



Familial Hypercholesterolaemia

Detection, diagnostic issues and collaboration between lipid clinics and general practice

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FAMILIAL HYPERCHOLESTEROLAEMIA

**DETECTION, DIAGNOSTIC ISSUES AND COLLABORATION
BETWEEN LIPID CLINICS AND GENERAL PRACTICE**

**BY
BERIT STORGAARD HEDEGAARD**

DISSERTATION SUBMITTED 2023



AALBORG UNIVERSITY
DENMARK

**FAMILIAL
HYPERCHOLESTEROLAEMIA:
Detection, diagnostic issues and
collaboration between lipid clinics and
general practice**

by

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AALBORG UNIVERSITY
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Berit Storgaard Hedegaard

March 2023

ENGLISH SUMMARY

Cardiovascular disease (CVD) is the most frequent cause of death worldwide. The main underlying cause of CVD is atherosclerosis, with elevated low-density lipoprotein cholesterol (LDL-C) playing a pivotal role. Familial hypercholesterolemia (FH) is considered a frequent monogenic inherited condition that causes elevated plasma LDL-C from birth. Individuals with FH have an increased risk of acquiring early atherosclerotic CVD (ASCVD), due to lifelong exposure to circulating high levels of LDL-C. Unfortunately, FH is underdiagnosed and undertreated in Denmark and most other countries.

Another hereditary cholesterol disorder includes elevated plasma lipoprotein(a) [Lp(a)], which is also associated with an increased risk of ASCVD.

The aims of the thesis were to investigate characteristics and diagnoses of patients referred to Danish lipid clinics on suspicion of FH. We also aimed to study the usefulness of the current referral criteria for suspected FH to lipid clinics, as well as the impact of genetic testing for a diagnosis of FH. Finally, we investigated the significance of plasma levels of Lp(a) for a diagnosis of FH and the risk associated with high Lp(a) for ASCVD and myocardial infarction (MI) in comparison with clinical and genetic FH.

Our hypotheses were that among the referrals approximately 20% would be diagnosed with FH and that genetic testing would considerably increase the number of individuals diagnosed with FH. We also hypothesized that high levels of plasma Lp(a) have an impact on the clinical diagnosis of FH, and that very high Lp(a) might be an important risk factor for MI and ASCVD similar to having FH.

Study I-II of this thesis were based on data from 1,488 adult patients referred on suspicion of FH to 15 lipid clinics distributed throughout Denmark. The inclusion period was from September 2020 to December 2021, and among those referred, more than 97% agreed to participate. The results showed that 26% were diagnosed with FH based on genetic examination and/or clinical criteria. Furthermore, we found that genetic testing, especially among the young, increased the number of individuals diagnosed with FH from 22% to 37% even though genetic testing was only done in 56% of the population. Interestingly, approximately 20% among those classified with unlikely or possible FH according to the Dutch Lipid Clinic Network criteria had a positive gene test. Consequently, they (and potentially their families) would not have been diagnosed with FH had they not been genetically tested for FH. If the referral

criteria had been changed to only apply to subjects with e.g. LDL-C \geq 6.5 mmol/L, a major proportion of those with FH would not have been referred and diagnosed.

Furthermore, individuals with high Lp(a) and because of this a high content of LDL cholesterol was instrumental for 27% of cases with clinical FH, and among those referred with early ASCVD for up to 32% of cases.

Study III was based on the Copenhagen General Population Study, with a total of 69,644 people included in this study. A very high Lp(a) \geq 180 mg/dL (389 nmol/L) corresponded to a risk for ASCVD similar to that associated with having genetic FH. This value was lower when the FH clinical diagnosis was based on Make Early Diagnoses Prevent Early Deaths Program (MEDPED) and Simon Broome criteria and higher when using Dutch Lipid Clinical Network criteria, criteria that beside LDL-C levels are based on family and personal history of early cardiovascular disease.

We conclude that the referral criteria to the Danish lipid clinics should remain unchanged. Genetic testing for FH should be more widespread among those referred on suspicion of FH. We also conclude that high Lp(a) may be responsible for a diagnosis of FH and may help explain some of the FH cases where a FH mutation cannot be demonstrated. We also observed that levels of Lp(a) at 67-402 mg/dL (142-873 nmol/L) corresponded to clinical and genetic FH. Furthermore, levels of plasma Lp(a) are important for CVD risk and Lp(a) should be measured in all patients suspected of FH.

Identification (and treatment) of persons and their families with FH should be intensified to reduce these persons high risk of cardiovascular disease.

DANSK RESUME

Hjerte-kar-sygdom er på verdensplan den hyppigste årsag til død og antallet af personer, der lever med en hjerte-kar-sygdom i Danmark er stigende. Den bagvedliggende årsag til hjerte-kar-sygdom er åreforkalkning og højt lav-densitet lipoprotein kolesterol (LDL-K) og spiller en afgørende rolle. Familiær hyperkolesterolæmi (FH) er en af de hyppigste monogene arvelige tilstande, der forårsager forhøjet plasma LDL-K allerede fra fødslen. Personer med FH har en højere risiko for at udvikle tidlig hjerte-kar-sygdom grundet livslang eksponering af højt LDL-K. FH er underdiagnosticeret og underbehandlet i det meste af verden, og dette gælder også i Danmark.

En anden arvelig hyperkolesterolæmi, forhøjet plasma lipoprotein(a), [Lp(a)] er ligeledes forbundet med øget risiko for hjerte-kar-sygdom.

Det overordnede formål med afhandlingen var at undersøge personer, der blev henvist på mistanke om FH til danske lipidklinikker og beregne procentdelen af de henviste som efterfølgende blev diagnosticeret med FH. Vi havde også til formål at undersøge de nuværende henvisningskriterier for mistænkt FH, og evaluere disse kriterier såvel som værdien af genetisk testning i udredningen af FH. Herudover undersøgte vi på betydningen af indholdet af Lp(a) i plasma for diagnosen af FH og risikoen forbundet med høje Lp(a)-niveauer for hjerte-kar-sygdom i sammenligning med risikoen ved klinisk og genetisk FH.

Vores hypoteser var, at blandt de henviste ville ca. 20% blive diagnosticeret med FH, og genetisk testning for FH ville øge antallet af personer diagnosticeret med FH betydeligt. Vi antog også, at høje niveauer af plasma Lp(a) har en indvirkning på den kliniske diagnose af FH, og at meget højt plasma Lp(a) var en vigtig risikofaktor for hjerte-kar-sygdom på samme niveau som FH.

Studie I+II var baseret på 1,488 henviste patienter ≥ 18 år med mistanke om FH i 15 lipidklinikker fordelt på hele Danmark. Inklusionsperioden var fra 1. september 2020 til 30. november 2021, og blandt alle henviste, accepterede mere end 97% at deltage i studiet. Vores undersøgelser viste, at 26% af de henviste blev diagnosticeret med FH. Hvis henvisningskriterierne blev indsnævret til kun at gælde personer med LDL-K ≥ 6.5 mmol/L ville en stor andel af de henviste ikke være blevet opdaget og diagnosticeret med FH. Endvidere fandt vi, at genetisk testning, især blandt de unge, selv med en lav klinisk score for FH, var afgørende for at etablere en FH diagnose. Højt plasma Lp(a) kunne forklare omkring 27% af de personer som opfyldte en klinisk

FH-diagnose, og blandt dem, der blev henvist med tidlig hjerte-kar-sygdom med næsten 32%.

Studie III var baseret på data fra Copenhagen General Population Study (Herlev-Østerbrounderundersøgelsen) en befolkningsundersøgelse med over 100.000 individer, hvoraf 69,644 personer blev inkluderet i denne undersøgelse. Personer med et meget højt Lp(a)-niveau på 180 mg/dL (389 nmol/L) havde samme risiko for udvikling af åreforkalkningsbetinget hjerte-kar-sygdom som det at have genetisk FH.

Vi konkluderer, at henvisningskriterierne til de danske lipidklinikker bør forblive uændrede. Flere bør gentestes for FH af de henviste, således at især unge får stillet diagnosen trods en lav klinisk FH-score. Det konkluderes også, at Lp(a) har en stor indflydelse på kliniske FH-diagnoser, og er en vigtig forklarende årsag i de tilfælde hvor en FH-mutation ikke kan påvises hos patienter med klinisk FH.

Vi anbefaler, at alle med mistanke om FH får målt Lp(a) for at give patienten den bedste risikovurdering og behandling.

Opsporing (og behandling) af personer og deres familier med FH bør intensiveres for at nedsætte disse personers høje risiko for hjerte-kar-sygdom.

LIST OF PAPERS

This thesis is based on the following three papers:

Paper I

Hedegaard BS, Bork CS, Kanstrup HL, Thomsen KK, Heitmann M, Bang LE, Henriksen FL, Andersen LJ, Gohr T, Mouridsen MR, Soja AMB, Elpert FP, Jakobsen TJ, Sjøøl A, Joensen AM, Nordestgaard BG, Klausen IC, Schmidt EB. Genetic testing increases the likelihood of a diagnosis of familial hypercholesterolaemia among people referred to lipid clinics: Danish national study[1]. Manuscript submitted.

Paper II

Hedegaard BS, Nordestgaard BG, Kanstrup HL, Thomsen KK, Bech J, Bang LE, Henriksen FL, Andersen LJ, Gohr T, Larsen LH, Soja AMB, Elpert FP, Jakobsen TJ, Sjøøl A, Joensen AM, Klausen IC, Schmidt EB, Bork CS. Lipoprotein(a) may explain 27% of diagnoses of clinical familial hypercholesterolemia in lipid clinics: Results from a Danish nationwide study[2]. Manuscript attached.

Paper III

Hedegaard BS, Bork CS, Kaltoft M, Klausen IC, Schmidt EB, Kamstrup PR, Langsted A, Nordestgaard BG. Equivalent Impact of Elevated Lipoprotein(a) and Familial Hypercholesterolemia in Patients With Atherosclerotic Cardiovascular Disease. *J Am Coll Cardiol.* 2022;80(21): 1998–2010.
<https://doi.org/10.1016/j.jacc.2022.09.021>[3].

ABBREVIATIONS

ASCVD, Atherosclerotic cardiovascular disease

Apo(a), apolipoprotein(a)

ApoB, apolipoprotein B

APOB, apolipoprotein B gene

CHD, Coronary heart disease

CVD, Cardiovascular disease

CGPS, Copenhagen General Population Study

HeFH, Heterozygote Familial hypercholesterolaemia

HoFH, Homozygote Familial hypercholesterolaemia

DLCN, Dutch Lipid Clinic Network

FH, Familial hypercholesterolaemia

FFH, Find Familial Hypercholesterolaemia

LDL-C, Low-density lipoprotein cholesterol

LDLR, Low-density lipoprotein cholesterol Receptor

Lp(a), Lipoprotein(a)

MEDPED, Make Early Diagnoses Prevent Early Deaths Program

MI, Myocardial infarction

PCSK9, proprotein convertase subtilisin/kexin type 9

PI, Principal Investigator

RKKP, Regionernes kliniske kvalitetsudviklingsprogram, The Danish Clinical Quality Program, National Clinical Registries

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CHAPTER 1. INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the world[4-8] and according to World Health Organization, about 15.3 million people died from heart attack or stroke in 2019[4].

In Denmark, CVD is the second most frequent cause of death surpassed only by cancer [9]. The number of deaths caused by CVD have decreased in the last twenty years in Denmark, but the number of individuals living with CVD has on the contrary increased with approximately 31% since 2004[9, 10]. This may be explained by better prevention and treatment of patients with CVD as well as an increased life-expectancy of the population[11]. More than half a million Danes are diagnosed with CVD, and more than 50.000 develop CVD in Denmark every year[12].

