Early-onset Coronary Artery Disease

Clinical and Hereditary Aspects

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PAPERS

The dissertation is based on the following papers:

Christiansen MK, Jensen JM, Brøndberg AK, Bøtker HE, Jensen HK. Cardiovascular risk factor control is insufficient in young patients with coronary artery disease. Vasc Health Risk Manag. 2016 May 25;12:219-27.

Christiansen MK, Jensen JM, Nørgaard BL, Dey D, Bøtker HE, Jensen HK. Coronary plaque burden and adverse plaque characteristics are increased in healthy relatives of patients with early-onset coronary artery disease. JACC Cardiovasc Imaging. 2017. Jan 12.

Christiansen MK, Nyegaard M, Pedersen LN, Larsen SB, Würtz M, Hjort J, Kristensen SD, Jensen HK. A 45-SNP genetic risk score is increased in early-onset coronary artery disease but independent of familial disease clustering. Atherosclerosis 2017;257:172–8.

ABBREVIATIONS

AOBP: automated office blood pressure
BMI: body mass index
CABG: coronary artery bypass graft
CAD: coronary artery disease
CI: confidence interval
CP: calcified plaque
CT: computed tomography
CTA: computed tomography angiography
DNA: deoxyribonucleic acid
GRS: genetic risk score
GWAS: genome-wide association study
HbA1c: hemoglobin a1c
HDL-C: high-density lipoprotein concentration
IQR: interquartile range
LDL-C: low-density lipoprotein concentration
LD-NCP: low-density non-calcified plaque
MI: myocardial infarction
NCP: non-calcified plaque
OR: odds ratio
PCI: percutaneous coronary intervention
PR: positive remodeling
RI: remodeling index
SD: standard deviation
SLFS: stratified log-rank family score
SNP: single-nucleotide polymorphism
WDHR: Western Denmark Heart Registry

1. BACKGROUND

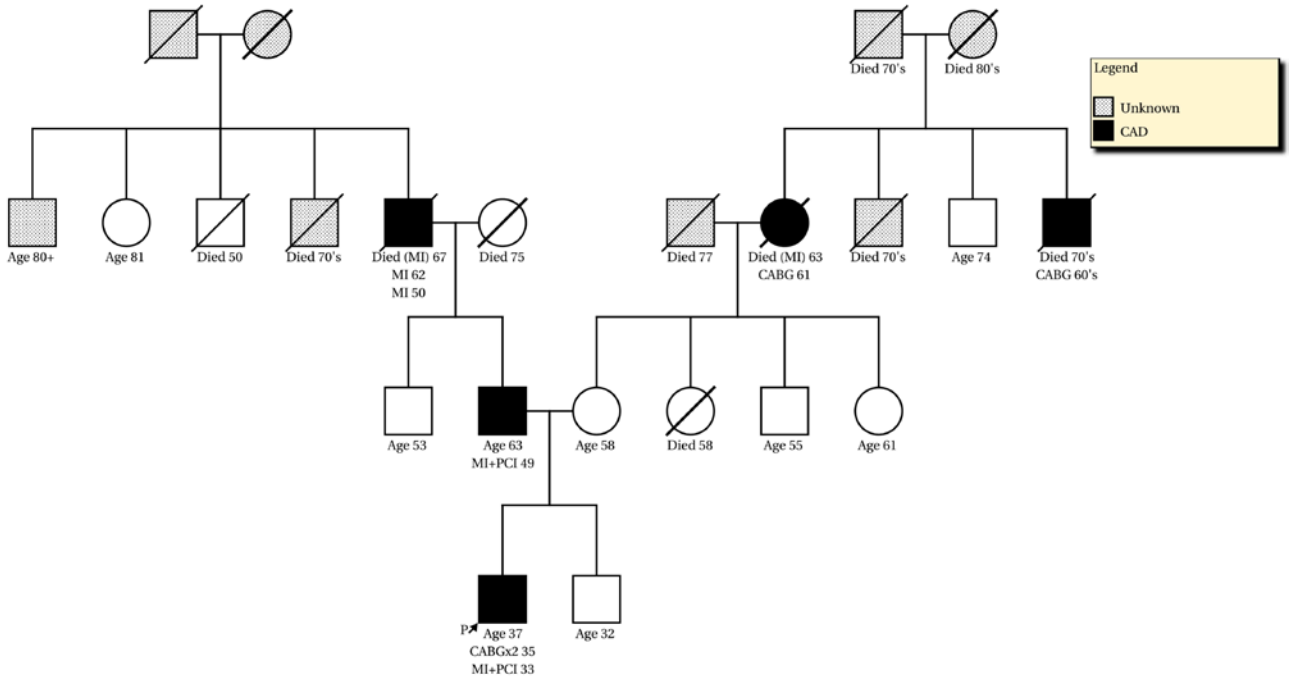
1.1. The importance of early-onset CAD

1.1.1. A case report

A 37-year-old patient with coronary artery disease (CAD) is referred to the outpatient clinic because of a family history of CAD at a young age. At 33 years, he had an anterior myocardial infarction (MI) treated with primary percutaneous coronary intervention (PCI). Two years later he underwent coronary artery bypass graft (CABG) surgery because of progressive angina and an invasive coronary angiography showing a restenosis at the left anterior descending and significant CAD in the circumflex artery.

The family pedigree is shown in Figure 1. Prior to CAD onset he was a smoker, but had no concurrent medical disease. He has quit smoking and is currently on treatment with aspirin, atorvastatin and ezetimibe. His blood pressure is 126/78 and his body mass index (BMI) is 26.4. Laboratory testing reveal a low-density lipoprotein concentration (LDL-C) of 1.7 mM on treatment, and normal levels of creatinine and hemoglobin a1c (HbA1c).
Several features may be noticed. A few conventional risk factors are present in the patient and a hereditary component prevails. A dominant pattern of inheritance might be present on the paternal side where individuals are affected at a young age, but several individuals suffer from CAD on both sides of the family suggesting a complex (i.e. non-Mendelian) mode of inheritance. Such interpretation, however, may potentially be hampered by a low penetrance due to the long subclinical disease course, or the presence of phenocopies because of the common nature of CAD. Despite absent signs or symptoms of CAD (hence unsupported by guidelines [1]), his mother and brother underwent coronary computed tomography (CT) angiography (CTA) revealing considerable diffuse coronary atherosclerosis in both individuals.

Figure 1: Family pedigree



Abbreviations: CABG, coronary artery bypass graft; MI, myocardial infarction; PCI, percutaneous coronary intervention.

1.1.2. Familial clustering of CAD

As illustrated by the case report, CAD may aggregate in families. It has been estimated that families with clustering of CAD represent 14% of the community, but account for 72% of young patients with CAD, which reflects the shared behavioral, environmental and genetic factors within families [2,3]. In general, a history of CAD in a first-degree family member is associated with a 1.5-3 fold increased risk of the disease [4-15], and the risk is inversely related to the age of onset of the affected family member [5,8,12,15,16]. Accordingly, the risk may be increased more than 10-fold if a brother is affected before the age of 45 years [5,16], but studies suggest a slightly increased risk even when a first-degree relative is diagnosed after the age of 70 years [5,8]. The size of the risk also depends on the number of family members affected. Some studies have found the odds to be a little less than 2-fold higher when one first-degree relative has CAD, but 3-to-4-fold higher when two first-degree relatives are affected [7,15]. Furthermore, when 2 or more first-degree relatives are affected from an early age the risk may be even higher [6,17].

1.1.3. Defining early-onset CAD

A number of definitions of early-onset CAD have been applied in the literature; typically defined by the onset before a cut-off of 40-65 years. Such differences complicate between-study comparison of family risk. Given the arbitrary cut-off and the fact that we aimed at investigating hereditary aspects of CAD, we selected patients with onset before 40 years for the present studies. In this thesis it is referred to as “early-onset CAD”, whereas the term “premature CAD” has been reserved for CAD onset <55 years in males and <65 years in females, as adopted by international societies [18,19]

1.2. Early-onset CAD and cardiovascular risk factors

Conventional modifiable risk factors (i.e. hypertension, hypercholesterolemia, smoking, obesity and diabetes) are frequent in young CAD patients. Almost all young cases carry one or more modifiable risk factors at onset, and the majority are affected by several [13,20,21]. Addressing and treating these risk factors are the cornerstone in the secondary prevention of CAD and substantially improve the clinical outcome [19,22-26].

The long-term prognosis of young CAD patients may have improved over the years, however, morbidity and mortality is still increased compared with the general population [20,27]. One explanation may be due to inadequate risk factor control. A number of previous studies have evaluated risk factor control in patients with established CAD and demonstrated major needs for improvement [28,29]. However, young patients were underrepresented in these studies, and large variation across countries may exist [28,29]. Given the young age and hence the many years at risk, adequate secondary prevention is of particular importance in early-onset CAD, but a further investigation must clarify the extend by which these targets are met.

