polygenic causes, plasma lipoprotein(a) levels and diet. High levels of lipoprotein(a) are frequent in individuals with clinical FH but without identifiable pathogenic variants [26]. Approximately 30-45% of the total mass of lipoprotein(a) is composed of LDL-C, and this content is not being accounted for when plasma LDL-C is calculated by the Friedewald formula or measured directly [73,104]. Therefore, given that FH is frequently diagnosed clinically, some individuals with high plasma lipoprotein(a) levels could pass the plasma LDL-C thresholds used to diagnose FH. Thus, one Danish study suggested that elevated lipoprotein(a) concentrations accounted for up to 25% of patients with clinically diagnosed FH [27]. Studies have also shown that high lipoprotein(a) levels increase the risk of developing CVD in subjects with FH [105,106]. Because we found strong associations between high levels of plasma lipoprotein(a) and clinical FH, lipoprotein(a) may have had a role in reaching the LDL-C threshold of an FH diagnosis of some of the individuals with clinical FH in our study.

Dietary measures

A healthy diet is recommended as a cornerstone of CVD prevention in all individuals [6]. Together with concomitant lipid-lowering therapy, lifestyle interventions are recommended to all subjects with elevated LDL-C levels in all risk categories [9]. The

dietary factors with the most significant effect on plasma LDL-C levels are SFAs and TFAs [9,107,108] and a reduced intake of these fatty acids may lower LDL-C by approximately 5-10% [9,109–111]. Dietary advice to reduce cardiovascular risk include a diet low in saturated fat with focus on whole-grains, vegetables, fruit and fish [6,9]. We found, that clinical FH cases had higher mean fat-scores according to self-reported dietary data indicating a lower intake of saturated fat compared to controls. One of the limitations with FFQs is that respondents often under-report consumption, and this practice is more common among those, who are overweight [76]. Thus, these results could be considered to be caused by information-bias. However, this was supported by the levels of SFAs in adipose tissue being lower in subjects with clinical FH. Also, they had higher levels of marine n-3 PUFAs and hence a higher intake of fatty fish compared to controls. Adipose tissue biopsy content of fatty acids is a biomarker of dietary intake over the last 1-2 years [76]. These findings could possibly be explained by the focus on eating more healthy when you are aware of your condition with highly elevated plasma LDL-C levels and as part of lifestyle interventions recommended by physicians. Differences in lifestyle did not explain the elevated LDL-C levels in our study, as dietary measures seemed to be more heart-healthy among clinical FH cases.

Coronary artery calcium

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 Houndsfield units, 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.

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 [112–114]. 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 [9,115]. This might suggest that some individuals may be less prone to develop early ASCVD despite a genetic susceptibility and lifelong exposure to significantly high LDL-C levels. Miname and Gallo [113,116] found a very low risk of ASCVD events in asymptomatic individuals with proven genetic diagnosis of FH and CAC scores of

0 after a short median of 3.7 and 2.7 years of follow-up, respectively. These studies may suggest that detection of CAC may qualify the risk stratification in subjects with FH.

In our study, we observed that subjects with clinical FH had higher levels of CAC compared to controls, which is supported by a few previous studies [117,118]. 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 [47,50,101]. 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 [47,102].

Our study subjects, however, did not undergo a coronary computed tomography angiography (CTTA) and thus we have no information on possible non-calcified plaques. Interestingly, a recent study among 948 Danish individuals with severe hypercholesterolemia found a prevalence of non-calcified plaque to be 22.8% in subjects with plasma LDL-C levels ≥4.9 mmol/L and a CAC score of zero [119]. A total of 9.6% had obstructive non-calcified plaques, obstructing more than 50% of the coronary lumen. These non-calcified plaques may have prognostic significance.