The risk of CVD increases with age, and men develop CVD at earlier age than women[9, 13]. The most important modifiable risk factors of CVD include elevated plasma cholesterol, smoking, hypertension, diabetes mellitus, sedentary lifestyle and obesity[5, 14]. Especially elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) has convincingly been shown to have a causal relationship with atherosclerosis and atherosclerotic cardiovascular disease (ASCVD)[5, 14, 15]. While age and sex are well-known non-modifiable risk factors for ASCVD, the importance of genetic variants is being increasingly recognized for their importance for early onset of ASCVD and cardiovascular death[14]. One of the most common monogenic diseases is familial hypercholesterolaemia (FH) which occurs in 1:250-300 individuals [16-18], but unfortunately FH is an underdiagnosed and undertreated condition[19, 20]. FH is characterized by elevated levels of LDL-C from birth and it is crucial to identify individuals with FH and to eliminate and treat modifiable risk factors in these patients to prevent or at least to reduce their risk of ASCVD later in life[21]. Therefore, a main focus of the present thesis was to investigate diagnostic issues of patients referred to Danish lipid clinics on suspicion of FH.

CHAPTER 2. BACKGROUND

The underlying cause of ASCVD is atherosclerosis not least characterized by arterial accumulation of cholesterol from apolipoprotein B (apoB) containing lipoproteins[22]. The process of atherosclerosis is extremely complex, have different phases and develops during decades[23-25], and is only very briefly dealt with in the following. The accumulation of cholesterol in the arterial intima may initiate inflammatory responses including monocyte recruitment and endothelial dysfunction which may result in atherosclerotic plaque formation [8, 15, 26]. All of the apoB containing lipoproteins are atherogenic and include LDL, very-low-density lipoprotein, intermediate-density lipoprotein and lipoprotein(a) [Lp(a)][27]. Cholesterol is taken up by monocyte/macrophages leading to foam cell formation a hall mark of the early fatty streak formation in atherosclerosis [28]. Macrophage inflammation results in enhanced oxidative stress and cytokine secretion, causing LDL-C oxidation, and endothelial cell dysfunction[8]. The formation of plaques is central for atherosclerosis and in particular plaques with inflammation and thin fibrous coats are prone to rupture with supervening thrombus formation, which may lead to acute cardiovascular events such as myocardial infarction (MI), ischemic stroke and sudden cardiac death[7, 8].

Atherosclerosis is a general disorder of the arteries and may give rise to clinical symptoms from the heart (e.g. angina pectoris or MI), the brain (e.g. ischemic stroke) and peripheral artery disease (e.g. intermittent claudication)[15, 18, 26]. LDL-C has a pivotal role in initiation and progression of atherosclerosis and has been shown to be causally associated with the risk of ASCVD[15]. High LDL-C may arise for several reasons including genetic disorders (typically FH), imprudent lifestyle - in particular a high intake of saturated fat - and because of conditions that may per se increase plasma cholesterol levels (e.g. thyroid, liver and renal diseases as well as the use of certain medications)[18, 29].

2.1 FAMILIAL HYPERCHOLESTEROLAEMIA

2.1.1 History

FH was first described in end of the 19th century by the Norwegian pathologist Francis Gottfred Harbitz and the Norwegian physician in Internal Medicine, Carl Arnoldus Müller[30]. They observed a correlation between xanthomas, hypercholesterolaemia and sudden CVD deaths in Norwegian families. This was also the reason why FH was called the Müller-Harbitz disease[30]. Several decades later in 1964, FH was clinically described in its heterozygous and homozygous forms by Khachadurian[24, 31, 32]. In their landmark studies in the 1970ties, Michael Brown and Joseph Goldstein were able to show that FH may be caused by defects in the gene coding for LDL receptors (LDLR) leading to reduced removal of LDL-C from the circulation and a new steady state with very high levels of LDL-C in plasma. For their important findings Brown and Goldstein were awarded the Noble Prize in Medicine in 1985[32]. The importance of the role of the LDL receptor for removal of cholesterol from the blood became even more interesting when it was shown that treatment with HMG-Coenzyme A Inhibitors (β -Hydroxy β -methylglutaryl-CoA) – often abbreviated statins - could inhibit cholesterol synthesis in the liver by inhibiting the rate limiting enzyme for conversion of acetyl Coenzyme A to cholesterol[33]. This reduced synthesis of cholesterol in the liver leads to secondary upgrading of the LDLR causing significantly enhanced removal of LDL cholesterol from the circulation and consequently a lowering of plasma LDL-C.

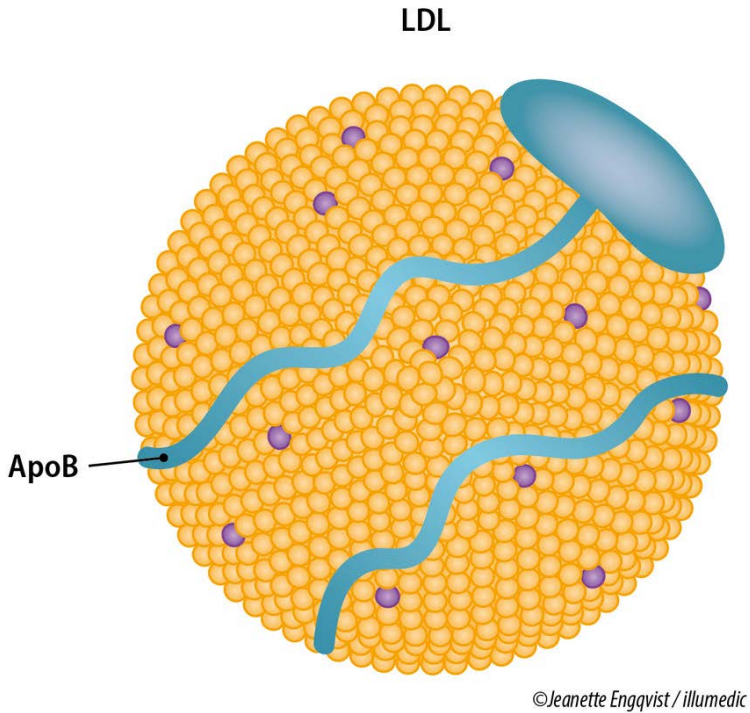


Figure 1: LDL particle with ApoB. Reproduced with permission from the author.

2.1.2 Genetics and FH

Today more than 2000 genetic variants in LDLR have been reported[32, 34, 35]. Also, genetic variants in the apolipoprotein B (APOB) gene, which is necessary for binding of ApoB containing lipoproteins to the LDLR, may cause FH with severe elevation of plasma LDL-C levels[36]. Further, gain of function mutations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene may cause FH by degrading the LDLR, while LDL adaptor protein 1 (LDLRAP1) may inhibit LDLR function and thereby cause high LDL-C and FH[15, 21, 37, 38].

FH is an autosomal dominant disorder, except from the LDLRAP1 mutations, which is characterized by autosomal recessive inheritance[37]. The most common pathogenic variants causing FH are LDLR mutations that accounts for >90% of the known pathogenic FH variants[18] while APOB variants causes approximately 5% of cases while pathogenic PCSK9,LDLRAP1 and other monogenic variants causing FH are very rare.

Genetic testing for FH sometimes reveals genetic variants with minor or no effects on LDL-C and these are therefore at present considered of unknown significance. Furthermore, no known pathogenic variants can be demonstrated in approximately one third of cases with clinical FH [19, 39]. These results are very puzzling and have led to suggestions that several genes in combination might create a phenotype of FH (polygenic hypercholesterolaemia) which may account for several cases of FH[40]. Individuals with clinically verified FH without pathogenic variants identified by genetic screening for FH may still have a hereditary disorder and be at substantial risk of ASCVD[35, 38].

Individuals with pathogenic FH variants usually have FH due to mutation in one allele, heterogenous FH (HeFH), but may also have compound HeFH with two or more mutations in different genes[41]. HeFH occurs in 1:250-300[16, 17] among most Caucasian populations, with higher occurrences in founder populations, for example among French Canadians, Lebanese and Afrikaaners [35, 42, 43].

Homozygote FH (HoFH) due to mutations in two alleles with defective LDL receptor function, occurs in only approximately 1:160.000[44] and are characterized by extremely elevated LDL-C levels[41], associated with high risk of early, progressive ASCVD and premature death[41].

In this Thesis, FH will be used equivalent to HeFH.

2.1.3 How to diagnose FH?

High plasma LDL-C does not give any symptoms per se, which of course may be good, but on the other hand individuals can have unnoticed high cholesterol levels considerably increasing their risk of atherosclerosis and ASCVD[45]. There is no regular screening for high cholesterol in Denmark and hypercholesterolaemia may therefore be detected by coincidence during a general health check-up. Other reasons for measurement of plasma cholesterol in an individual might be awareness by the

patient and/or the doctor of the significance of cholesterol for CVD, or high cholesterol and/or early CVD in the family. Very occasionally the observation of xanthelasmata and even more rarely the findings of xanthomas or arcus cornealis might lead to testing for high cholesterol. Diagnosis and treatment of FH therefore rely on awareness of the diagnosis and appropriate response to finding high plasma LDL-C in an individual.

A diagnosis of FH can be made when a causative pathogenic FH variant is identified. However, if genetic testing is not available or negative, FH can be established by clinical scoring systems. Among the most frequently used scoring systems are the Simon Broome Register Diagnostic criteria (Table 1) from United Kingdom[46], the Make Early Diagnoses Prevent Early Deaths Program (MEDPED) from the US (Table 2)[47, 48] and the Dutch Lipid Clinic Network (DLCN) criteria from the Netherlands (Table 3)[14]. The DLCN criteria is the most used clinical scoring system to diagnose FH in Europe.

The clinical criteria are based on elevated total cholesterol or LDL-C levels, personal history of early ASCVD, occurrence of xanthomas or arcus cornealis before the age of 45 years, and a history of early ASCVD and/or high cholesterol in the family[21, 49].