1.3. Imaging the hereditary aspect of early-onset CAD

Coronary atherosclerosis is the most common cause of CAD [30]. The disease may become clinically evident in case of plaque-rupture leading to coronary thrombosis and MI [31]. Alternatively, atherosclerotic plaque formation may cause increasing luminal obstruction, myocardial ischemia and angina pectoris [31] a presentation less common among young patients [32]. The silent development of atherosclerosis takes place over many years and affects the majority of individuals even from early adulthood [31,33,34]. Therefore, it is intriguing why some families without other risk factors have more rapidly progressing atherosclerosis than others.

Certain features characterize obstructive plaques as well as ruptured plaques. Particularly, ruptured plaques are characterized by the presence of a lipid-rich core, a thin fibrous cap, and expansive plaque remodeling [31]. These features may be identified by coronary CTA [35-39]. It has been demonstrated that coronary artery calcium, a marker of atherosclerosis and a predictor of future coronary events [40,41]. is increased in healthy individuals with a family history of premature CAD [42,43]. However, coronary artery calcium in itself is considered a late manifestation of

stable plaques, and only reflects a minor ingredient of the coronary atherosclerosis. One study reported on the plaque burden and composition in individuals with a family history of CAD before the age of 60 years [44]. The study showed that almost half of these individuals had plaque on coronary CTA of which the vast majority was non-calcified [44]. However, they did not specify plaque features and there was no control group for comparison. Therefore, it is unknown whether a family history of CAD is associated with an increased plaque burden or any specific plaque features.

1.4. Genetics of CAD

1.4.1. Genome-wide association studies and genetic risk scores

Recent advances in genotyping assays and next-generation sequencing techniques have led to increasing efforts in uncovering the genetic basis of CAD. Modern genotyping arrays simultaneously genotype up to around a million genetic variants at a time (mostly single-nucleotide polymorphisms [SNPs]), which are spread throughout the genome. This technique is applied in genome-wide association studies (GWASs) to establish associations between genetic loci and a given disease of interest by comparing the allele frequencies of all the genotyped SNPs between affected cases and healthy controls [45]. An association is established, if an allele is significantly more common in cases compared with controls, taking into account the multiple statistical tests performed [45].

The first GWASs in CAD were published in 2007 by two independent groups reporting an association with common variants at chromosome 9p21 [46,47]. Subsequently, several large-scale GWASs have established associations between CAD and common variants at a number of different loci [48-55]. In general, each variant is only associated with a small increased risk of CAD, and therefore, the variants are not suitable for predicting risk, individually [56]. However, assuming additive genetic effects, the risk may be added up in genetic risk scores (GRSs), which is typically performed by summing the number of risk alleles, of variants identified from GWASs, weighted by their effect size. This approach has shown to predict incident cardiovascular events in various cohorts free of CAD at baseline [57-67], of which some suggest an improved risk classification beyond current risk assessment models [60-64,67]. Furthermore, GRSs may predict recurrent CAD events [66,68-72], and possibly even identify individuals, who derive maximum absolute benefit of lifestyle changes or preventive medical treatment [66,73].

1.4.2. Common genetic risk-variants in early-onset CAD

Heritability is defined as the proportion of phenotypic variance that can be attributed to genetics [74]. Sibling studies consistently show that heritability in CAD is around 50%, with a higher genetic contribution in earlier phases of life [75-77]. Increasing evidence suggest that the genetic effect is primarily caused by many common genetic variants that individually have a small effect on the phenotype [55]. Jointly, however, a combination of several genetic risk variants (i.e. polygenic burden) may cause disease by crossing a threshold of susceptibility [55].

A few previous studies have examined the role of common risk variants on the age of CAD onset [68,78]. Using an 11-SNP and a 30-SNP GRS, respectively, two studies demonstrated that younger MI patients suffered a higher polygenic burden than older MI patients [68,78]. Accordingly, the age of onset and the extent of familial clustering in early-onset CAD may potentially be determined by the inherited polygenic burden. This would be consistent with the fact that early-onset CAD often clusters in families in a non-Mendelian fashion. However, whether such relationship exists remains unknown.

2. HYPOTHESES AND AIMS

The objectives of the present thesis were:

2.1. Study I

Hypothesis: Control of cardiovascular risk factors is inadequate in early-onset CAD.

Aim: To estimate the prevalence and control of risk factors in early-onset CAD patients.

2.2. Study II

Hypothesis: A strong family history is associated with a high coronary plaque burden and adverse plaque features.

Aim: To characterize and quantify subclinical atherosclerosis by coronary CTA in 1st degree relatives of patients with early-onset CAD compared with controls without a familial predisposition.

2.3. Study III

Hypothesis: A high polygenic burden is associated with age of CAD onset and familial clustering in early-onset CAD.

Aim: To quantify the polygenic burden (measured as a 45-SNP GRS) in early-onset CAD patients compared with older CAD patients and to investigate whether early-onset individuals with a strong familial clustering of CAD have a larger polygenic burden. Furthermore, to examine whether these measures of heritability are associated with CAD severity.

3. METHODS

3.1. Study populations

The methods used in study I-III are presented in the following. Additional descriptions may be found in the appended papers.

3.1.1. Early-onset CAD (study I+III)

Early-onset CAD patients treated at Aarhus University Hospital from January 2002 to December 2013 were recruited from the Western Denmark Heart Registry (WDHR) [79]. Early-onset CAD was defined as having a coronary revascularization procedure performed before the age of 40 years. A total of 358 early-onset CAD patients were registered in the WDHR, of whom 283 were considered eligible and 143 were included in study I (Figure 2). All patients were stable at the time of recruitment (no revascularization procedure within 6 months prior to enrollment).

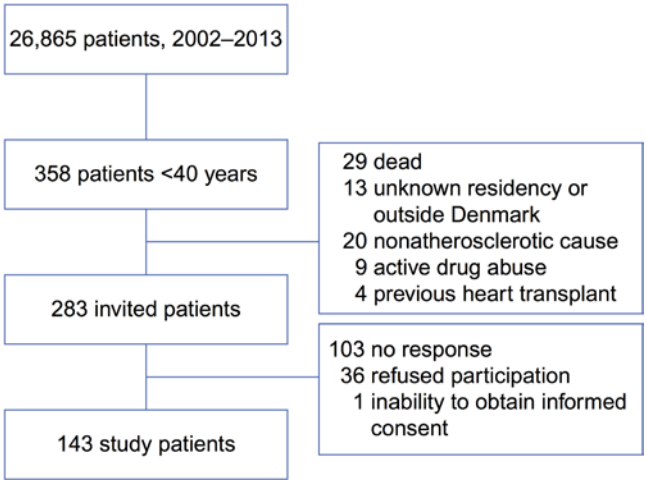
For study III, we used the same study population, however, excluding patients with genetically verified familial hypercholesterolemia (i.e. a mutation in the LDLR, PCSK9 or APOB genes considered pathogenic) and only including one individual per family (by selecting the individual with the youngest age at CAD onset). Therefore, 134 early-onset CAD patients were included in study III.

3.1.2. Relatives of patients with early-onset CAD and matched controls (study II)

Early-onset CAD patients from study I, without genetically verified familial hypercholesterolemia, were used as a link to recruit, first-

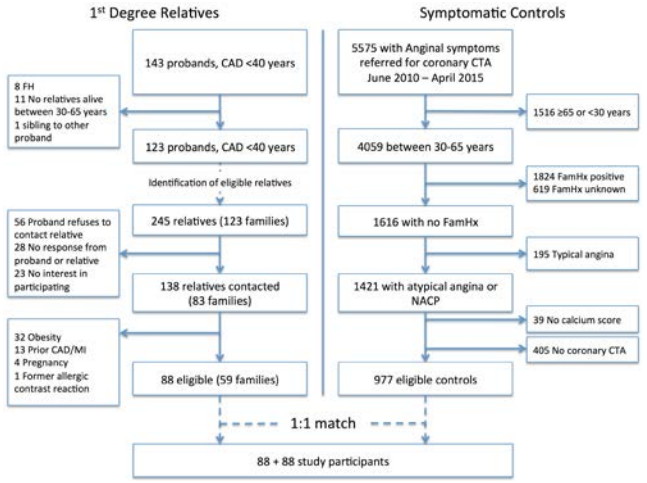
degree relatives between 30-65 years for study II. In total 88 relatives with no prior CAD were included (Figure 3). Control patients were identified from the Western Denmark Cardiac Computed Tomography Registry [80]. The control group comprised patients without known CAD and without a family history of CAD who underwent calcium scoring and CTA at our department on a suspicion of CAD. For each relative, one control patient with the same age and sex was randomly chosen among eligible controls.

Figure 2: Selection of patients in study I



Reproduced from paper I.

Figure 3: Selection of patients in study II



Abbreviations: CAD, coronary artery disease; CTA, computed tomography angiography; FamHx, family history; FH, familial hypercholesterolemia; MI, myocardial infarction; NACP, non-anginal chest pain. Reproduced from paper II.

3.1.3. Late-onset CAD and healthy controls (study III)
Between November 2007 and January 2011, 900 patients were recruited from the WDHR for studies exploring the antiplatelet effect of aspirin [81]. The patients were stable at the time of recruitment and were on mono-antiplatelet therapy with aspirin. Additionally, 90 healthy volunteers with no sign of CAD had been recruited through local advertisement [82]. Late-onset CAD patients, defined by the first coronary revascularization procedure 55 years in males and ≥65 years in females, and healthy controls were included in our studies.