CHAPTER 7. CONCLUSIONS AND PERSPECTIVES

In conclusion, we found distinct differences in lipid and lipoprotein distribution according to age and sex in the Faroese population. Also, we found a very high prevalence of possible FH in the Faroe Islands with 1 in 8 having an LDL-C level of 5 mmol/L or above and 1 in 142 fulfilled a definite clinical FH diagnosis according to the MEDPED criteria. This led us to believe, that the Faroe Islands could be a new FH founder population given the genetic history of the Faroese population with isolation and genetic drift. However, the proportion of a monogenic pathogenic FH mutation was as low as 2.5% in subjects with clinical FH and highly elevated levels of plasma LDL-C. A rare mutation in the *LDLR* gene was found in approximately 10% of those, who were genetically tested. However, this mutation is classified as a variant of unknown significance and further investigation of this mutation is needed before any conclusions can be made on its possible role for FH in the Faroe Islands. Some rare mutations in the APOB and ABCG8 gene were found in a few of the individuals. However, after literature research, these mutations seem to be associated with higher plasma LDL-C levels, although not causative of FH per se. Interestingly, we found a very high prevalence of over 60% of polygenic FH defined as PRS >80th percentile. The SNPs causing polygenic FH can be inherited in families but in another pattern than monogenic FH. We examined a selected group of participants with extremely high plasma LDL-C levels genetically, so the possibility of finding a mutation, that we have not yet encountered is of course plausible, but not very likely.

We conducted an extensive examination of the participants included in our case-control study. When it was clear, that only a very few monogenic mutations would be found, we started investigating other possible explanations for the highly elevated cholesterol levels in the Faroe Islands. The traditional Faroese food is high in saturated fat, the dietary habits, however, have shifted to a more western like diet in the recent years. In fact, we found that subjects with clinical FH and highly elevated LDL-C levels reported a more heart-healthy diet compared to healthy controls. This could possibly be explained by awareness of their condition and also as a part of lifestyle interventions proposed by physicians or dietitians. The amount of SFAs in adipose tissue biopsies, however, was also significantly lower in clinical FH cases. Also, they had higher levels of marine n-3 PUFAs in adipose tissue, suggesting a higher intake of fatty fish. The significance of dietary habits in FH in the Faroe Islands needs further investigation. Our findings, however, do not suggest that diet has significant influence. We found high levels of lipoprotein(a) in cases compared to controls, and lipoprotein(a) could have contributed to the FH diagnosis in some of our clinical FH

cases. We conclude, however, that the very high levels of LDL-C observed in the Faroese population mainly are due to polygenic causes and to a lesser extent other genetic factors and elevated plasma lipoprotein(a) levels.

We found higher levels of CAC as a biomarker for subclinical atherosclerosis in subjects with clinical FH compared to controls. This was expected and was also our hypothesis. When analyzing subgroups, we found the highest level of CAC in those with a polygenic cause of FH.

Lipids and lipoproteins are recognized as the most important modifiable risk factor for CVD. The investigation of lipids, lipoproteins and FH in the Faroese population has been initiated but there are still areas that need further investigation. A more comprehensive investigation of the genetic causes of FH is warranted and especially further examination of the frequent variant found in many of the clinical FH cases included in our study. Also, since the estimated prevalence of FH in the Faroe Islands is so high, an investigation of the incidence of CVD in the Faroese population would be of interest. Further, studies investigating potential CVD risk differences among individuals fulfilling a clinical diagnosis of FH compared to polygenic and monogenic causes of FH would be of major interest.

ENGLISH SUMMARY

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death globally. Low-density lipoprotein cholesterol (LDL-C) is a causal factor for the development of atherosclerosis, and subjects with familial hypercholesterolemia (FH) are characterized by severely elevated LDL-C levels and hence a considerably increased risk of premature ASCVD and death. The worldwide prevalence of FH has been estimated to be 1 in 300, but an even higher prevalence can be seen in certain FH founder populations. FH is underdiagnosed and undertreated in most parts of the world.

The Faroe Islands are an isolated archipelago, and the Faroese population has been affected by genetic drift and is considered the genetically most homogenous population in the North Atlantic region. Several genetic disorders are very prevalent in the Faroe Islands. The prevalence of FH in the Faroe Islands is unknown, and neither the genetic basis nor the clinical characteristics of FH in the Faroe Islands have previously been studied.