MEDPED is a clinical scoring system that relies on total cholesterol and LDL-C levels in the general population or relatives in the family, whereas Simon Broome and DLCN criteria are based on a combination of clinical history, physical findings, family history and genetic test results[21].

| Simon Broome criteria |
|--|
| <p>Definite FH is defined as:</p> <p>Total cholesterol >6.7 mmol/L or LDL-C >4.0 mmol/L in a child aged <16 years or total cholesterol >7.5 mmol/L or LDL-C >4.9 mmol/L in an adult</p> <p>Plus:</p> <p>Tendon xanthomas in patient or in a 1st degree relative or in a 2nd degree relative</p> <p>Or:</p> <p>DNA-based evidence of an LDL receptor mutation or familial defective apoB-100 or a PCSK9 mutation</p> |
| <p>Possible FH is defined as:</p> <p>Total cholesterol >6.7 mmol/L or LDL-C >4.0 mmol/L in a child aged <16 years or total cholesterol >7.5 mmol/L or LDL-C >4.9 mmol/L in an adult</p> <p>and at least one of the following:</p> <p>Family history of myocardial infarction before 50 years of age in a 2nd degree relative or before 60 in a 1st degree relative</p> <p>Or:</p> <p>Family history of raised cholesterol >7.5 mmol/L in adult 1st or 2nd degree relative or >6.7 mmol/L in children or siblings aged <16 years</p> |

Table 1: Simon Broome FH Criteria[48, 50].

| Make Early Diagnosis to Prevent Early Deaths (MEDPED) diagnostic criteria for HeFH | | | | |
|---|---|------------------------|-----------------------|--------------------|
| | Total Cholesterol (LDL-C) concentrations, mmol/L | | | |
| Age | First-degree relative | Second-degree relative | Third-degree relative | General population |
| <20 | >5.7 (4.0) | >5.9 (4.3) | >6.2 (4.4) | >7.0 (5.2) |
| 20-29 | >6.2 (4.4) | >6.5 (4.7) | >6.7 (4.8) | >7.5 (5.7) |
| 30-39 | >7.0 (4.9) | >7.2 (5.2) | >7.5 (5.4) | >8.8 (6.2) |
| ≥40 | >7.5 (5.3) | >7.8 (5.6) | >8.0 (5.8) | >9.3 (6.7) |

Table 2: MEDPED diagnosis of FH[47].

| Dutch Lipid Clinic Network (DLCN) criteria | |
|--|-------|
| Family history (maximum of 2 point) | Score |
| First-degree relative with premature* ASCVD <u>or</u> First-degree relative with known LDL-C $\geq 95^{\text{th}}$ percentile for age and sex | 1 |
| First-degree relative with tendinous xanthomata and/or arcus cornealis <u>or</u> Children aged <18 years with known LDL-C $\geq 95^{\text{th}}$ percentile for age and sex | 2 |
| Clinical history (maximum of 2 point) | |
| Premature* myocardial infarction | 2 |
| Premature* cerebral or peripheral vascular disease | 1 |
| Physical examination (maximum of 6 point) | |
| Tendinous xanthomata | 6 |
| Arcus cornealis, before age 45 years | 4 |
| LDL-C level | |
| LDL-C ≥ 8.5 mmol/L | 8 |
| LDL-C 6.5-8.4 mmol/L | 5 |
| LDL-C 5.0-6.4 mmol/L | 3 |
| LDL-C 4.0-4.9 mmol/L | 1 |
| Genetic testing | |
| Causal FH mutation, LDLR, APOB, PCSK9 | 8 |
| Familial hypercholesterolaemia stratification | |
| Definite FH | > 8 |
| Probable FH | 6-8 |
| Possible FH | 3-5 |
| Unlikely FH | < 3 |
| *Premature=Men <55 years and women <60 years. | |

Table 3: DLCN criteria of FH[18].

The DLCN score is based on the total number of points in the following categories: Family history (maximum of 2 points), clinical history (maximum of 2 points), physical examination (maximum of 6 points) and LDL-C level (maximum of 8 points). A score of <3 points defines unlikely FH, 3-5 points possible FH, while a diagnosis of probable- and definite FH requires 6-8 points and >8 points, respectively[18].

In Denmark, the Simon Broome criteria were used for many years in clinical practice for establishing an FH diagnosis, but in recent years there has been consensus on the use of DLCN criteria in the Danish lipid clinics[40].

Patients with FH are mainly diagnosed in lipid clinics that receive referred patients for dyslipidemia including FH from primarily general practitioners but also from hospital departments. The lipid clinics are placed in Departments of Cardiology at 15 hospitals spread around the country. Investigations for FH in the lipid clinics are carried out by a multidisciplinary staff often consisting of physicians, nurses, and clinical dietitians in collaboration with Departments of Clinical Biochemistry.

In 2013, a Consensus Statement of the European Atherosclerosis Society stated that FH is an underdiagnosed and undertreated disease in most countries, including Denmark[19]. Furthermore, not only was the detection rate poor, but treatment efforts to lower LDL-C levels were insufficient. Similar findings were noted in most other countries in Europe based on findings from the EAS Familial Hypercholesterolaemia Studies Collaboration group[20, 51]. The highest detection rates of FH were reported from The Netherlands and Norway, with 71% and 43%, respectively diagnosed of those assumed having FH, respectively[19]. The high success rate for detection of FH in these countries probably arise from national screening programs with support from public authorities. This included widespread use of genetic testing and cascade screening together with support by dedicated healthcare professionals, patient associations and public information material on FH written for lay persons and patients[52, 53].

According to an internal status report from the Danish Ministry of Health[54] published in 2017, it was estimated that only 20% of adults with FH were diagnosed in Denmark. Therefore, a sum of money was allocated by the Ministry of Health in the years 2016-2020 to strengthen efforts to improve detection and treatment of individuals with FH in Denmark. Therefore, The Danish Clinical Quality Program,

National Clinical Registries (RKKP) in 2017 established a database on FH with focus on monitoring detection of new cases of FH, and to evaluate the treatment quality in the lipid clinics[55, 56]. Next, from the beginning of 2019, a cross-regional working group was set up with representation from all Danish Regions and relevant medical societies with the aim of preparing solutions to improve efforts concerning practical issues in the management of FH. This group submitted its report to the Danish Regions in 2020[57] with suggestions for improved collaboration between lipid clinics and general practitioners as well as improvement of education of patients and health professionals in the lipid clinics.

To improve detection of FH it is of outmost importance with a good collaboration between lipid clinics and general practitioners, who may identify the patients with hypercholesterolaemia and individuals suspected of FH. In addition, there should be clear guidelines for referral of patients with suspected FH to local lipid clinics. Finally, the group also strongly advocated for a national study to examine the proportion of patients diagnosed with FH among those referred on suspicion of FH to all the lipid clinics[57]. Another aim was to evaluate whether the current referral criteria for FH to lipid clinics were optimal for detection of as many individuals as possible with FH and at the same time secure, that the resources available were used optimally[57]. We planned and organized such a nationwide study described in Paper I of this thesis.

2.1.4 Treatment of FH

Prevention and treatment of high LDL-C in FH consists of a heart-healthy lifestyle along with lipid-lowering drug therapy to reduce the lifelong burden of LDL-C and to slow the progression of atherosclerosis to reduce the risk of cardiovascular events[18, 19, 21, 58, 59]. Statin treatment is the cornerstone of medical treatment of high LDL-C in individuals with FH[58], but it is often necessary to add ezetimibe to meet the treatment goal of LDL-C in subjects with FH[18]. In case this is not achieved with the use of these drugs in the maximally tolerated dose, then PCSK-9 inhibitors or small-interfering-RNA-based therapeutics may be indicated. These drugs are expensive and rules for their use are given by Danish Medicines Council in Denmark[60]. Patients with extremely high LDL-C despite the aforementioned treatments notably patients with HoFH may be offered treatment with lomitapide, mipomersen or LDL-C apheresis[44].

2.1.5 Children and FH

The high cholesterol level is usually present from birth in subjects with FH, and a diagnosis of FH can in principle be made at that time. This has led to suggestions of screening for FH at birth, but this has not been done so far in any country and despite gains need careful ethical considerations. However, in Slovenia there has been established universal screening for FH in children at age 5-6[61]. The atherosclerotic process starts very early[58] and with the increasing knowledge and data of safety and very few side effects to pharmaceutical treatment with primarily statins, guidelines have during recent years advocated for earlier detection and treatment of FH[18, 58]. In all children with FH it is mandatory to secure a heart-healthy lifestyle, but medications are also often needed.

2.2 LIPOPROTEIN(a)

In the beginning of the 1960'es the Norwegian scientist Kåre Berg was searching for hereditary variants of LDL containing particles, and in 1963 he reported the finding of a new lipoprotein named Lp(a)[62]. Later, Lp(a) was described as an LDL-particle with a central core of cholesterol esters and triglycerides, free cholesterol and phospholipids carried by Apo B100 and bound via a disulfide bridge to a glycoprotein, apolipoprotein(a)[63-65]. Apolipoprotein(a) contains a series of loop structures called kringles and have an inactive protease domain, kringles type IV and V, whereas kringle IV has 10 types. Kringle IV type 2 varies with multiple copies from two to more than 40 and the number of kringle IV type 2 repeats affects plasma levels of Lp(a)[64, 66]. The heritability associated with levels of Lp(a) was described by Utermann in the late 1980s[63]. His studies showed that Lp(a) levels in plasma were inversely correlated to size variants of apolipoprotein(a). The higher the plasma Lp(a) level, the fewer repetitions in kringle IV[63]. Plasma levels of Lp(a) are for more than 90% determined by genetic variability in the LPA gene[64, 67].

Later, McLean et al. succeeded in cloning the apolipoprotein(a) gene and found significant homology to plasminogen an important component of the coagulation and fibrinolytic system[68]. Therefore, Lp(a) was initially hypothesized to increase the

risk of thrombosis, but later studies showed that no causal relation of the thrombotic processes in humans with impaired fibrinolysis and enhanced coagulation has yet been convincingly demonstrated in patients with high Lp(a)[69, 70].

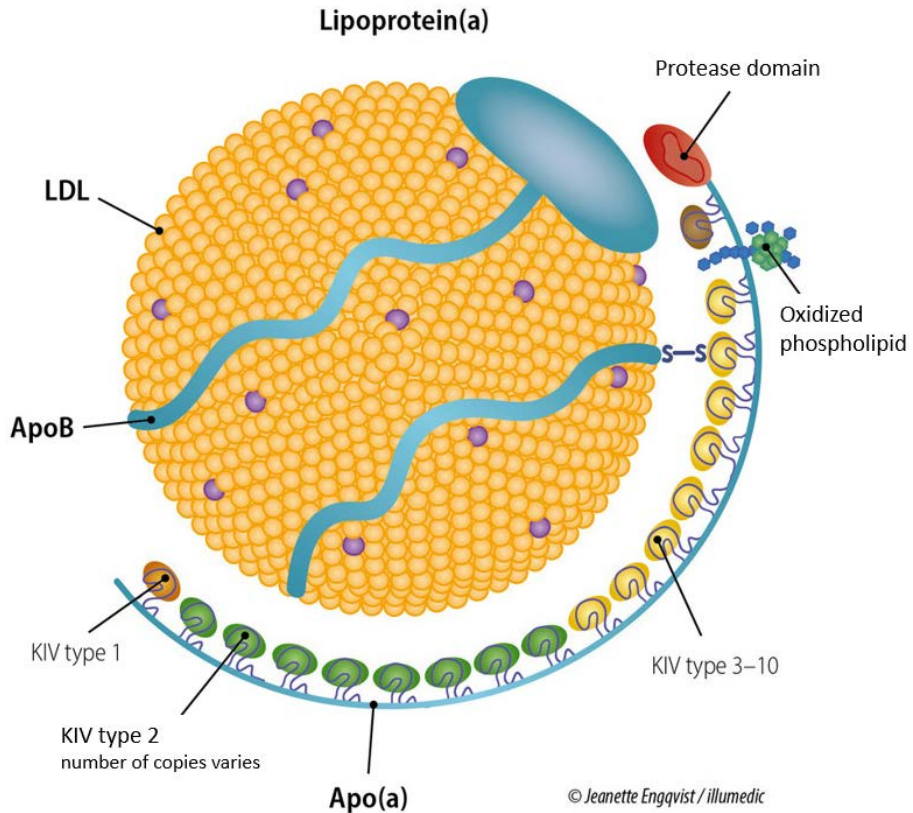


Figure 2: Lp(a) particle. Lp(a) is an LDL particle attached to apolipoprotein(a) which consists of kringles. Apolipoprotein(a) size is determined by the number of copies of kringle IV type 2 (KIV type 2) and is inversely correlated with levels of Lp(a). Reproduced with permission from the author.