3.2. Data sources

3.2.1. Health examination and risk factor evaluation (study I)
Early-onset CAD patients underwent a thorough interview and the medical records were reviewed. Patients were classified as current smokers (i.e. smoking within the last month), former smokers (prior smoking exceeding one pack-year) and never smokers. Physical activity was defined based on questions from the Danish Health and Morbidity Survey [83]. An automated office blood pressure (AOBP) measurement was performed using the BpTRU device [84]. Height, weight and waist were measured and body mass index (BMI) was calculated. Creatinine, HbA1c, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured on non-fasting venous blood samples, and LDL-C was calculated in case of triglycerides <4 mM.

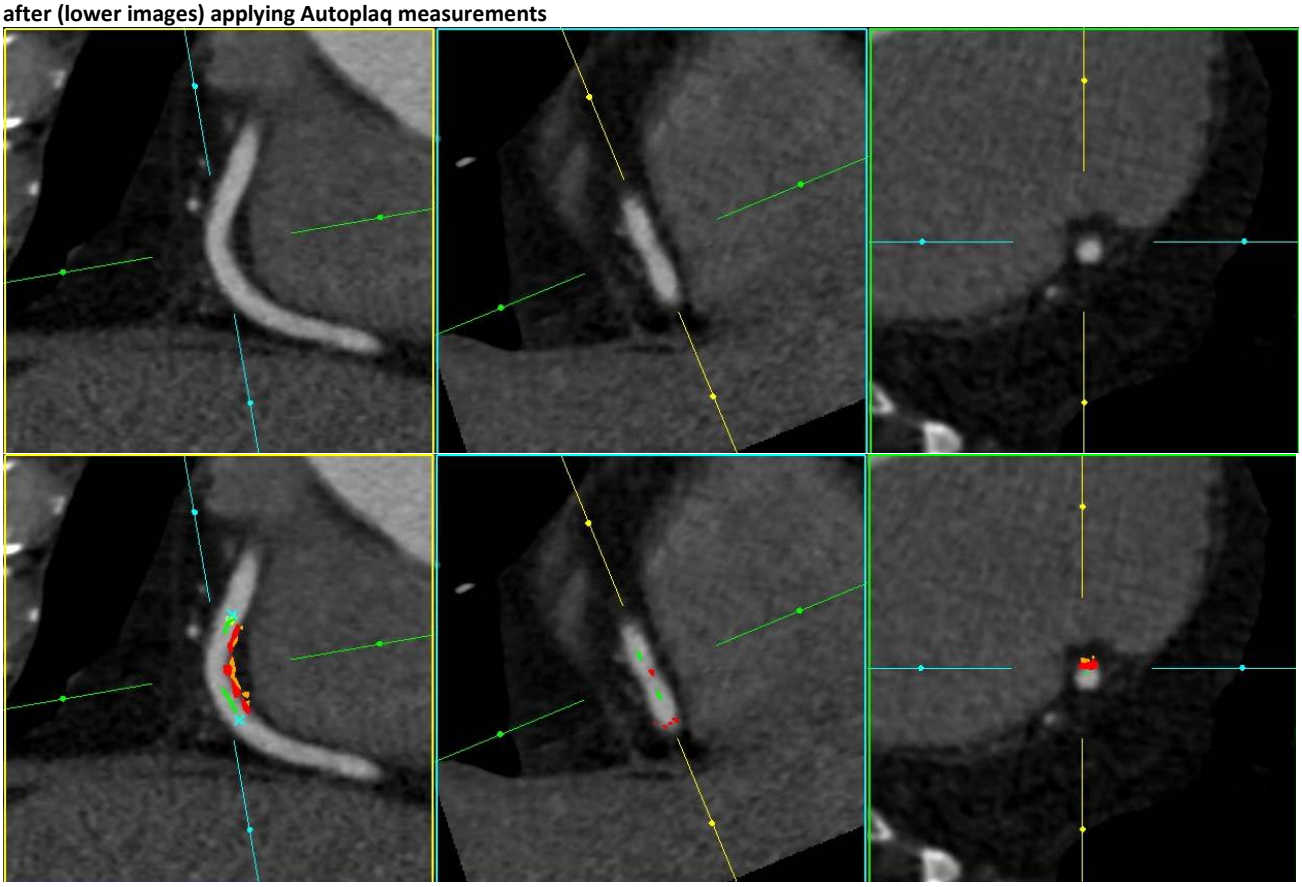
Recommendations from the European guidelines on cardiovascular disease prevention were used to define risk factor control [85]. Accordingly, optimal risk factor control was defined as: (1) a BMI <25 kg/m2, (2) waist circumference <102 cm in males and <88 cm in females, (3) moderate or vigorous intensity exercise for at least 30 minutes ≥3 times a week, (4) no current smoking, (5) a systolic blood pressure of <140 mmHg and a diastolic blood pressure of <90 mmHg (in diabetics blood pressure was considered elevated if ≥140/80 mmHg), (6) a LDL-C <1.8 mM or, if available, a reduction of at least 50% from the untreated value, and (7) a HbA1c value <53 mmol/mol.

3.2.2. Coronary computed tomography angiography (study II)
Coronary CTA was performed on a dual-source CT scanner (SOMATOM Definition Flash; Siemens, Forchheim, Germany). Initially, a non-enhanced 120 kV high-pitch spiral CT-scan was performed for coronary calcium scoring. Sublingual nitroglycerin (0.8 mg) was administered prior to the contrast scan in all patients for optimal image quality. Oral and/or intravenous betablockers were administered if necessary, targeting a heart rate <60 beats/min. The contrast-enhanced CT-scan was performed using prospective electrocardiographic triggering. In patients weighing ≤70 kg data acquisition was performed with 100 kV tube voltage, whereas 120 kV was used in patients >70 kg.

CT images were manually evaluated blinded to the clinical data. The calcium score was recorded using Agatston's method [86], and the coronary CTA analysis was performed on segments ≥2 mm using an 18-segment model [87,88]. The number of evaluable segments was recorded and segments with plaque were identified. A visual stenosis >50% was considered obstructive. Proximal CAD was defined as any CAD in the left main artery or any of the proximal segments of the left anterior descending, circumflexus or right coronary arteries (segments 1, 5, 6 or 11) [87].

A semi-automated plaque analysis was performed using the Autoplaq software (Autoplaq version 9.7, Cedars-Sinai Medical Center, Los Angeles, CA, USA) [89]. For each lesion the proximal- and distal center points of the plaque were manually identified in Autoplaq followed by automated segmentation of plaque and vessel borders (Figure 4). Hence, vessel volume and volumes of calcified plaque (CP), non-calcified plaque (NCP; i.e. plaque with attenuation <150 Hounsfield Units), and low-density NCP (LD-NCP, i.e. NCP with attenuation <30 Hounsfield Units) were computed. Remodeling index (RI) was defined as the maximum vessel

Figure 4: Case example showing a multiplanar reconstruction of a plaque on the right coronary artery before (upper images) and after (lower images) applying Autoplaq measurements



Red marks non-calcified plaque (NCP) and orange represents low-density-NCP.

area at any point across the centerline divided by the vessel area at the proximal plaque-free center point. Positive remodeling (PR) was defined as an RI ≥1.1 [90].

3.2.3. Family history of early-onset CAD and the stratified log-rank family score (study III)

Early-onset CAD patients were requested to obtain a cardiovascular disease history from 1st and 2nd degree relatives ≥18 years and a family pedigree (exemplified in Figure 1) was drawn upon attendance. A family history of CAD was considered present if a patient reported a history of MI or any coronary revascularization procedure in a 1st or 2nd degree relative.

The family pedigrees were used to compute a stratified log-rank family score (SLFS) as a continuous measure of familial clustering based on age, number of family members, familial relations, and CAD status among 1st and 2nd degree family members in the pedigree [91]. Briefly, the score is calculated as follows: For a given family member type (in the following we consider fathers) the age of CAD onset (i.e. observed events) of all affected fathers is used to construct time intervals. Each time interval is assigned a log-rank score based on the number of observed events divided by the number of observed and censored events in the period. The score of the father is the negative value of the cumulated log-rank scores of the time intervals up to the time of his event (CAD onset) or censoring (current age or death). A value of one is added to the score of the father in case he is affected by CAD. The SLFS for a given family is calculated as the sum of the scores of all family members in that family.

3.2.4. Genotyping and construction of a multi-locus genetic risk score (study III)

A review on 46 loci genome-wide significantly associated with CAD/MI in populations of European ancestry was used to select the SNPs (or relevant proxies) for genotyping [92]. Genomic deoxyribonucleic acid (DNA) was extracted from whole blood and genotyping was performed on a Fluidigm BioMark HD (Fluidigm Corp., South San Francisco, CA, USA). One SNP (rs17114036) was excluded due to poor clustering on all chips, and four samples with less than 50% of SNPs successfully genotyped were excluded. Therefore, the final dataset consisted of 45 SNPs and 669 samples.