The objective of this thesis was to describe the lipid and lipoprotein distribution in the Faroese Islands and to estimate the prevalence of FH here. Furthermore, we aimed to examine the genetic and clinical characteristics and potential causes of FH in the Faroe Islands and to investigate whether coronary artery calcium deposition as a marker of subclinical atherosclerosis was more prevalent in subjects with FH compared to healthy controls.

The data in this thesis were based on blood samples from approximately 60% of the Faroese population over a 14-year period (2006-2020). Based on these blood samples, we described the lipid and lipoprotein distribution according to sex and age and estimated the prevalence of FH in the Faroese population. We conducted a case-control study to investigate genetics, clinical characteristics and possible causes of FH. Thus, we recruited subjects that fulfilled a clinical diagnosis of FH and age- and sex-matched healthy controls from the background population. We collected self-reported data on medical history and lifestyle factors, including diet, using a detailed background questionnaire and a food frequency questionnaire. All subjects underwent clinical and physical examination, and blood samples were collected for genetic analyses and measurement of plasma lipoprotein(a) levels. Also, adipose tissue biopsies were collected to assess the content of fatty acids as a biomarker of dietary intake. Coronary artery calcium as a marker of subclinical atherosclerosis was quantified using a non-contrast cardiac CT scan.

We found we found a very high FH prevalence of 1 in 142, which is equivalent to the prevalence found in certain FH founder populations. Also, distinct differences in lipid and lipoprotein distribution according to age and sex were found. In the case-control study, we found a very low prevalence of pathogenic monogenic FH causing mutations of 2.5% in subjects with highly elevated LDL-C levels and phenotypically FH. However, we found a very high proportion of polygenic FH of over 60%. Also, we found strong associations between high levels of plasma lipoprotein(a) and clinical FH. We found significant positive associations between a dietary fat-score and the content of marine n-3 polyunsaturated fatty acids in adipose tissue biopsies, while the content of saturated fatty acids in adipose tissue was inversely associated with clinical FH. Finally, we found strong associations between coronary artery calcium levels and clinical FH, and clinical FH cases with a likely polygenic cause had high levels of coronary artery calcium compared to controls.

In conclusion, the very high levels of LDL-C observed in the Faroese population were likely explained by polygenic causes and, to a lesser extent, other genetic factors and elevated plasma lipoprotein(a) levels. Differences in lifestyle did not explain the elevated LDL-C levels; if anything, we measured a more healthy diet among clinical FH cases compared to controls. Also, clinical FH cases and especially those with a likely polygenic cause had high levels of coronary artery calcium indicating subclinical atherosclerosis.

DANSK RESUMÉ

Hjertekarsygdom er fortsat den primære årsag til død på verdensplan. Lav-densitets lipoprotein kolesterol (LDL-kolesterol) spiller en væsentlig rolle i udviklingen af åreforkalkning. Familiær hyperkolesterolæmi (FH) er en medfødt sygdom som er karakteriseret ved svært forhøjet LDL-kolesterol i blodet med heraf følgende høj risiko for udvikling af tidligt indsættende hjertekarsygdom og død. FH menes at forekomme hos 1 ud af 300 personer på verdensplan, men endnu højere forekomster kan ses i befolkningsgrupper etableret af et mindre antal grundlæggere (founder-populationer).

Færøerne er en isoleret øgruppe og den færøske befolkning betragtes som den genetisk mest homogene befolkning i den Nordatlantiske region. Flere genetiske sygdomme er således vist at være hyppigere på Færøerne end andre steder i verden, men forekomsten af FH er ukendt og de genetiske årsager hertil samt kliniske karakteristika ved FH er ikke tidligere blevet undersøgt på Færøerne.