Early reports including a paper from Seed et al.[71] suggested that patients with FH had an even more increased risk of ASCVD, if they also had high plasma Lp(a) levels which fuelled a lot of enthusiasm for the significance of Lp(a) in ASCVD. However, in the following years mixed results regarding Lp(a) was published and dampened for

a period the interest in Lp(a), perhaps also because plasma levels of Lp(a) were not reduced by diet, lifestyle and the medications used at that time, including statins[72-75] and also of issues related to measurement of Lp(a)[76]. However, new data not least obtained from the Copenhagen General Population Study by Kamstrup, Nordestgaard et al.[65, 72, 76-78] but also from other groups[79-81] convincingly have shown the significance of Lp(a) for ASCVD and aortic stenosis. An obvious reason for this relates to the fact that Lp(a) contains approximately 30-45% LDL-C[82]. As elevated LDL-C plays a major role in the diagnosis of FH, this may imply that individuals with high plasma Lp(a) levels may meet diagnostic LDL-C criteria for FH without having classical autosomal dominant FH. In fact, Langsted et al.[74, 77, 82, 83] and Chan et al.[84] reported that approximately 25% patients with clinical FH may be diagnosed with FH because of elevated plasma Lp(a). However, it is possible to correct for the contribution of LDL-C from Lp(a)[69], which is of clinical importance also because of a less efficient response to statins and ezetimibe on LDL-C in patients with highly elevated Lp(a). In the lipid clinics, Lp(a) corrected LDL-C is not used in the diagnosis of FH, and the clinical significance of this is uncertain. We therefore aimed to investigate this in a study included in the thesis (Paper II).

Studies have reported that plasma levels of Lp(a) are an independent risk factor for ASCVD[14, 85, 86]. The importance of Lp(a) levels compared to having FH was investigated in this thesis (Paper III).

2.2.1 Treatment of elevated Lp(a)

Guidelines recommend to assess the absolute risk for ASCVD and to ensure a healthy lifestyle in individuals with elevated Lp(a) and if deemed unacceptable, statin treatment may be used to reduce overall risk despite having no beneficial effect on Lp(a)[18, 69, 87].

PCSK9 inhibitors have been shown to reduce Lp(a) levels by up to 25% in post-hoc analysis among patients in secondary prevention, but they are not used solely for this indication. Finally, lipoprotein apheresis may reduce Lp(a) by 60-70%[88], but is a time-consuming and very expensive treatment not advocated in Denmark (but in few other countries including Germany) for this purpose[89].

There have recently been developed drugs that reduce plasma Lp(a) by approximately 80%, and some of these are now investigated in large clinical trials for assessment of tolerability, safety and potential reduction of ASCVD[90].

CHAPTER 3. AIMS AND HYPOTHESES

The overall aims of the thesis were to determine individuals referred on suspicion of FH to Danish lipid clinics, and to calculate the percentage of individuals who eventually was diagnosed with FH. Further we wanted to describe characteristics of these patients regarding LDL-C levels, personal and family history of hypercholesterolaemia and ASCVD. We also aimed to investigate the current referral criteria for suspected FH, and to evaluate whether these criteria should be changed as well as the impact of genetic testing for a diagnosis of FH. Finally, we focused on the significance of plasma levels of Lp(a) with respect to a diagnosis of FH and the risk associated with high Lp(a) levels for ASCVD in comparison with a diagnosis of FH.

Our hypotheses were that approximately 20% of those referred had FH; that genetic testing would increase the number of individuals diagnosed with FH considerably; that high levels of plasma Lp(a) might lead to a diagnosis of FH otherwise not given, and that very high plasma Lp(a) was an important risk factor for ASCVD in the same order as having monogenic FH.

These aims and hypotheses were investigated in three studies mentioned below.

3.1 PAPER I

A study of all individuals referred to the Danish lipid clinics (n=15) during a period of at least one year and until at least 1,000 individuals were recruited. These patients were investigated for FH and for clinical and laboratory characteristics.

Genetic testing increases the likelihood of a diagnosis of familial hypercholesterolaemia among people referred to lipid clinics: Danish national study. (Paper I)[1].

3.2 PAPER II

A study of the significance of plasma Lp(a) for a diagnosis of FH, when values of plasma LDL-C were corrected for the content of cholesterol in Lp(a).

Lipoprotein(a) may explain 27% of diagnoses of clinical familial hypercholesterolaemia in lipid clinics: Results from a Danish nationwide study (Paper II)[2].

3.3 PAPER III

A study comparing the risk for MI and ASCVD associated with high Lp(a) levels or with FH diagnosed by genetic testing or by clinical criteria (MEDPED, Simon-Broome or DLCN). This was investigated using data from the Copenhagen General Population Study (CGPS).

Equivalent Impact of Elevated Lipoprotein(a) and Familial Hypercholesterolaemia in Patients With Atherosclerotic Cardiovascular Disease (Paper III)[3].

CHAPTER 4. METHODS

The methods used have been described in detail in paper I[1], II[2] and III[3] included in this thesis and will be given in brief below.

4.1 Find Familial Hypercholesterolaemia (Study I+II)

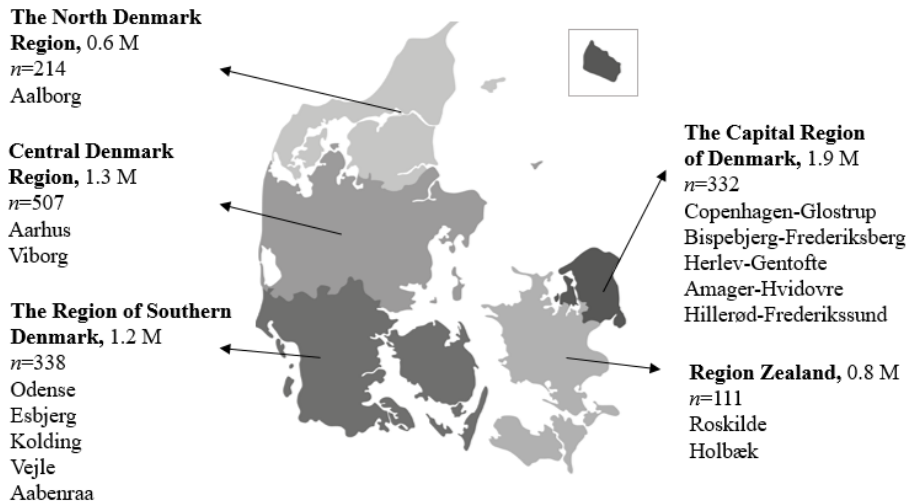
The project, which was named Find Familial Hypercholesterolaemia (FFH) was introduced in February 2020. All lipid clinics in Denmark (Figure 3) were invited to participate, and all accepted the invitation. All patients referred on suspicion of FH according to standardized criteria (Table 4) to the lipid clinics were eligible provided that secondary dyslipidemia was excluded. No reliable power calculations could be performed, but the inclusion period was decided to last for at least one year, and at least 1,000 patients should be included (both criteria should be fulfilled).

Referral Criteria

1. LDL-C ≥ 5 mmol/L and age above 40 years
2. LDL-C ≥ 4 mmol/L and age 18 to 40 years
3. LDL-C ≥ 4 mmol/L and premature (men < 55 years, women < 60 years) ASCVD
4. Cascade screening after detected FH in a first-degree relative

Table 4: Referral criteria to the lipid clinics in Denmark on suspicion of FH as described in paper I+II).

Each of the 15 lipid clinics in Denmark participated in an on-site start-up meeting, where those involved in the study were introduced by the primary investigator (PI) to the protocol, its definitions (e.g. registration of smoking and family data), consent forms, data worksheets (Table 5a and 5b) and instruction in the use of the study database.



Map of Denmark, photo: The North Region of Denmark

Figure 3: Map of lipid clinics in Denmark divided into 5 regions with populations given in millions (M) and the number of patients included per region.

There were two or more on-site visits by the PI during the inclusion period with data entry and review. For the whole period lipid clinics could contact the PI by telephone calls, mails or texts for solving questions related to the study. After the inclusion period had ended, all lipid clinics were revisited by the PI to finalize registration of available data. The remaining questions, mainly related to delay of answers from genetically tests were dealt with individually.

Inclusion was started September 1st 2020 and ended November 30th 2021. A total of 1,527 individuals were invited and 2,6% (n=39) declined to participate[1, 2]. Exclusions were made for both study I and II at the time of data analysis, and the participants were divided into three referral criteria (Figure 4).

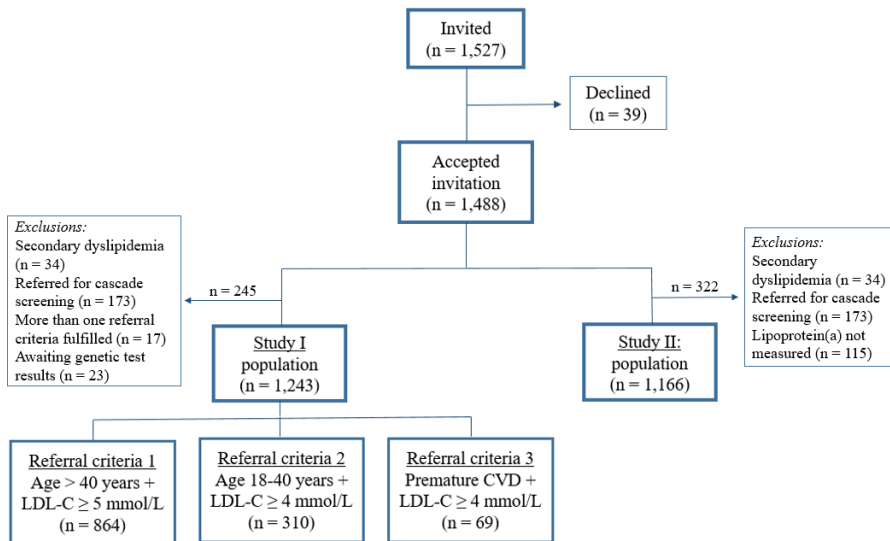


Figure 4: FFH Study, flowchart study population I+II and distribution of the patients according to the referral criteria.

4.2 Ethics

Before the start of the FFH project, approval was obtained from the Data Protection Authority. The Scientific Ethics Committee and the Danish Health Authority were contacted and stated, that the project only had to be approved by the Danish Data Protection Authority. All participating patients were given oral and written information about the study and signed an informed consent form.

We created an encrypted database Research Electronic Data Capture (REDCap) database at Aalborg University for data collection. The database was approved by the Danish Data Protection Agency (J.nr. 2019-899 / 10-0584) and fulfilled the authorities' security requirements. Each lipid clinic had a database created with access only to own patient data.

4.3 Data collection

The patients were invited to participate in the study at their first visit in the lipid clinic. The study was reviewed together with the patient, and eligible patients who signed informed consent forms were included.

Personal history of ASCVD, diabetes mellitus, hypertension and information regarding lipid lowering treatment were collected from the patient and from their medical records.

Patients were asked for family history of ASCVD and family history of elevated LDL-C levels. Self-reported data on smoking habits and alcohol consumption were collected together with dietary data achieved from the Danish Heart Diet Questionnaire[91, 92].

Anthropometric measurements (height, weight and waist circumference) were obtained by the medical staff at the visit. An objective examination of patients for xanthomas, arcus cornealis and xanthelasmata was also carried out.

Data from the enrolled patients were registered at the worksheets handed out to the lipid clinics (Tables 5a and 5b) including the DLCN scoring scheme.

The definition of secondary dyslipidemia was clinically significant hypothyroidism, dysregulated diabetes mellitus, nephrotic syndrome and/or chronic renal insufficiency, primary biliary cirrhosis, use of medications inducing hypercholesterolaemia (e.g. high-dose glucocorticoids, cyclosporine and psychotropic drugs) and extreme diets like anorexia and low carb high fat diets.

The definition of smoking was never smoker (never smoked or who had smoked less than 100 cigarettes in lifetime), former smoker (smoked more than 100 cigarettes in lifetime and not smoked within the last 28 days) and current smoker (currently smoking ≥ 1 cigarette on average per day and had smoked more than 100 cigarettes during the lifetime).