A weighted multi-locus GRS was calculated for each patient as the sum of the number of risk alleles (0–2) weighted by the log of the odds ratio (OR) for each SNP. The ORs were retrieved from the respective original discovery papers [48-54]. To avoid a value of zero (in the rare case of a missing genotype) the value for that particular SNP was set to the group-specific average.

3.3. Statistical analysis

Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]) or number (percentage), unless otherwise specified. Two-sided p-values ≤0.05 are considered statistically significant. All statistical analyses were performed using Stata/IC 13.1 (StataCorp., College Station, TX 77845, USA).

3.3.1. Study I

Differences between males and females were assessed using Fischer's exact test, $\chi 2$ test, Wilcoxon rank-sum test, or Student's t-test as appropriate.

3.3.2. Study II

Differences in patient characteristics between groups were compared using logistic, ordinal or linear regression models, with robust variance estimation to account for the possible family clustering effect or using Somers' D with the clustering option specified. CAD metrics were analyzed on a per-individual level by pooling the plaque measures within each individual. Ordinal plaque variables were analyzed using ordinal logistic regression with robust variance estimation. Continuous variables were log-transformed as log (variable + 0.5). For binary and continuous plaque variables mixed-effects models were used taking into account a possible family-clustering effect. The odds ratios (OR) or the median ratios (as a measure of the relative difference between groups) were compared.

3.3.3. Study III

For statistical analyses the GRS and SLFS were standardized. Associations between continuous outcome variables and explanatory variables were assessed using multivariable linear regression and one-way analysis of variance (when divided into groups). The relationship between the SLFS and GRS, respectively, and CAD severity was evaluated by ordinal logistic regression.

4. SUMMARY OF RESULTS

The main results of the studies are provided below, whereas a detailed description is presented in the appended papers.

4.1. Study I

4.1.1. Risk factors at onset

In total, 143 patients with early-onset CAD were included of whom 110 (76.9%) were males. At the time of CAD onset, the median age was 37 years (34-38). One-hundred-thirteen (79.0%) presented with acute MI and 107 (74.8%) had one vessel disease. Dyslipidemia, hypertension, and diabetes had been diagnosed prior to CAD onset in 40 (28.0%), 23 (16.1%), and 12 (8.4%) patients, respectively, and 104 (72.7%) patients were active smokers. Eight (5.6%) patients had a pathogenic mutation in the LDLR gene, consistent with a diagnosis of familial hypercholesterolemia.

4.1.2. Risk factors at interview

Patient characteristics at interview are presented in Table 1. Patients were interviewed after a median of 5.6 years past a coronary intervention. Median age was 44 (41-47) years. Regular CAD risk factor consultations with the general practitioner were reported by 69 (48.3%) patients, whereas 29 patients (20.3%) were regularly seen at a hospital, and 10 patients (7.0%) stated both.

Control of risk factor items is presented in Table 2. Uncontrolled lifestyle-related risk factors were common with the majority being overweight, displaying abdominal obesity and exercising less than recommended.

Table 1: Characteristics of the participants at study interview

	Total	Male	Female	p-value
Age	44 (41-47)	43 (41-47)	45 (40-48)	0.40
Years since last coronary revascularization	5.6 (2.1-8.9)	5.6 (2.4-8.7)	6.4 (1.9-10.5)	0.55
Diseased vessels				0.05
- 1 VD	91 (63.6)	64 (58.2)	27 (81.8)	
- 2 VD	26 (18.2)	23 (20.9)	3 (9.1)	
- 3 VD	26 (18.2)	23 (20.9)	3 (9.1)	
Last estimate of LVEF (%)	60 (50-60)	60 (50-60)	60 (50-60)	0.98
Vascular co-morbidity				
- Prior MI	120 (83.9)	92 (83.6)	28 (84.9)	1.00
- Prior stroke	11 (7.7)	7 (6.4)	4 (12.1)	0.28
- PAD	3 (2.1)	2 (1.8)	1 (3.0)	0.55
Other co-morbidity				
- Metabolic syndrome	68 (47.6)	51 (46.4)	17 (51.5)	0.60
- Diabetes	29 (20.3)	18 (16.4)	11 (33.3)	0.03
- FH	8 (5.6)	6 (5.5)	2 (6.1)	1.00
Systolic BP (mmHg)	122 ± 14	122 ± 14	123 ± 13	0.49
Diastolic BP (mmHg)	82 ± 9	83 ± 10	81 ± 8	0.41
BMI (kg/m2)				0.01
- <18.5	1 (0.7)	1 (0.9)	0 (0)	
- 18.5-25	29 (20.3)	16 (14.6)	13 (39.4)	
- 25-30	53 (37.1)	46 (41.8)	7 (21.2)	
- ≥30	60 (42.0)	47 (42.7)	13 (39.4)	
Waist (cm)	101.4 (92.1-112.2)	103.9 (93.2-114.3)	96.6 (82.3-103.2)	<0.01
Biochemistry				
- TC-C (mM)	4.1 (3.5-5.1)	4.1 (3.4-5.1)	4.0 (3.6-4.8)	0.81
- LDL-C (mM)[a]	2.2 (1.5-2.7)	2.2 (1.5-2.7)	2.0 (1.5-2.7)	0.40
- Triglycerides (mM)	1.5 (1.0-2.2)	1.6 (1.0-2.2)	1.4 (1.0-2.2)	0.60
- Hemoglobin A1c (mmol/mol)	39 (36-42)	38 (36-40)	41 (38-50)	<0.01
- Creatinine (μM)	78 (68-87)	79 (72-87)	66 (59-71)	<0.01

Values are expressed as n (%), median (interquartile range) or mean ± standard deviation. [a] LDL-C was calculated in 133 participants. Abbreviations: BMI, body mass index; BP, blood pressure; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PAD, peripheral artery disease; TC-C, total cholesterol; VD, vessel disease. Reproduced in a modified form from paper I.

Table 2: Uncontrolled risk factors at study interview

	Total	Male	Female	p-value
Treatment goals				
- High BP[a]	37 (25.9)	29 (26.4)	8 (24.2)	0.81
- Low HDL-C[b]	48 (33.6)	38 (34.6)	10 (30.3)	0.65
- High LDL-C[c]	77 (57.9)	59 (58.4)	18 (56.3)	0.83
- High Triglycerides[d]	67 (46.9)	52 (47.3)	15 (45.5)	0.85
Lifestyle goals				
- Elevated BMI[e]	113 (79.0)	93 (84.6)	20 (60.6)	<0.01
- Abd. obesity[f]	76 (53.2)	57 (51.8)	19 (57.6)	0.56
- Sedentary lifestyle[g]	78 (54.6)	58 (52.7)	20 (60.6)	0.43
- Current smoking[h]	53 (37.1)	33 (30.0)	20 (60.6)	<0.01

Values are expressed as n (%). [a] BP threshold is ≥140/90 mmHg except ≥140/80 mmHg in diagnosed diabetics. [b] HDL-C <1.0/1.2 mM (M/F). [c] LDL-C ≥1.8 mM & <50% of untreated value. Values were calculated in 133 participants and untreated values were available in 75 participants. [d] Triglycerides ≥1.7 mM. [e] BMI ≥25 kg/m^2. [f] Waist circumference ≥102/88 cm in males/females, respectively. [g] Exercising ≥30 minutes <3 times per week. [h] Smoking within the last month. Abbreviations: BP, blood pressure; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; Abd. obesity, abdominal obesity. Reproduced in a modified form from paper I.

Blood pressure was above target level in 37 (25.9%) patients of whom the larger part had grade 1 hypertension. Among patients above target level, 7 (18.9%) did not receive any antihypertensive medication, whereas 18 (48.7%) were medicated with at least two antihypertensive drug regimens. Seventy-seven (57.9%) patients did not reach the LDL-C target level, of whom 17 (22.1%) did not receive any lipid-lowering medication and 52 (67.5%) were only on statin therapy. Twenty-nine (20.3%) patients had diabetes and 68 (47.6%) patients met the criteria of the metabolic syndrome. Even when omitting patients in whom diabetes was diagnosed upon interview, 15 (65.2%) of patients with diabetes had an HbA1c value of >53 mmol/mol.

The median number of uncontrolled risk factor items was 2 (2-4). Control of all items was achieved in 7 (4.9%) patients. By comparing patients who did and did not attend regular CAD risk factor consultation there was no difference in the number of uncontrolled items (p=0.88).

4.2. Study II

We included 88 relatives (of 59 patients from study I) and 88 matched controls in the study. The mean age was 47.8 ± 7.9 years of which 53% were males. There was a trend for lipid-lowering therapy to be more common in controls than in relatives, and total cholesterol and HDL-C were higher in relatives. Other patient characteristics were comparable between relatives and controls.

The visual assessment of CAD is presented in Table 3. CAD was more prevalent in relatives (70%) compared with controls (51%), p=0.016. Relatives had higher calcium scores, and CAD was more often obstructive and present in the proximal coronary segments compared with controls.