Formålet med denne afhandling var at beskrive kolesterolniveauerne i blodet i den færøske befolkning med fokus på LDL-kolesterol samt at beregne forekomsten af FH på Færøerne baseret på tidligere målte kolesteroltal. Derudover ønskede vi at undersøge genetiske og kliniske karakteristika og mulige årsager til FH på Færøerne ved at lave gentest på personer med FH og at sammenligne kostvaner og andre livsstilsfaktorer hos personer med FH med raske kontrolpersoner. Herudover ønskede vi at undersøge om forekomsten af kalk i kranspulsårerne, som regnes for at være et tidligt tegn på åreforkalkning, var mere hyppig hos personer med FH sammenlignet med raske kontrolpersoner.

Denne afhandling er baseret på data fra blodprøver fra cirka 60% af den færøske befolkning indsamlet i perioden 2006 til 2020. Baseret på disse tidligere blodprøver har vi beskrevet kolesterolniveauerne i blodet opdelt på alder og køn og efterfølgende beregnet forekomsten af FH i den færøske befolkning. Herefter inviterede vi personer med svært forhøjede kolesterolværdier forenelige med FH sammen med tilfældigt udvalgte raske kontrolpersoner til at indgå i et case-kontrolstudie med henblik på at undersøge genetiske årsager, kliniske karakteristika og mulige årsager til FH på Færøerne. Alle deltagere gennemgik en objektiv undersøgelse og vi indsamlede data om deres sygdomshistorie og livsstilsfaktorer inklusiv deres kostvaner. Blodprøver blev taget til genetiske analyser og måling af indholdet af lipoprotein(a) niveauet i blodet. Derudover fik alle deltagere taget fedtvævsbiopsier til at undersøge indholdet

af fedtsyrer heri, der repræsenterer et objektivt mål for indtaget af fedtsyrer i kosten. Kalkindholdet i kranspulsårerne blev undersøgt ved hjælp af en hjerte-CT skanning.

Vi fandt væsentlige forskelle i kolesterolniveauerne hos mænd og kvinder og på tværs af aldersgrupper. Herudover fandt vi en meget høj forekomst af klinisk FH på 1 ud af 142, hvilket er sammenligneligt med forekomsten fundet i flere kendte founder-populationer. I case-kontrolstudiet fandt vi imidlertid en meget lav forekomst af sygdomsfremkaldende monogene FH mutationer på kun 2.5% blandt personer som opfyldte diagnostiske kriterier for klinisk FH. I forbindelse med mere detaljerede genetiske undersøgelser fandt vi imidlertid at mere end 60% havde en sandsynlig polygen årsag til deres FH diagnose. Det vil sige, at de havde flere ganske små genetiske afvigelser i flere gener, som tilsammen kan have betydning for kolesterolniveauet. Herudover fandt vi, at personer med FH angav et lavere indtag af mættet fedt via kosten og havde lavere indhold af mættet fedt i deres fedtvæv sammenlignet med de raske kontrolpersoner. I tillæg hertil fandt vi, at personer med FH havde et højere indhold af fiskeolie (n-3 fedtsyrer) i fedtvæv som udtryk for et højere indtag af fisk sammenlignet med raske kontrolpersoner. Endelig fandt vi en sammenhæng mellem indholdet af lipoprotein(a) i blodet og klinisk FH samt mellem niveauet af kalk i kranspulsårerne og klinisk FH, og at deltagere med en sandsynlig polygen årsag til FH havde høje niveauer af kalk i kranspulsårerne sammenlignet med raske kontrolpersoner.

Konklusionerne af dette studie er derfor, at den høje forekomst af FH observeret i den færøske befolkning overvejende skyldes polygene årsager og i en mindre grad andre genetiske årsager samt forhøjet plasma lipoprotein(a). Forskelle i kost-livsstilsfaktorer forklarede ikke de høje kolesterolværdier og det tydede på, at personer med FH efterlevede en mere hjertevenlig kost sammenlignet med kontrolpersonerne. Herudover fandt vi, at personer med FH og især personer med en polygen årsag til FH havde høje niveauer af kalk i kranspulsårerne.

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ISSN (online): 2246-1302
ISBN (online): 978-87-7573-855-7

AALBORG UNIVERSITY PRESS