Hypertension was defined as treatment with antihypertensive medication.

Coronary heart disease (CHD) was defined as MI, percutaneous coronary intervention) and/or coronary artery bypass graft surgery. Cerebral disease was defined as an ischemic stroke or a medically treated event of transient cerebral

ischaemia. Peripheral artery disease was defined by relevant symptoms compatible with this and an ankle-brachial index below 0.9 or revascularization of a peripheral artery.

Projekt FFH

Udfyldt af: _____

Dato: ____/____/____ (dd/mm/åå)

Navn _____

CPR _____

REDCap ID _____

Udgave 3/December 2020

Henvisningsårsag (sæt kryds(er)):

- Alder > 40 år og LDL-kolesterol (LDL-k) \geq 5 mmol/l
 Alder 18 - 40 år og LDL-k \geq 4 mmol/l
 LDL-k \geq 4 mmol/l og tidlig personlig hjertekarsygdom (mænd < 55 år, kvinder < 60 år)

Antal LDL-k værdier der opfylder det givne henvisningskriterie: 0 1 2 >2

Henvist efter påvist FH hos slægtning

| | Ja | Nej |
|---|--------------------------|--------------------------|
| Sekundær dyslipidæmi udelukket som årsag til hyperkolesterolemia | <input type="checkbox"/> | <input type="checkbox"/> |
| Hvis nej, angiv type | | |

Højde (cm) _____ Vægt (kg) _____ Livvidde (cm) _____

Fedt-score _____ FiskFrugtGrønt-score _____

Rygning Aldrig Tidligere Aktuel Pakkeår _____

Alkohol (genstande pr. uge) 0-7 8-14 \geq 15

| | Ja | Nej |
|--|---|---------------------------------|
| Hjertekarsygdom | <input type="checkbox"/> | <input type="checkbox"/> |
| Hvis ja, hvilke(n) | | |
| <input type="checkbox"/> Angina pectoris <input type="checkbox"/> AMI <input type="checkbox"/> PCI <input type="checkbox"/> CABG <input type="checkbox"/> Påvist aortastenose <input type="checkbox"/> Iskæmisk apopleksi/behandlet TCI <input type="checkbox"/> PAD (Symptombigivende + ABI < 0,9 og/eller revaskularisering) | <input type="checkbox"/> Aterosklerose/iskæmi påvist ved <input type="checkbox"/> KAG (diffus, insignifikante og signifikante stenoser) <input type="checkbox"/> CT-angio <input type="checkbox"/> Hjerte-CT, CAC score..... <input type="checkbox"/> Ultralyd af karotider <input type="checkbox"/> Anden billeddiagnostik (fx stress MR, RbPET, myokardieskintigrafi m.f.) | |
| Diabetes Mellitus | <input type="checkbox"/> | <input type="checkbox"/> |
| Hvis ja, angiv type | Type 1 <input type="checkbox"/> | Type 2 <input type="checkbox"/> |
| | Anden type <input type="checkbox"/> | |
| I behandling for hypertension | <input type="checkbox"/> | <input type="checkbox"/> |

Kolesterolværdier ved henvisningstidspunktet (mmol/L)

Behandlet* Ubehandlet

Total-k _____ HDL-k _____ LDL-k _____ Triglycerid _____

* Hvis behandlet, angiv type, dosis og frekvens:

Præparat 1: _____ dosis: _____ frekvens: _____

Præparat 2: _____ dosis: _____ frekvens: _____

Lp(a) målt i lipidklinikken: _____ mg/L; ved anden måleenhed angiv denne: _____

Table 5a: Template for collecting data to FFH in the lipid clinics.

| Dutch Lipid Clinic Network Score for FH | | Point | Kriterie opfyldt |
|---|---|--------------------------|-------------------------|
| 1. Familiehistorie | | | |
| Førstegradsslægtning med præmatur (mænd < 55 år, kvinder < 60 år) kardiovaskulær sygdom <i>eller</i> førstegradsslægtning med LDL-k over 95. percentilen for alder og køn ^{se tabel 1} | 1 | <input type="checkbox"/> | |
| Førstegradsslægtning med senexanthomer og/eller arcus cornealis <i>eller</i> børn < 18 år med LDL-k over 95. percentilen for alder og køn ^{se tabel 1} | 2 | <input type="checkbox"/> | |
| 2. Klinisk anamnese | | | |
| Præmatur koronararteriesygdom (mænd < 55 år, kvinder < 60 år) | 2 | <input type="checkbox"/> | |
| Præmatur cerebral eller perifer arteriesygdom (mænd < 55 år, kvinder < 60 år) | 1 | <input type="checkbox"/> | |
| 3. Objektiv undersøgelse | | | |
| Senexanthomer | 6 | <input type="checkbox"/> | |
| Arcus cornealis før 45 års alderen | 4 | <input type="checkbox"/> | |
| Xanthelasmata | 0 | <input type="checkbox"/> | |
| 4. LDL-k niveau | | | |
| Højeste ubehandlede LDL-k* _____ mmol/L | | | |
| LDL-k ≥ 8.5 mmol/L | 8 | <input type="checkbox"/> | |
| LDL-k 6.5–8.4 mmol/L | 5 | <input type="checkbox"/> | |
| LDL-k 5.0–6.4 mmol/L | 3 | <input type="checkbox"/> | |
| LDL-k 4.0–4.9 mmol/L | 1 | <input type="checkbox"/> | |
| 5. DNA analyse | | | |
| Er gentest foretaget? Ja <input type="checkbox"/> Nej <input type="checkbox"/> | | | |
| Hvis ja, afventer svar <input type="checkbox"/> | | | |
| Sygdomsassocieret mutation påvist (LDL-receptor-, ApoB- eller PCSK9 gen) | 8 | <input type="checkbox"/> | |
| <i>Kun højeste score tæller indenfor hver af de 5 områder</i> | | | |
| Angiv total DLCN Score | | | |
| <small>Definitiv FH (>8 points), Sandsynlig FH (6–8 points), Mulig FH (3–5 points)</small> | | | |

*foreligger der ikke en ubehandlet LDL-k værdi, angives den højeste LDL-k på den mest LDL-reducerende behandling mhp. senere beregning af korrigeret LDL-k værdi:

Behandlet LDL-k (mmol/L): Præparat: dosis: frekvens:

Tabel 1. 95% percentilen af LDL-kolesterol i Herlev-Østerbrouundersøgelsen

| Alder | Mænd | Kvinder |
|-------|------|---------|
| < 30 | 4.2 | 3.9 |
| 30-34 | 4.7 | 4.0 |
| 35-39 | 4.9 | 4.1 |
| 40-44 | 5.0 | 4.3 |
| 45-49 | 5.0 | 4.6 |
| 50-54 | 5.1 | 4.9 |
| 55-59 | 5.1 | 5.1 |
| 60-64 | 5.0 | 5.2 |
| 65-69 | 4.9 | 5.2 |
| 70-74 | 4.8 | 5.2 |
| 75-79 | 4.7 | 5.1 |
| 80-84 | 4.6 | 5.1 |

Evt. yderligere kommentarer:

Table 5b: Template for collecting data on DLCN criteria to FFH in the lipid clinics.

4.4 Laboratory measures

All patients had blood samples taken in connection with the referral as a standard local procedure when referring to lipid clinics. Measurements of total cholesterol, HDL (high-density lipoprotein)-cholesterol, and triglycerides with calculation of LDL-C by the Friedewald formula or in a few instances by direct measurement of LDL-C based on local practice. Before referral it was attempted, that all patients should have lipid levels measured at least twice for confirming of elevated LDL-C.

In order to rule out secondary dyslipidemia levels of serum creatinine, glomerular filtration rate, alanine transaminase, alkaline phosphatase, thyroid stimulating hormone and hemoglobin A1c were determined by standard methods used locally.

The highest available LDL-C value was noted in the DLCN scheme.

Genetic testing included pathogenic FH variants in the LDLR, APOB and PCSK9 genes and was performed at five different specialized laboratories in Denmark (Aalborg, Aarhus, Odense, Roskilde and Copenhagen). There were some differences in methods of analyzing the genetic tests as three of the laboratories used next generation sequencing and two used Sanger sequencing for investigation of causative pathogenic FH variants.

4.5 Diagnosis of FH in FFH

The diagnosis of FH in the FFH project was defined as a DLCN score ≥ 6 points corresponding to probable or definite FH (Table 3). Patients were classified according to clinical DLCN criteria with and without taking into account the results of genetic testing for FH. We categorized individuals with probable or definite FH (without taking into account genetic test results) as clinical FH cases, while individuals that carried a pathogenic FH variant were classified as genetic FH cases. Individuals with probable or definite FH according to DLCN criteria after genetic test results, were classified as clinical/genetic FH cases.

4.6 Statistical analyses

We described continuous covariates as medians, while percentages were used to describe categorical variables. We calculated the fraction of individuals with clinical FH, genetic FH and clinical/genetic FH among individuals referred and stratified according to pre-specified characteristics and referral criteria. Subsequently, we evaluated the impact of genetic testing for FH by comparing the fraction of patients with probable or definite FH before and after genetic test results were taken into account. Data were analysed using Stata/MP version 17.

CHAPTER 5. STUDY I

5.1 Study population

Study I was based on data from newly referred patients on suspicion of FH to the lipid clinics fulfilling the nationwide referral criteria (Table 4). All individuals referred (n=1,527) to one of the lipid clinics were invited for study participation and >97% (n=1,488) accepted (Figure 4)[1].

The majority of the individuals referred (n=864) were referred due to referral criteria 1 (LDL-C \geq 5 mmol/L and age >40 years). Referral due to criteria 2 (LDL \geq 4 mmol/L and age 18-40 years) accounted for 310 patients, while 69 were referred on the basis of criteria 3 (LDL \geq 4 mmol/L and premature ASCVD) (Figure 4)[1].

5.2 Main results

A total of 1,243 individuals referred for hypercholesterolaemia on suspicion of FH were included. Genetic testing for pathogenic FH variants was performed in 705 of the referred individuals and increased the probability of FH from 22% to 37%[1].

Compared to the total study population those ultimately diagnosed with FH had similar age and sex, but a higher percentage with FH had a personal and family history of ASCVD (Table 6). We found a higher proportion of tendon xanthomas (14.6 vs. 3.8%), higher untreated highest plasma LDL-C (6.5 vs 5.5 mmol/L) and slightly more received lipid-lowering treatment (34.8 vs 27.2 %) in those with FH compared to the whole study population (Table 6).

| Selected characteristics from the FFH population and the subpopulation with FH | | |
|--|-------------------------|----------------------|
| | Study population | FH population |
| Total | 1,243 | 322 |
| Men | 590 (47.5) | 154 (47.8) |
| Women | 653 (52.5) | 168 (52.2) |
| Age | | |
| Age ≤ 40 years | 292 (23.5) | 73 (22.7) |
| Age > 40 years | 951 (76.5) | 249 (77.3) |
| Family history | | |
| First-degree relative with premature ASCVD | 384 (30.9) | 135 (41.9) |
| First-degree relative with elevated LDL-C | 346 (27.8) | 134 (41.6) |
| Clinical history | | |
| History of premature ASCVD | 117 (9.4) | 56 (17.4) |
| History of ASCVD | 227 (18.3) | 89 (27.6) |
| Physical examination | | |
| Tendinous xanthomata | 47 (3.8) | 47 (14.6) |
| Highest untreated LDL-C mmol/L | 5.5 (4.9-6.9) | 6.5 (5.1-8.1) |
| Lipid-lowering treatment | 338 (27.2) | 112 (34.8) |
| Data are n (%) for categorical variables and medians (10th; 90th percentile) for continuous variables. | | |

Table 6: Selected characteristics from the FFH population and the subpopulation with FH.