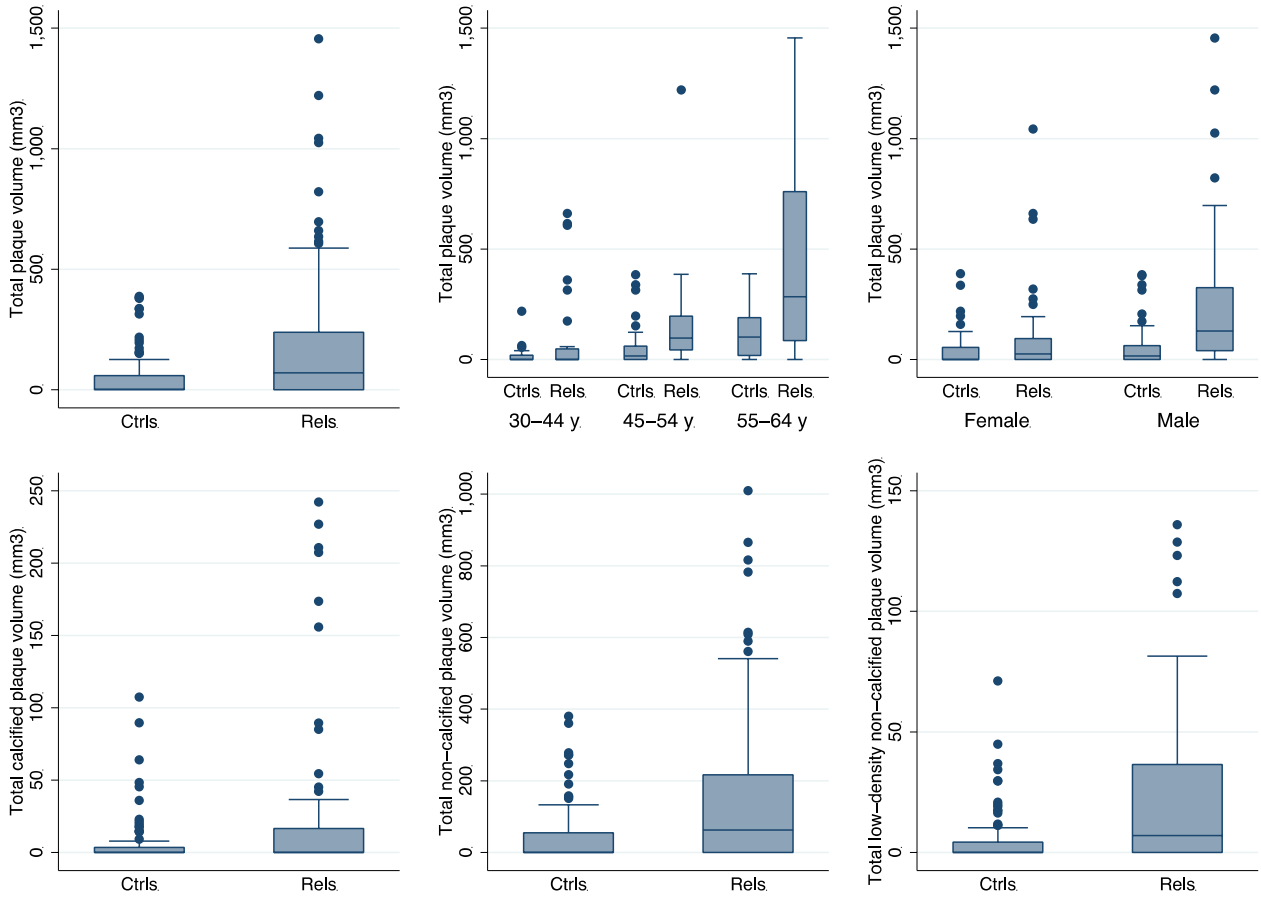
The semi-automated plaque analyses are presented in Figure 5 and Table 4. The total plaque volume was significantly increased in relatives compared with controls. This was driven by higher

Table 3: Visual assessment of CAD

	Relatives	Controls	p-value
Affected coronary segments, n (%)			0.001
- 0 segments	26 (30)	43 (49)	
- 1-2 segments	24 (27)	28 (32)	
- 3-4 segments	16 (18)	5 (6)	
- ≥5 segments	22 (25)	12 (14)	
CAD severity, n (%)			0.017
- No CAD	26 (30)	43 (49)	
- Non-obstructive CAD[a]	49 (56)	36 (41)	
- Obstructive CAD[b]	13 (15)	9 (10)	
Proximal CAD[c], n (%)			0.011
- No proximal CAD	38 (43)	55 (63)	
- Non-obstructive proximal CAD	42 (48)	29 (33)	
- Obstructive proximal CAD	8 (9)	4 (5)	
Calcium Score, median (95% CI)	4.1 (1.9-8.0)	1.0 (0.5-1.8)	0.004

Calcium scores were derived from log (Agatston score + 0.5)-transformed values to account for the skewed distributions and zero's. [a] Stenosis severity ≤50% based on expert reader visual assessment. [b] Stenosis severity >50% based on expert reader visual assessment. [c] CAD involving the left main artery or any of the proximal segments of the left anterior descending, circumflexus or right coronary arteries (segments 1, 5, 6 and 11). Abbreviations: CAD, coronary artery disease. Reproduced from paper II.

Figure 5: Distribution of total plaque burden in relatives and controls



Boxes indicate quartiles and whiskers display adjacent values. Values outside range of adjacent values are plotted as outliers. For illustrative purposes, one outlier was removed from the graph displaying the total calcified plaque volume (Relative; total calcified plaque volume: 590.2 mm3). Abbreviations: Ctrls, controls; Rels, relatives; y, years. Reproduced from paper II.

volumes of CP, NCP, and LD-NCP. One or more plaques with PR (crude OR [95% CI]: 2.4 [1.3-4.5], p=0.004; adjusted OR [95% CI]: 4.2 [1.2-14.0], p=0.021) and one or more plaques containing LD-NCP (crude OR [95% CI]: 2.5 [1.3-5.0], p=0.008; adjusted OR [95% CI]: 4.2 [1.9-9.5], p=0.001) were also more commonly observed among relatives.

Table 4: Total plaque burden

	Crude				Adjusted			
	Relatives median (95% CI)	Controls median (95% CI)	Median ratio (95% CI)	p-value	Relatives median (95% CI)	Controls median (95% CI)	Median ratio (95% CI)	p-value
Total plaque volume (mm3)	27.0 (14.2-51.0)	5.3 (2.9-9.5)	4.7 (2.1-10.8)	<0.001	29.2 (17.8-47.6)	4.7 (2.6-8.2)	5.8 (2.8-11.9)	<0.001
Total plaque length (mm)	8.6 (5.2-14.0)	2.4 (1.5-3.8)	3.2 (1.7-5.8)	<0.001	9.1 (6.3-13.2)	2.2 (1.3-3.4)	3.6 (2.1-6.1)	<0.001
Total CP (mm3)	2.6 (1.4-4.7)	0.9 (0.5-1.4)	2.3 (1.2-4.2)	0.009	2.8 (1.6-4.6)	0.8 (0.4-1.2)	2.6 (1.5-4.5)	<0.001
Total NCP (mm3)	24.3 (13.1-44.9)	4.8 (2.6-8.4)	4.7 (2.1-10.6)	<0.001	26.3 (16.2-42.5)	4.1 (2.2-7.2)	5.8 (2.9-12.0)	<0.001
Total LD-NCP (mm3)	5.2 (3.1-8.6)	1.2 (0.7-1.8)	3.4 (2.0-6.0)	<0.001	5.5 (3.6-8.2)	1.0 (0.6-1.5)	4.0 (2.5-6.6)	<0.001

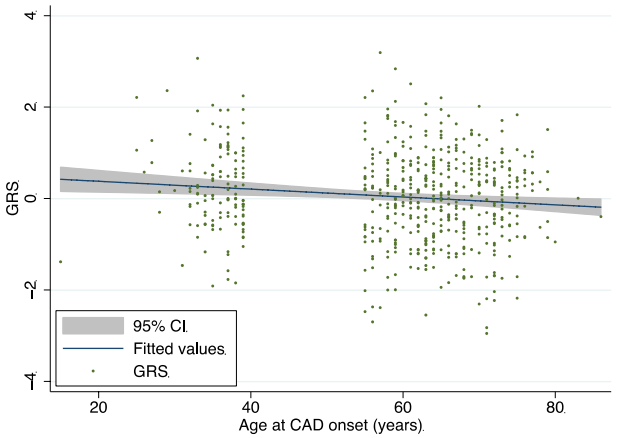
Adjusted for age, gender, active smoking, hypertension, dyslipidemia, LDL-C, and number of evaluable segments. Estimates are derived from log (variable + 0.5)-transformed values to account for the skewed distributions and zero's. Abbreviations: CP, calcified plaque; LD-NCP, low-density non-calcified plaque; NCP, non-calcified plaque. Reproduced from paper II.

4.3. Study III

A total of 669 subjects were included, of which 134 had early-onset CAD, 446 had late-onset CAD, and 89 were healthy controls. Early-onset CAD patients more often had prior CABG surgery and reduced renal function compared with late-onset CAD patients, and they were more often smokers and overweight. Conversely, antihypertensive and statin therapy were more common in late-onset CAD patients.