The number of individuals referred according to referral criteria with clinical and genetic diagnosis is shown in Table 7[1]. The majority of patients (n=864) was referred via criteria 1, while the highest percentage with FH was seen in individuals from referral criteria 3[1].

| | Referral criteria 1 | Referral criteria 2 | Referral criteria 3 |
|----------------------------|---|---|--|
| | LDL-C \geq 5.0 mmol/L and age > 40 years | LDL-C \geq 4.0 mmol/L and age 18 to 40 years | LDL-C \geq 4.0 mmol/L and premature ASCVD |
| Individuals | <i>n</i> =864 | <i>n</i> =310 | <i>n</i> =69 |
| Clinical FH | 18.4% | 11.3% | 30.4% |
| Genetic tested | 55.2% | 56.8% | 75.4% |
| Genetic FH | 21.4% | 27.8% | 17.3% |
| Genetic/clinical FH | 26.0% | 23.6% | 34.8% |

Table 7: Individuals divided by referral criteria diagnosed according to clinical FH, genetic FH, and genetic and/or clinical FH.

Among the referrals on suspicion of FH to Danish lipid clinics, 26% (322/1,243) were diagnosed with genetic and/or clinical FH according to DLCN criteria. If the referral criteria had been changed to apply only to individuals with LDL-C levels >6.5 mmol/L and a family history of early ASCVD or elevated LDL-C, the probability of having FH among referrals would have been higher. However, at the same time the detection had been much lower and only 42% ($n=134/322$) would have been diagnosed with FH[1].

In individuals from referral criteria 1, the percentage of patients with genetic and/or clinical FH increased from 26% (225/864) to 39% (173/440) when information regarding a family history of premature CVD or a family history of elevated LDL-C was added, whereas the percentage of patients with genetic and/or clinical FH was 76% (124/164)[1].

5.3 Major strengths and limitations

A major strength was the study size with 1,527 consecutive patients invited and acceptance of study participation by 97.4% of the referred patients[1]. Further, the

study was nationwide with participation of all Danish lipid clinics and with predefined definitions, standardized examinations and registration of patient data. Major limitations of the study were that diets were unknown when the highest measured plasma LDL-C levels were registered. Furthermore, only 57% were genetically tested leading to underestimation of the number of patients with FH[1].

5.4 Main conclusions

We found that 26% of the referrals were diagnosed with FH. The current referral criteria on suspicion of FH were suitable as many with FH would have been missed if LDL-C ≥ 6.5 mmol/L and family history of ASCVD and family history of hypercholesterolemia had been the referral criteria[1]. Genetic testing markedly increased the diagnosis of FH independent of the clinical diagnosis, and we therefore recommend a more widespread use of genetic testing in particularly in the young. Notable, among those with unlikely or possible FH, 20% would not have been diagnosed with FH, if genetic testing had not been undertaken[1].

Chapter 6. STUDY II

6.1 Study population

Study II was also based on data from the FHH project[2]. At the time, when results from study II were calculated all the genetic test results were available, but Lp(a) measurements were missing in 115 patients. The study population for study II therefore consisted of 1,166 patients (Figure 4)[2].

6.2 Measurements of plasma Lp(a)

Lp(a) was determined in the vast majority of cases at the baseline visit. Lp(a) was measured at three different hospitals (Hjørring, Herlev and Rigshospitalet) using the same Denka assay in all three laboratories[2].

For the conversion of Lp(a) in mg/dL to nmol/L, we used the equation $2.18 \cdot \text{Lp(a)} - 3.83$. We calculated values of corrected LDL-C for content of cholesterol of Lp(a) by subtraction 30% of total Lp(a) mass from LDL-C[2].

6.3 Diagnosis of Lp(a)-FH

The diagnosis of FH was given according to DLCN criteria, and we adjusted the highest measured LDL-C in each individual for Lp(a) content by subtracting 30% of Lp(a) total mass and reclassified all individuals according to the DLCN criteria. We determined the fraction of individuals fulfilling a diagnosis of FH, when LDL-C was corrected for Lp(a) and at the same time calculated how many was diagnosed with FH due to elevated plasma Lp(a) and defined it as Lp(a)-FH[2].

6.4 Statistical analyses

We calculated the fraction of individuals fulfilling a diagnosis of clinical FH without taking into account genetic test results as well as the fraction with a clinical/genetic FH diagnosis before and after correction for the content of LDL-C in Lp(a)[2].

6.5 Main results

Among referrals (n=1,166) the median Lp(a) was 15 mg/dL (29 nmol/L) compared to a median Lp(a) of 10 mg/dL (18 nmol/L) in the CGPS (n=69,644). In our dataset the 95th percentile was 142 mg/dL (306 nmol/L) and the 99th percentile was 221 mg/dL (478 nmol/L), whereas in the CGPS the 99th percentile was 140 mg/dL (301 nmol/L)[2].

A total of 206 individuals had clinical FH, while 151 individuals had clinical FH after correction for the contribution of Lp(a). Thus, 27% (55/206) had clinical FH due to elevated Lp(a)[2].

6.6 Major strengths and limitations

The FFH study covered the whole country, and 15 lipid clinics invited 1,527 patients to participate and only 2.6% declined rejected the invitation to participate[2].

A weakness was that measurement of Lp(a) was not done in 115 participants and these were therefore excluded from study II. Also, we corrected the LDL-C for content of cholesterol of Lp(a) by subtraction 30% of total Lp(a) mass from LDL-C, but the most appropriate correction factor is uncertain and remains to be determined[2].

6.7 Main conclusions

In conclusion, the diagnosis of clinical FH in 27% was likely due to elevated Lp(a). This should be considered when patients with high plasma Lp(a) are given a diagnosis of FH, and further studies are warranted to determine whether LDL-C should be corrected for Lp(a) cholesterol in patients with high Lp(a)[2]. It is also plausible that some cases of clinical FH without pathogenic variants in the FH gene is caused by high LDL-C in Lp(a)[2].

CHAPTER 7. STUDY III

Study III was part of an external cooperation with the Department of Clinical Biochemistry, Copenhagen University Hospital, Herlev-Gentofte[3].

The methods for this study have been described in detail in paper III and will only be mentioned briefly below.

The aim of study III was to compare the importance of high Lp(a) for a diagnosis of FH on the risk of MI and ASCVD[3].

7.1 Study population

Study III was based on data from CGPS[3, 36, 93, 94], a prospective cohort study from the general population of Copenhagen, Denmark with a 42 years median follow up using record linkage with the nationwide Danish registries[3]. The individuals in CGPS were randomly invited from the area around Copenhagen[3].

For this study we included 69,644 individuals with Lp(a) measurements available allowing for classification according to modified FH criteria [3].

Diagnoses of ASCVD and MI were based on the national Danish Register of Causes of Death and the Danish National Patient Registry[3, 95, 96] and classified due to International Classification of Diseases ICD-8 and ICD-10 codes. Individuals were followed until 2018[3].

7.2 Laboratory measures

The plasma lipid profile including total cholesterol, HDL-C and triglycerides measured on fresh samples[3]. LDL-C was calculated with Friedewalds formula or measured with a direct method if plasma triglycerides were >4 mmol/L. Plasma Lp(a) was measured in mg/dL using the Denka assay[3, 77].

The study population were genotyped for FH using TaqMan assays for the four most common causal LDLR and APOB mutations in Denmark[3, 36].

7.3 Diagnosis of FH

The diagnosis of FH was made using the Simon Broome[50], MEDPED[47] and DLCN[14] criteria. Due to missing information regarding xanthomas and arcus cornealis and levels of LDL-C in first degree children, the criteria were slightly modified as done previously[3, 93, 94]. Family history of early ASCVD was defined as onset of ASCVD before the age of 60 years in women and before the age of 55 in men and siblings[3].

7.4 Ethics

The CGPS was approved by the Danish ethical committee (H-KF-01-144/0) and Herlev and Gentofte Hospital[3]. All individuals had a signed written consent obtained at enrolment[3].

7.5 Statistical analyses

We investigated the risk of MI and ASCVD measured as hazard rate ratios associated with levels of Lp(a). Subsequently, we estimated the risk of MI and ASCVD among individuals fulfilling a diagnosis of FH according to genetic FH, Simon Broome, MEDPED and DLCN criteria. Following, we determined the exact Lp(a) level that conferred the same hazard rate ratio as the FH criteria studied[3]. Also, we investigated interaction between plasma Lp(a) levels and FH or a family history of MI.

We used Cox proportional hazard regression with attained age as the underlying timescale and delayed entry. Participants were considered at risk from initiation of the Danish National Patient Registry in 1977 or at birth, whichever came latest[3].

7.6 Main results

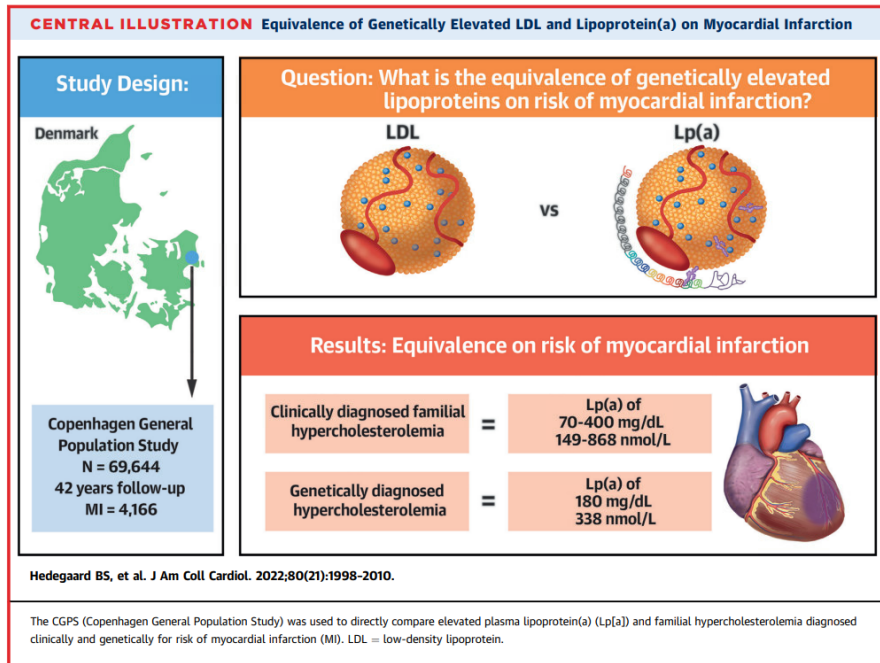
The median follow-up time was 42 years and 4,166 developed MI and 11,464 were diagnosed with ASCVD. The risk of having MI or ASCVD increased with higher levels of Lp(a), and when both FH and elevated Lp(a) were present, the risk was even higher[3]. The Lp(a) levels equivalent risk of MI to clinical FH according to Simon Broome, MEDPED and DLCN criteria were 67 to 402 mg/dL and 180 mg/dL for genetic FH. For the risk of having ASCVD, the comparable level of Lp(a) was 130-391 mg/dL according to clinical FH criteria and 175 mg/dL in genetically verified FH (Figures 6 and 7)[3].

7.7 Major strengths and limitations

Major strengths include the large sample size, the quality of Danish health registries for providing information on MI and ASCVD and measurement of Lp(a) in the same laboratory[3]. Limitations include that only the four most common FH mutations were investigated, and information on clinical and familial data of the patients were incomplete which may have affected results in diagnosis of FH patients determined by Simon Broome or DLCN criteria[3].