The GRS was higher CAD patients compared with healthy controls, and early-onset CAD patients had a higher GRS than late-onset CAD patients (Overall p<0.0001. Healthy controls vs. late-onset CAD: p=0.002. Late-onset CAD vs. early-onset CAD: p=0.02. Healthy controls vs. early-onset CAD: p<0.0001). In crude regression analyses, one SD increase in the GRS was associated with 1.7 years (95% CI 0.5-2.8, p=0.004) earlier CAD onset (Figure 6). Similarly, a 1.2 years (95% CI 0.1-2.2, p=0.028) earlier onset of CAD was observed per SD increase in the GRS in the adjusted model.

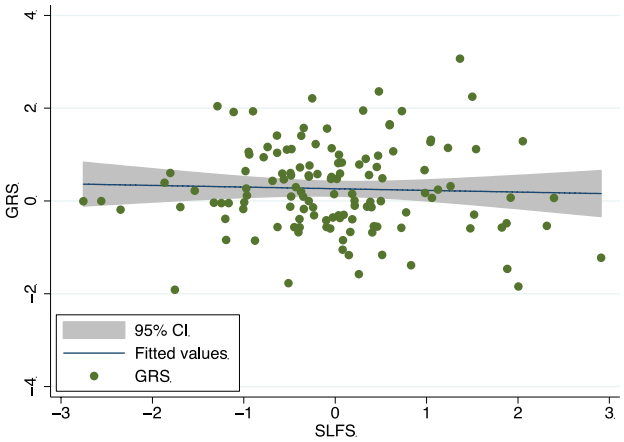
Figure 6: Association between the GRS and age at CAD onset



The GRS is standardized (i.e. one unit on the y-axis corresponds to 1 standard deviation of the GRS). Abbreviations: CAD, coronary artery disease; GRS, genetic risk score. Reproduced from paper III.

The SLFS was calculated based on family pedigrees from 131 early-onset CAD patients encompassing information about 487 1st degree relatives and 1098 2nd degree relatives. The GRS was not associated with the SLFS in either crude or adjusted analyses (Figure 7 and Table 5). However, antihypertensive treatment and BMI were associated with the SLFS, of which BMI remained significantly associated in the adjusted analyses.

Figure 7: Association between the genetic risk score (GRS) and the stratified log-rank family score (SLFS) in early-onset coronary artery disease patients



The SLFS and the GRS were standardized (i.e. one unit corresponds to 1 standard deviation). Reproduced from paper III.

We next evaluated the association between the GRS, the SLFS, and CAD severity. The crude OR (95% CI) of an increased number of affected coronary vessels was 2.0 (1.4-2.9), p<0.001, per SD increase in the SLFS, whereas the crude OR (95% CI) per SD increase in the GRS was 1.2 (0.8-1.8), p=0.29. The estimates did not significantly change after both variables were added to the model (OR [95% CI] per SD increase in the SLFS: 2.0 [1.4-3.0], p<0.001. OR [95% CI] per SD increase in the GRS: 1.3 [0.9-1.9], p=0.17).

5. A CRITICAL REVIEW OF METHODS AND RESULTS

5.1. The cross-sectional study design

All our studies were cross-sectional and it is important to consider the advantages and disadvantages of the design. Considering the low prevalence of early-onset CAD, a cross-sectional design was a reasonable, time-, and cost-effective approach to maximize recruitment of patients and determine the prevalence of risk factor control and plaque metrics [93].

Table 5. Univariable and multivariable linear regression analyses of the SLFS

	Unadjusted			Adjusted	
	Beta (95% CI)	p-value	R^2	Beta (95% CI)	p-value
Age	0.02 (-0.05-0.09)	0.60	<0.01	0.03 (-0.04-0.10)	0.46
Male sex	0.03 (-0.88-0.82)	0.94	<0.01	0.03 (-0.86-0.93)	0.94
BP treatment	1.04 (0.19-1.89)	0.016	0.04	0.67 (-0.29-1.62)	0.17
Statin treatment	0.48 (-0.44-1.40)	0.31	<0.01	0.24 (-0.73-1.22)	0.62
Diabetes	0.53 (-0.35-1.42)	0.24	0.01	0.21 (-0.70-1.12)	0.65
Current smoking	0.09 (-0.63-0.82)	0.80	<0.01	0.32 (-0.42-1.07)	0.39
BMI	0.09 (0.03-0.14)	0.002	0.07	0.08 (0.03-0.14)	0.005
GRS	-0.14 (-0.52-0.24)	0.47	<0.01	-0.16 (-0.53-0.22)	0.41

Adjusted for sex, antihypertensive treatment, statin treatment, diabetes, current smoking, BMI, and GRS, which were added simultaneously. The GRS is standardized (i.e. one unit corresponds to 1 standard deviation of the GRS). R^2 = 0.12 for the full model. Abbreviations: BMI, body mass index; BP, antihypertensive treatment; GRS, genetic risk score; SLFS, stratified log-rank family score. Reproduced from paper III.

A cross-sectional study cannot prove causality, but may be extended to investigate associations between exposures and outcomes, assuming that exposure variables are constant over the time period of interest [93]. In study III, this may be a challenge when analyzing factors affecting the time of CAD onset and the SLFS. Since the primary exposure variable (the GRS) is unchanged over time, a cross-sectional design is not a concern. However, incorporating cardiovascular risk factors as covariates in a meaningful way may be a challenge. Using physician-based diagnoses prior to CAD onset, silent diseases like hypertension and hypercholesterolemia are presumably heavily underdiagnosed in young individuals. Upon CAD onset, measurements performed are likely unreliable for diagnosing because of the acute setting in which they were measured. After CAD onset, secondary preventive treatment is often routinely initiated. Measurements of blood pressure or lipid levels do therefore not reflect natural levels and diagnoses based on treatment will likely inflate the prevalence. Therefore, the value of these factors as covariates may be modest. However, we chose to include them in the adjusted analyses since they are important risk factors for CAD development, and theoretically could influence the estimates.

5.2. Measuring risk factor control

The prevalence of risk factor control relies on the way it is measured. Cardiovascular researchers have generally adopted interviews and self-report questionnaires to capture lifestyle-related health measures [29,94,95], but it is important to recognize the limitations. Both smoking and measures of a less definite character (like physical activity) may exhibit a weak agreement with direct measures [96,97]. In particular among patients in whom a healthy lifestyle is of special importance, underreporting of an undesirable lifestyle may be slightly more common [96]. This may potentially lead to an underestimation of risk factor control. Another concern is the fact that measures of risk factor control in the setting of a research study may not reflect that of the clinical practice. In particular, blood pressure may vary. Therefore, treatment decisions are generally recommended on the basis of several measurements or an automated ambulatory measurement [85]. The AOBP is a relatively new method to measure blood pressure. Compared with the automated ambulatory blood pressure measurement as the golden standard, the technique is accurate and almost eliminates the presence of white-coat hypertension [84]. Considering the feasibility and accuracy of the AOBP we chose to use this method for our study.

5.3. Coronary computed tomography angiography for plaque detection

Coronary CTA is a well-established, non-invasive imaging technique and the diagnostic modality of choice in patients with low-intermediate stable chest pain symptoms [22]. In recent years, the technical evolution has lead to lower radiation exposures and at the same time the spatial and temporal resolution has substantially increased [98]. Consequently, image quality has been improved and coronary CTA is increasingly used in research and clinical practice [99].

Image quality is of major importance for the interpretation of coronary CTA, particularly in smaller vessels [98]. In case of low image quality, coronary segments may be adjudicated as unfit for evaluation [98]. In our study the number of evaluable segments was generally high. However, some individuals with low-quality images may potentially have caused an underestimation of the prevalence of CAD.

Image quality is sensitive to body composition and heart rhythm irregularity [98]. Therefore, obesity and chronic atrial fibrillation were chosen as part of the study exclusion criteria in the selection of relatives for our study. In the clinical setting, from which the control population was drawn, these criteria are not absolute contraindications. This selection caused a slight difference in the observed distribution of BMI, which could potentially bias our estimates. However, it is important to note that the number of evaluable segments was comparable in relatives and controls, and this number was also included as a covariate in the adjusted analyses. Therefore, it is unlikely that image quality has affected the comparisons made.

5.4. Capturing heritability in genetic risk scores

Constructing a GRS is a sensible method to combine the effects of many risk variants into one predictive measure of genetic risk. Maximizing capture of the polygenic burden is challenging. The majority of prior studies have build their GRSs based on replicated variants reaching genome-wide significance in large GWAS (i.e. meeting a Bonferroni-corrected threshold of significance at $p = 5\times10^{-8}$). Compared with earlier candidate-gene driven methods, GWASs have provided largely unbiased associations between risk variants and CAD (with regard to prior knowledge on biological pathways) [100]. Additionally, specific 'exome arrays' have been developed to detect rare coding variants affecting the risk of CAD [101]. However, the variants discovered are generally all common since GWASs are not suitable to detect rare or private variants, which may contain larger effect sizes. Another disadvantage is that many truly associated variants may not reach the stringent Bonferroni-corrected criteria of significance. One study suggested that a 46-SNP GRS (similar to the one we used) provided the optimal threshold of prediction and discrimination for incident CAD [63]. Furthermore, they reported that adding SNPs genome-wide significantly associated with intermediate traits (cholesterol, diabetes etc.) did not improve prediction [63]. Another study found that incorporating 7387 SNPs meeting a GWAS significance level of $p<0.001$ was superior in terms of prediction and discrimination [102].

When the estimated effect sizes of the risk variants differ, one may improve genetic prediction by weighting the GRS, but improper weighting may potentially bias the estimates and reduce power [103]. Obtaining the weights externally from large GWAS has generally been accepted as the strategy of choice. We used the weights derived from the original discovery GWAS, as was the method adapted by Ripatti et al [59], because it was a simple and unbiased way of selecting from the several GWAS variant replications. Others argue that choosing estimates from the largest available meta-analysis might be preferable [104].

6. DISCUSSION

6.1. Cardiovascular risk factors in early-onset CAD

Risk factor patterns differ among younger and older patients. Overweight/obesity, smoking, dyslipidemia, and a family history of CAD are typically seen in young CAD patients, whereas hypertension and diabetes are more common in older patients [105-108]. This may reflect ageing per se, although an alternative explanation may be that some risk factors exert different effects on the development of CAD at different points in life [13,109].

Dyslipidemia has an important role in early-onset CAD [13,109]. One study investigated the prevalence of lipid-disorders in early-onset MI patients compared with matched controls [110]. In consistence with our findings, the study demonstrated that only 8% of patients with early-onset MI had probable/definite familial hypercholesterolemia according to the Simon-Broome criteria [110]. Conversely, they estimated that 38% displayed a familial combined hyperlipidemia phenotype by using criteria based on levels of total cholesterol, triglycerides, and Apo B100. This corresponded to an estimated 24-fold increased OR of early-onset MI [110]. Since we did not measure levels of Apo B100, a direct comparison is not possible. However, a large proportion of patients in our study had high levels of total cholesterol and triglycerides. This was particularly the case in patients with the metabolic syndrome, which may underline the overlap between the two phenotypes [111].

The benefits of pursuing a reduction of risk factors in the primary and secondary prevention of cardiovascular disease are evident. In 2010 the American Heart Association defined national recommendations with the goal of improving cardiovascular health and reducing death from cardiovascular disease by 20% over a 10-year period [112]. They recommended aiming for an "ideal cardiovascular health" including a normal BMI, regular physical activity, a healthy diet, refraining from smoking, and obtaining normal levels of blood pressure, cholesterol, and glucose [112]. Although the same term has not been implemented in Europe, the items included in European recommendations are similar [19,85]. Considering that early-onset CAD patients are at very high risk of subsequent events throughout their lifetime, it is striking that only 4.9% of the study patients met the goals of optimal risk factor control.

A number of prior studies have addressed risk factor control in patients with and without overt CAD. Although young patients have not been specifically addressed, the most recent Euroaspire IV investigation presented similar findings in the strata of young patients [29]. When compared with our results the proportions being overweight (79.5% vs. 79.0%), smoking (33.6% vs 37.1%) and displaying blood pressure above target (26.3% vs. 25.9%) were similar, although we found LDL-C above target to be less common (83.3% vs. 57.9%).

Different factors may have affected the limited success of risk factor control. Lifestyle-related items were most commonly uncontrolled. This likely reflects the challenges of changing lifestyles but may underscore the need for further promotion and support of behavioral change [113]. However, a considerable proportion of patients did not reach blood pressure or lipid targets either, even though the majority attended regular health care visits and received antithrombotic, antihypertensive, and lipid-lowering therapy. It may be that physicians do not react on deviations from blood pressure or cholesterol targets since a significant proportion of patients not on target had mildly elevated levels. Given the well-documented benefits of an aggressive treatment strategy [24,25], such a strategy should be pursued.

6.2. Plaque burden in patients with a family history of early-onset CAD

To our knowledge, the present study is the first to evaluate the detailed associations between a family history of CAD and coronary plaque burden and composition. We found that coronary plaque burden was significantly increased in patients with a familial history compared with controls with no familial predisposition. This difference was observed in spite of the fact that control patients underwent CTA on a clinical suspicion of CAD.

Previous studies have investigated the effect of a family history of CAD on coronary CTA findings; most commonly by using dichotomized plaque measures. A report from the CONFIRM (COronary CT Angiography EvaluatioN For Clinical Outcomes: An InteRnational Multicenter) registry studied patients referred for CTA due to suspected CAD [114]. The authors reported that a family history of premature CAD in a first-degree relative was associated with an increased risk of any CAD (40% vs. 30%; relative risk 1.33, p<0.001), more segments affected (p<0.001) and obstructive CAD (11% vs. 7%; adjusted OR 1.71 [95% CI 1.42-2.07]), comparable to the findings of our study [114]. A smaller single-center study also demonstrated an association with a family history of premature CAD and any CAD (79% vs. 65%; adjusted OR 2.54 [95% CI 1.44-4.48]) and obstructive CAD (54% vs 40%; adjusted OR 2.01 [95% CI 1.24-3.26]) in patients undergoing CTA [115]. On the other hand, a large study on self-referred asymptomatic subjects in Korea did not support an association between a family history of premature CAD and the presence of CAD (OR 0.73 [95% CI 0.37-1.44]) [116].

Through many years, pathologists have provided evidence that specific plaque characteristics are associated with vulnerability (i.e. risk of rupture) [31]. These plaques have been characterized as fibroatheromas identified by the presence of a lipid-rich necrotic core covered by a luminal fibrous cap. In particular, a large lipid-core, a thin fibrous cap, and expansive plaque remodeling have been identified as risk factors for plaque rupture [31,117], and in-vivo assessment of these features has proven to predict adverse coronary events [118-120]. On the other hand, in the absence of a lipid-core, there is no cap to rupture, and consequently, the risk of adverse coronary events is low [121].
In coronary CTA studies, LD-NCP has been shown to represent lipid-cores [36]. Furthermore, the extent of expansive plaque remodeling is reflected in the RI with values ≥1.1 determined as an optimal cut-off for PR compared with intravascular ultrasound as the reference [90]. In the present study, we observed that both LD-NCP and PR were particularly more common in relatives compared with controls. Although outcome studies are warranted, these findings may potentially explain the increased risk of coronary events in patients with a family history of early-onset CAD. Obstructive CAD, LD-NCP and PR independently predict myocardial ischemia [88,122]. Therefore, it may be intriguing that these features were higher in relatives compared with controls referred for CTA due to CAD symptoms. This may be in accordance with a previous study reporting that almost one in five healthy siblings of patients with CAD onset <60 years had silent myocardial ischemia on nuclear perfusion imaging [123].

Our findings may explain the increased risk of adverse coronary events in patients with a family history of early-onset CAD, but they do not explain the underlying mechanisms. Furthermore, they do not clarify whether the findings reflect a more advanced disease stage or perhaps the development of a more 'high-risk' type of atherosclerosis. One study evaluated coronary angiograms of 882 siblings from 401 families with CAD onset before the age of 60 years [77]. They found that heritability was particularly high for CAD present in the left main and proximal coronary arteries as well as for ectasies and calcifications [77]. An appreciable heritability in atherosclerotic calcifications has also been demonstrated in studies using CT [124,125]. Although these studies are not proof that patients with a family history of CAD are particularly prone to a certain type of atherosclerosis, they suggest that family history plays a role in the way atherosclerosis develops.

6.3. Common risk variants and familial clustering in early-onset CAD

In consistence with previous studies [46,68,78], we found that early-onset CAD patients had a higher GRS compared with late-onset CAD patients and healthy control subjects. However, the effect on early-onset CAD patients was modest as illustrated by the small effect on the age at onset.

A few studies have examined the relationship between a GRS and family history of CAD. In subanalyses of studies focusing on other endpoints, some have reported significant associations [70,126], whereas another have not [68]. One study specifically examined the relationship between self-reported family history (<45 and <55 years in male and female first-degree relatives, respectively) and found it to be independent of a 30-SNP GRS, but both factors jointly increased the risk of multivessel disease by coronary angiography [127]. A prospective study on the Malmö Diet and Cancer cohort investigated the effects of self-reported family history and two GRSs on the risk of incident CAD. The authors found that family history and GRSs predicted CAD independently, but with additive effects [128]. Of note, the observed effects were largest among young individuals [128]. Our study adds to these findings by demonstrating that the GRS acts independently of the extent of familial clustering in early-onset CAD. The absence of an association between the GRS and familial clustering of CAD may be intriguing. However, family history is not only the result of genetic inheritance. Genetic, behavioral and environmental factors (of which some can be captured in known risk factors) may all cluster in families and lead to overt CAD [9,21,94]. A community-based study in individuals >45 years from the REGARDS (the REasons for Geographic And Racial Differences in Stroke) cohort used the SLFS to study the relationship between familial clustering of MI and traditional cardiovascular risk factors. They found a graded and highly significant relationship between the SLFS and hypertension, diabetes, dyslipidemia, smoking, and obesity [129]. In our study, BMI was associated with the SLFS and also explained most of the variation in age of CAD onset. These results suggests that traditional risk factors may play a more prominent role in familial clustering of early-onset CAD than the polygenic burden of the CAD risk variants identified this far.