7.8 Main conclusions

The equivalence on risk of having ASCVD in genetic FH was comparable with plasma Lp(a) levels of 180 mg/dL (389 nmol/L) and 175 mg/dL (378 nmol/L) for MI, respectively (Figure 5)[3]. Regarding levels of Lp(a) for clinical criteria of FH, the equivalent risk of MI was: Simon Broome, 110 mg/dL (236 nmol/L), MEDPED, 67 mg/dL (142 nmol/L) and 402 mg/dL (873 nmol/L) for DLCN[3]. This shows that high Lp(a) is an important risk factor for ASCVD and MI comparable to the risk of having FH[3].



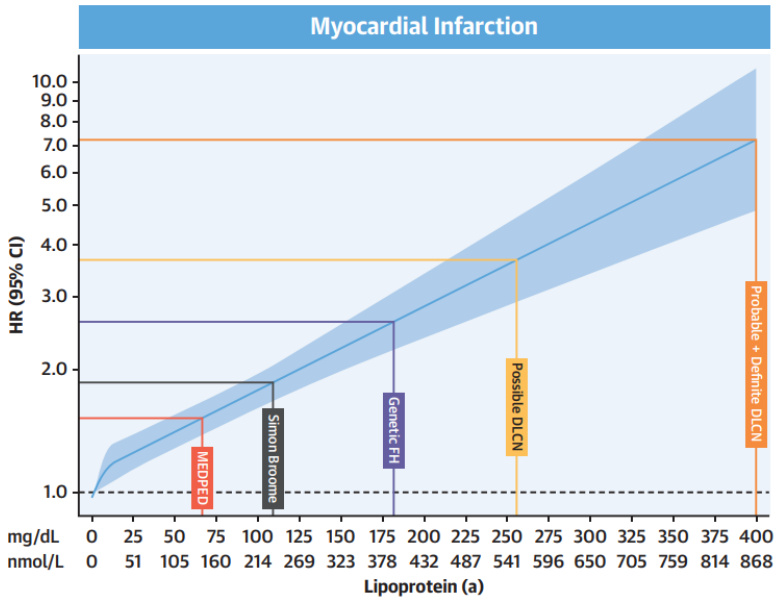


Figure 6: *Lp(a)* and FH in relation to risk of MI[3]. Permission from JACC.

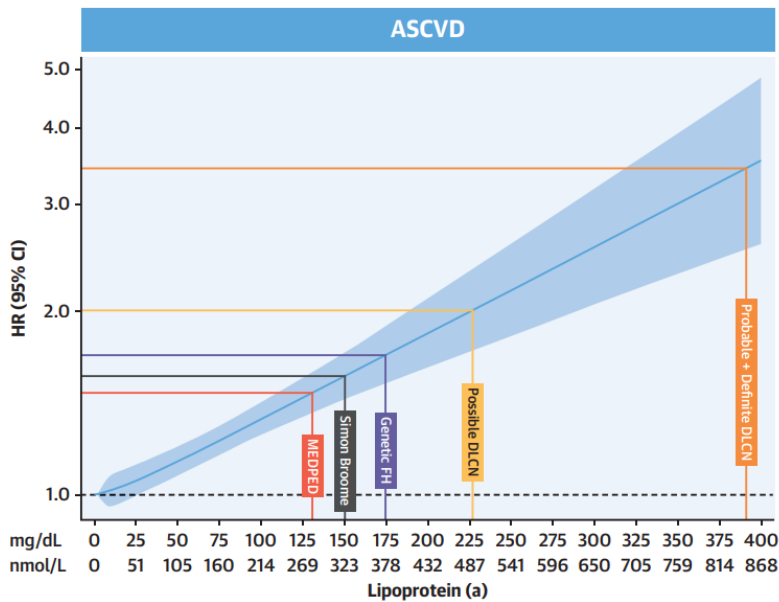


Figure 7: *Lp(a)* and FH in relation to risk of ASCVD[3]. Permission from JACC.

CHAPTER 8. DISCUSSION

8.1 Comments to the studies

The aims and hypotheses of the thesis were formulated in Chapter 3, but the intention was to characterize and study individuals referred to Danish lipid clinics on suspicion of FH[1]. Furthermore, we aimed to study the significance of plasma Lp(a) levels for a diagnosis of FH[2] and their impact on the risk of MI and ASCVD[3].

8.1.1 Study I

In paper I, the main findings were that a total of 25.9% of the study participants were diagnosed with FH; that 21.7% were diagnosed with FH before genetic testing a figure that increased to 36.9% after genetic testing was undertaken in 705 patients (56.7%)[1]. Interestingly, approximately 20% with unlikely and possible FH carried a pathogenic FH variant. Our hypotheses for study I were therefore fairly correct.

It is worth noticing that 18% of the referred individuals had CVD and only 27% received lipid-lowering drug treatment. Focusing only on those with FH these figures were moderately higher as 28% had a history of CVD and 35% received lipid-lowering drug treatment. This clearly suggests pharmaceutical undertreatment of this group.

Another remarkable finding from FFH was the low occurrence of tendon xanthomas and arcus cornealis. The occurrence of tendon xanthoma provides 6 points using DLCN criteria and is a prerequisite for establishing a definite diagnosis of clinical FH according to DLCN criteria and also according to the Simon Broome FH criteria. The presence of arcus cornealis contributes with 4 points in the DLCN score among subjects below 45 years of age. Xanthomas and arcus cornealis are therefore important for diagnosis and classification of clinical FH, but could only be demonstrated in approximately 15% of our population diagnosed with clinical/genetic FH. Others have reported a prevalence of 20-25% of both tendon xanthomas and arcus cornealis in patients with FH, highest in those with a pathogenic FH variant[97]. Surprisingly, arcus cornealis was only reported in 0.4% (5/1,243) of our study population[1]. Thus, arcus cornealis was present in 28% with genetic FH and 14% with mutation negative

clinical FH in a study of 753 Brazilian FH patients[97]. Important explanations for the differences between studies might include the use of earlier and more aggressive lipid-lowering treatment during recent years.

In the FFH study population approximately 26% were diagnosed with FH among the referrals[1]. This percentage was higher compared to an earlier published Danish study among referrals to the lipid clinic at Viborg Regional Hospital[98]. In that study of referrals all with LDL-C ≥ 5 mmol/L, only 18% (n=68/384) were diagnosed with FH over a 5 year period[98].

In a study from the CGPS (n=69,209) the positive predictive value for FH was calculated according to levels of LDL-C. Interestingly, plasma LDL-C ≥ 8.5 mmol/L had a positive predictive value of 100% for FH, whereas it was only 11% among individuals with LDL-C ≥ 5 mmol/L[17]. This is in line with another study from Copenhagen reporting that recognition of FH in general practices was rather poor, despite known high LDL-C levels in the patients[99]. Also, in the first annual report published from RKKP in 2023, there was a very low fraction of all individuals in Denmark with LDL ≥ 5 mmol/L, that was referred to a lipid clinic[56].

In a Danish study among referrals (n=408) with high LDL-C at Aarhus University Hospital recruited between 1995 and 2003, the prevalence of identified pathogenic FH variants in patients with probable or definite FH was as high as 48.1% (90/187)[100]. The mean LDL-C levels among patients with no pathogenic FH variants identified was 6.3 mmol/L, whereas for the LDLR and APOB mutations the mean LDL-C was 7.3 mmol/L and 6.4 mmol/L, respectively. The high frequency of FH mutations can at least in part be explained by the high LDL-C levels in the population investigated, as the risk of a pathogenic FH variant in general is higher the higher LDL-C as reported in the FFH project[1] and other studies[97, 101].

In a Spanish cohort of 5,430 index cases with a clinical diagnosis of FH 41% had genetic FH[101]. Genetic FH was also found in 16.4% with unlikely FH and 23.9% with possible FH (n=156), respectively. As observed in the FHH study, the percentage of genetic verified FH was higher among individuals with clinical FH) increasing from approximately 16% in those with unlikely to 54% in those with definite FH[101].

In a multicentre study from Catalonia including 967 patients suspected of FH, a pathogenic FH variant could be demonstrated in 38.6% of the individuals[102]. Also, in a study from Brazil the percentage of genetic FH among individuals suspected of

FH (n=753) was 34.1%[97]. These results were quite similar to ours as we found the percentage of genetic FH was 34.6% among those with clinical FH that were genetically tested.

In a study from Italy[103] a different approach was taken and adult patients with genetically verified FH (n=1,377) were classified according to the DLCN criteria, although only 57% had all data required for full DLCN scoring[103]. The authors reported that 5.3% had unlikely, 28.3% possible, 28.5% probable and 37.9% had definite FH. Thus, approximately 66% would have been classified with clinical FH, and one third would not have been diagnosed with FH if genetic testing had not been undertaken[103].

We conclude that the percentage of patients referred on suspicion of FH who have a pathogenic FH variant varies considerably between studies, likely because of differences in patient populations, but figures like ours are not uncommon. It also seems evident that the higher the LDL-C level and the more DLCN points obtained, the higher is the likelihood of finding a pathogenic FH variant. Importantly, these are not independent measures as higher LDL-C levels are reflected by a higher DLCN score.

Still, in a substantial fraction of patients with clinical (even definite) FH it is not possible to find a pathogenic FH mutation. Some of these cases may have high Lp(a) as found in FFH[1] or be caused by polygenic hypercholesterolaemia[35, 104, 105].

8.1.2 Study II

In paper II the main findings were that median plasma Lp(a) was 15 mg/dL; that 324 (28%) of the participants had high Lp(a) ≥ 50 mg/dL; that 18% with high Lp(a) had genetic FH; and that in approximately 27% the high Lp(a) was believed to be instrumental for high LDL-C levels and for a diagnosis of clinical FH confirming our hypothesis[2].

In a study population (n=330) suspected of FH with Lp(a) ≥ 50 mg/dL from Australia[84], the diagnosis of FH was established by DLCN and Simon Broome criteria. When levels of LDL-C were corrected for 30% of Lp(a) mass, the overall down classification in DLCN groups was 36.1%. This percentage decreased to 24.7% when only applied to individuals (n=166) with Lp(a) between 50 and 100 mg/dL[84].

A study from the Netherlands conducted among 1,507 subjects with LDL-C levels ≥ 5 mmol/L (mean 6.3 mmol/L), referred to genetic testing for FH, reported that 9.1% were classified from possible to unlikely FH according to the DLCN criteria[106] when LDL-C was corrected for 17.3% of Lp(a) mass calculated on the basis of genetics of LPA[106]. In a sensitivity analysis, the authors showed that 18.4% were reclassified from possible to unlikely FH[106] when LDL-C was corrected for 30% of Lp(a) mass.

It has been argued by Yeang et al.[107], that the correction value for Lp(a)-cholesterol content in LDL-C should be lower than 30%, and also argued that direct measurement of Lp(a)-cholesterol in relation to the mass is subject to significant variations. In the FFH project, we decided to correct LDL-C for 30% of Lp(a) mass[2]. In the European Atherosclerosis Society consensus statement about Lp(a)[69] it was recommended to correct LDL-C for Lp(a) cholesterol in individuals suspected of FH, but not on a routine basis[69]. At present there is no consensus for the percentage calculation regarding Lp(a)-cholesterol content in calculated LDL-C.

The prevalence of elevated Lp(a) ≥ 50 mg/dL among individuals with clinical FH in study II[2] was 18% (58/324) and 25% (81/324) among those with clinical/genetic FH. Compared to other studies the percentage was lower than observed in the CGPS (20%), while some studies have reported that approximately 40% with FH had Lp(a) ≥ 50 mg/dL[79, 108, 109].

8.1.3 Study III

In paper III the main findings were that high plasma Lp(a) was an important risk factor for MI and ASCVD and comparable to the risk of having FH depending on by which clinical criteria (MEDPED, Simon Broome or DLCN) or genetically the diagnosis of FH was established. Thus, our hypothesis that a high plasma Lp(a) may be associated with a comparable relative risk for MI and ASCVD as FH was confirmed.

The risk of ASCVD is markedly increased in individuals with FH[17-19, 45, 94, 110]. Likewise, elevated levels of Lp(a) are also genetically determined and independently increase the risk of ASCVD[18, 76, 77, 86, 111-114]. In the European guidelines for the management of dyslipidaemia it is recommended to measure plasma Lp(a)[18] to identify individuals with elevated Lp(a) values, and especially those with Lp(a) level >180 mg/dL (389 nmol/L), which might correspond to a condition of FH in terms of risk of ASCVD[18, 115]. Our study confirmed the risk of having genetic FH was

equivalent to the risk of MI with Lp(a) ≥ 180 mg/dL, and 175 mg/dL to the risk of ASCVD[3].

Patients with both FH and elevated Lp(a) have a very high risk of ASCVD when both conditions are present[116].

We know from studies in the general population, that approximately 20% has Lp(a) levels above 50 mg/dL (105 nmol/L)[76, 86], which is far higher percentage than those with FH occurring in 1:250-300[16]. This shows the importance of Lp(a) on a population level.

The International Classification of Diseases (ICD)-10 code for elevated Lp(a) E78.41 have not yet been implemented in Denmark, but this should indeed be considered for registration of patients with high Lp(a).

8.2 How to identify more patients with FH

The prevalence of FH may differ between populations[19-21], but estimates suggest a prevalence of 1:250-1:300 in Denmark. Therefore, approximately 25.000 Danes may have FH[54, 55] with perhaps less than 30% of those diagnosed[54]. Individuals with FH have a significantly increased risk of ASCVD[19, 21, 58], which is potentially preventable if FH is diagnosed and treated at early age[14, 17, 21, 41, 59, 117]. In addition, with an expected prevalence of hoFH of 1:160.000 individuals a total of approximately 50 Danish subjects would be expected to have hoFH, but very few individuals with hoFH are currently diagnosed[54].

There is an urgent need to diagnose and treat more individuals with FH. Some initiatives have been undertaken[54, 55] and suggested[57] and these are likely to improve detection of more patients with FH, but further initiatives are needed.

One problem has also been the lack of and inconsistent use of International Classification of Diseases coding for FH (DE78.0B). When patients are referred with hypercholesterolemia, the diagnosis code DE78.0 has been used as a common term for hypercholesterolaemia[56], but in some cases the coding may not be specified with the use of B (DE78.0B) in subjects with FH[54]. The clinical FH diagnosis for registration purposes is given to individuals who meet the clinical DLCN criteria of probable and definite FH but also to individuals that have inherited a pathogenic FH variant. The prevalence and incidence of FH in Denmark is monitored by the RKKP

using record linkage with the Danish nationwide registries and the Database for familial hypercholesterolemia[55, 56]. In the first annual report from the RKKP published in 2023, a total of 7,998 individuals had been registered with a diagnosis of FH in Denmark[56]. According to this, the detection rate had been markedly increased recently. However, there is uncertainty about the diagnostic coding and validation of coding is much needed[56].

In Denmark all lipid clinics are required to use a web-based family tree program (PROGENY) which allows for registration of individuals with FH (and their relatives) across the country and classification according to DLCN criteria. This should help improve finding patients with FH and open up for more effective cascade screening for FH.

Overall, awareness of the diagnosis by the public and health professionals and an organisation with sufficient resources that can evaluate, diagnose, set standards for treatment and follow-up are necessary. In the following three important aspects for improvement will be discussed: Screening for FH, the use of genetic testing, and collaboration between general practitioners and lipid clinics.

8.2.1 Screening for FH

Treatment of elevated LDL-C and elimination of other risk factors, in patients with FH might prevent ASCVD. In Denmark there has been opportunistic screening mainly of individuals with ASCVD or with suggestions of high cholesterol or premature CVD in the family. However, in many countries there have been developed screening strategies for FH[20, 118].

A genetic diagnosis of monogenic FH can be made at birth. The benefit of an early diagnosis is obvious and might increase early initiating of treatment, already from 8-10 years of age in those affected[39, 58]. However, there are important ethical issues to consider including the right not to know and the fact that a diagnosis of FH cannot be ruled out by a negative gene test. Genetic testing for FH at birth has not been performed in any country so far, but in Slovenia[61] there is an study investigating universal screening for FH at pre-school age of 5-6 years with test of plasma total cholesterol [61]. Children with total cholesterol >6 mmol/L or between 5-6 mmol/L and with a family history of hypercholesterolemia or presence of early ASCVD, are referred to a lipid clinic[61].

The two countries that have been most active in screening for FH have been Holland and Norway, where the highest percentages of the population with FH also have been detected[19]. In Holland there has been an extensive government supported cascade screening program[53]. Another screening strategy has been used in Norway, where general practitioners have the possibility to order a genetic test for FH. If a causal mutation for FH is found, patients are referred to a lipid clinic primarily in Oslo. The detection of FH in Norway is 10.000/25.000 (40%) according to the Norwegian National Center of Competence[119]. In Sweden there has recently been introduced a digital screening platform in the area around Stockholm for first-degree relatives to individuals with FH[120, 121]. When an FH causal mutation is found, this is registered in an electronic platform and the proband has access via bank ID to invite first-degree relatives to receive a link to order a genetic test for FH. The relatives then receive appointments for blood sampling and subsequent answers in lipid clinics. In new cases of FH, cascade screening continues[121].

The FH screening, FAMCAT[122] used in the United Kingdom National Health Service Systematically is an automatic electronic search in health records in primary care. The system is based on a model stratified by more information than included in DLCN such as information regarding a diagnosis of diabetes mellitus or kidney disease and triglyceride levels[122, 123]. This technology tool may identify patients at high risk of having FH with advice to refer those fulfilling certain criteria to a lipid clinic.

8.2.2 The use of genetic testing in FH

The choice of whether to perform a genetic test for FH or not in Denmark is decided by the treating physician in the lipid clinics. Local availability of test methods, patient preferences, lack of clinical relevance of the test result (e.g. no family members), ethical and insurance considerations could all be reasons for not offering a genetic test to individuals suspected of FH. However, the relatively expensive price of testing is probably of major importance for a decision not to perform a genetic test for FH.

In our study 25.9% of those referred on suspicion on FH was diagnosed with FH[1]. In those genetically tested, 21.7% had clinical FH before genetic testing, whereas 36.9% had FH after genetic testing[1]. This was observed despite the fact that only 56.7% of the referrals were genetically tested for FH. The results from our nationwide study suggest that a more widespread use of genetic testing should be offered[1]. Thus, it could be argued that the majority of patients referred on suspicion of FH and

fulfilling the Danish referral criteria should be genetically tested as approximately 20% classified with either unlikely or possible FH by DLCN criteria had a pathogenic FH variant[1]. Such patients would usually not have been diagnosed as having clinical FH and family testing rarely been advised.

It is of utmost importance that identification of an individual with FH leads to investigation of FH within the family, initially in relevant first-degree family members, as FH is an autosomal dominantly inherited disorder. However, it must be strongly emphasized that only approximately two thirds with clinical FH have a positive gene test[19]. Therefore, a negative gene test does not rule out FH. However, a positive gene test in a proband makes family testing (cascade screening) easier, cheaper (searching only for one specific pathogenic FH variant) and the diagnosis more reliable. Also, the finding of a positive gene test in an individual might help motivate this person to adherence to a healthy lifestyle and to pharmacological treatment. Finally, it has been reported in some studies, that the risk of ASCVD is higher in patients with FH carrying a pathogenic mutation compared to individuals without mutations[38, 39] although findings have been inconsistent[3].

8.2.3 Collaboration between general practitioners and lipid clinics

General practitioners are essential for diagnosing more individuals with FH. It is often here high cholesterol levels are first measured and a suspicion of FH may be raised also because of the physician's knowledge of premature CVD and/or hypercholesterolaemia within the family. However, this require that general practitioners are aware of FH, receive education and that relevant literature regarding FH is accessible[124-126]. The general practitioners should have easy access to lipid clinics both for discussion of cases and for referrals. Patients suspected of FH should be referred to lipid clinics for evaluation, often including genetic testing and measurement of plasma Lp(a). Our study showed that the referral criteria shown in Table 4 are useful, but more individuals fulfilling the referral criteria, still need to be referred. According to the results from the annual report of RKKP[56], most individuals with LDL-C levels above 5 mmol/L still is not referred to lipid clinics.

In the FFH study population, elevated LDL-C according to referral criteria, should be measured twice and secondary dyslipidemias ruled out before referral[1]. This might help to avoid referrals of individuals without FH and reduce costs.

CHAPTER 9. CONCLUSIONS AND PERSPECTIVES

The aim of the thesis was to investigate adult patients referred on suspicion of FH to Danish lipid clinics and to study the impact of genetic testing for the diagnosis. We also investigated the impact of high plasma Lp(a) for a diagnosis of clinical FH and compared the risk of MI and ASCVD associated with high levels of Lp(a) to the risk associated with FH. The major findings were that of the 1,243 included individuals, 26% (n=322) had clinical/genetic FH. Interestingly, in those genetically tested 22% was diagnosed with clinical FH before genetic testing, a figure that increased to 37% after testing (performed in 57% of the study population). Patients were categorized according to DLCN criteria and even in subjects classified with unlikely or possible clinical FH as many as 20% carried a pathogenic FH variant. Our findings therefore suggest a more widespread use of genetic testing for FH in the lipid clinics.

The commonly used referral criteria in Denmark were found to be appropriate, and most of the subjects (n=864) were referred because of LDL-C ≥ 5 mmol/L. The estimated content of LDL-C in Lp(a) may contribute substantially to plasma LDL-C and more than one quarter of individuals with a clinical diagnosis of FH was likely due to elevated Lp(a). Finally, we found that very high plasma levels of Lp(a) carried a risk in the same order of magnitude for future MI and ASCVD as a diagnosis of FH. We therefore conclude that plasma Lp(a) should be measured and taken in consideration for both diagnostic and risk evaluation of ASCVD in individuals suspected of FH.

Many individuals in Denmark are unaware of their cholesterol levels, and many with known elevated LDL-C, are insufficiently investigated and treated. The consequences of undetected or insufficiently treated FH may be devastating for the individual and the family, but premature ASCVD and death may also be costly for society. It would therefore be of great interest to study health economy issues related to gains of early detection and treatment of FH and costs of the organization, lipid clinics, genetic testing and other initiatives as suggested here.

For detection and treatment of individuals with FH some steps have recently been taken as previously described, but there are other approaches that should also be considered. First, public awareness of the importance of cholesterol and FH for health and disease need to be strengthened. Next, general practitioners need to be aware of FH and to have easy access to local lipid clinics for consultation and referral of individuals suspected of FH. Thirdly, lipid clinics with resources to examine, detect, and treat patients with FH are pivotal with organizing screening of family members (cascade screening) for FH as an important part of their role.

Organization of nationwide initiatives through a national center, like in Norway, may facilitate detection and treatment of FH and support detection and awareness of FH through social medias, public campaigns, brochures, should be considered a future perspective and possibility. Finally, the importance of an active and strong patient organization should not be underestimated for a beneficial role in patients with FH and their relatives.

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