Another explanation for the modest effect of the GRS on age and the lack of association with familial clustering may be the fact that the incorporated genetic variants explain little of overall CAD heritability. One calculation estimated the fraction of heritability explained by variants (broadly similar to the ones in our GRS) was 10% [54]. A more recent GWAS of CAD was published in 2015, after the beginning of study III [55]. This study reported the discovery of 10 new loci taking the fraction of heritability explained to 13% [55]. Additionally, they estimated that 28% of CAD heritability could be explained by considering 202 variants that were associated with CAD of which the majority did not meet strict genome-wide significance (accepting a false discovery rate of 0.05). Only 15 of the 202 were low-frequency variants (which jointly explained only 2% of the total heritability). This and other emerging evidence suggest that most of the overall heritability of complex traits may be caused by common variants with low effect sizes [130,131]. Importantly, however, GWASs based on genotyping arrays are not suited to detect private or rare variants with large effect-sizes. Such variants may potentially play a larger role in younger CAD patients with familial disease clustering [132-134]. The first CAD exome-wide association study (i.e. based on exome sequencing data) has recently been published [135]. In an attempt to discover rare coding variants contributing to CAD development they sequenced the exomes of cases with MI ≤50/60 years in males/females and older CAD-free controls. They found an association between CAD risk and rare variants in two lipid-related genes (LDLR and APOA5). The study was likely severely underpowered [135,136], hence more novel rare variants may arise as the sizes of sequencing databases increase.

6.4. Clinical implications

Current guidelines recommend cardiovascular risk factors are evaluated in relatives of patients with premature CAD to establish if primary preventive treatment should be initialized [19]. However, the systematic coronary risk evaluation tool may underestimate risk in individuals with a strong family history [19], and hence, preventive treatment may not be implemented despite a potential benefit. We have provided evidence that measures of plaque burden are increased in relatives of patients with early-onset CAD, and possibly, such measures might have a role in the aid of decision-making. However, further studies, in particular intervention studies to modify plaque progression and/or composition, are needed to clarify a true usefulness.

The modest effect of the GRS on the age of onset and the lack of association with familial clustering in patients with early-onset CAD may reflect a lack of clinical utility of current GRSs in the setting of early-onset CAD [19]. In particular, these risk variants cannot be used to explain that some patients are affected from a very early age, and they do not explain the familial aggregation in some of these families. However, in the light of the significant heritability in early-onset CAD, the value of genetic testing may possibly improve as the understanding of the genetic contributions is likely to increase in the upcoming years.

7. LIMITATIONS

7.1. General limitations

All three studies are limited by the sample size, which is reflected in the wide confidence intervals of the estimates. In particular in study III, valid family history on patients with late-onset CAD was not available. This is a major limitation, as it reduces the power to observe weak associations between the GRS and family clustering. Furthermore, we cannot detect any association between the GRS, familial clustering and CAD severity in late-onset CAD, where risk factors may differ from those of early-onset CAD patients.

7.2. Specific limitations of study I

Of patients considered eligible for inclusion in study I only 143 out of 283 were included. This may potentially have introduced selection bias. To address this issue we evaluated characteristics entered into the Western Denmark Heart Registry upon the first coronary intervention, which was overall similar among eligible participants (n=143) and non-participants (n=140). Additionally, 29 patients were considered non-eligible due to death prior to enrollment; individuals who were likely more severely diseased than the average of the eligible population. Another important consideration is the fact that risk factor control may vary across regions due to different cultures or medical practices. Therefore, caution should be used when our results are applied outside the region from which the study patients resided.

7.3. Specific limitations of study II

The study was a single-center study, which may compromise the generalizability. There was a trend towards lipid-lowering treatment being more common in controls, and accordingly, the lipid levels differed between the groups. These differences may potentially have influenced our findings. The controls were all referred

to CTA due to a suspicion of CAD. The plaque burden in these patients may likely be higher than that of an asymptomatic control population, which may have influenced the size of the difference measured.

7.4. Specific limitations of study III

We did not include patients in the age-interval between early- and late-onset CAD. Therefore, the effect of the GRS on age in the regression model should be interpreted with caution. The effect should illustrate the average effect on age, which might not apply to patients in that interval. Familial clustering is an arbitrary measure and therefore the exact weighing of age, numbers, relationships and disease status used in the definition may be argued. We chose to use the SLFS as a measure of familial clustering because it is a relatively simple measure of family history severity. The SLFS has the advantage that it differentiates between families with no events by taking into account the number of relatives and time at risk, and the SLFS considers the age of onset and the number of relatives affected, thereby differentiating between families with events. Importantly, the SLFS has shown to improve risk prediction of clinical outcomes compared with a dichotomous measure of family history [91]. However, it should be noted that validation studies were performed in datasets significantly larger than our study sample [91]. Nevertheless, our sample size was large enough to observe an association between the SLFS and CAD severity, which was not the case for the GRS.

8. CONCLUSIONS

8.1. Study I

Among early-onset CAD patients, cardiovascular risk factors are common. A substantial potential for improvement of risk factor control remains.

8.2. Study II

First-degree relatives of patients with early-onset CAD have a high coronary plaque burden compared with controls with no familial predisposition. The plaques display characteristics associated with myocardial ischemia and adverse coronary events.

8.3. Study III

Early-onset CAD patients have a modestly increased polygenic burden (measured as a 45-SNP GRS) compared with late-onset CAD patients and healthy control individuals. The familial clustering in early-onset CAD does not associate with the polygenic burden. Furthermore, only familial clustering significantly predicted CAD severity.

9. PERSPECTIVES

The present thesis provides a detailed characterization of early-onset CAD. The results emphasize the burden of risk factors in these patients and demonstrate the substantial hereditary component of coronary atherosclerosis with notable adverse features present in predisposed individuals. While the yet identified common genetic risk variants may provide some clinical value in selected populations, our results support current guidelines that they should currently not be applied in the clinical setting of early-onset CAD [19].

Several of the results in the present thesis need further investigation. First, we demonstrated that risk factor control is inadequate, however, we provide no answers to optimize. Second, our findings of an increased plaque burden in patients with a family history of early-onset CAD may provide valuable insights into the hereditary aspects of CAD. However, larger longitudinal studies are needed to clarify whether the observed features may explain the increased risk of coronary events in such patients. Third, although the GRS did not associate with familial clustering or CAD severity in early-onset CAD, we did not have any data on late-onset CAD patients. Such a relationship might exist in older patients, in whom genetic contribution to disease risk and traditional risk factors are likely to differ from that in early-onset CAD patients, but this remains to be clarified.

Until now, genetic studies have focused on CAD as a dichotomous phenotype, although the underlying pathology (i.e. coronary atherosclerosis) is a quantitative trait. Coronary CTA is a unique method to obtain an in-vivo quantification of coronary atherosclerosis, and combined with the fast-evolving genetic techniques, it may provide an opportunity to further characterize the heritability of CAD.

10. SUMMARY

A family history of coronary artery disease (CAD) is an important risk factor for adverse coronary events, in particular if the disease has an early onset. The risk of CAD is influenced by genetic and environmental factors with a greater genetic contribution earlier in life. Through recent years the advances in genetic techniques has led to an increased understanding of the genetic background of CAD, which may potentially be translated into clinical use.

The studies of this thesis aimed to investigate the burden of conventional risk factors and control in early-onset CAD (i.e. <40 years), and to characterize and quantify subclinical atherosclerosis in their relatives. Furthermore, the aim was to explore the impact of common genetic risk variants on the age of onset, familial clustering and disease severity.

In study I, 143 patients with early-onset CAD were recruited from the Western Denmark Heart Registry and risk factor control was evaluated. The study revealed that risk factors are common in early-onset CAD and that a large room for risk factor improvement remains.

In study II, we used coronary computed tomography angiography to compare the coronary plaque burden and characteristics between 88 first-degree relatives of patients with early-onset CAD and 88 controls with no familial predisposition. Relatives had a significantly increased coronary plaque burden, which displayed characteristics associated with myocardial ischemia and adverse coronary events.

In study III, 134 patients with early-onset CAD, a cohort of 446 late-onset CAD patients (onset >55/65 years in males/females), and 89 healthy controls were genotyped for 45 common genetic risk variants and a genetic risk score was calculated as a measure of the polygenetic burden. Early-onset CAD patients had a modestly increased genetic burden compared with late-onset CAD patients and healthy controls; however, the burden did not associate with familial clustering of CAD. Additionally, familial clustering seemed to be stronger associated with CAD disease severity than the polygenetic burden.

Our findings emphasize the hereditary component of coronary atherosclerosis and underpin the need for risk factor optimization in early-onset CAD. Furthermore, our data support that yet identified common risk variants may have little clinical relevance in the clinical setting of early-onset CAD.

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