ASSESSMENT OF CORONARY ARTERY CALCIUM AND ITS ASSOCIATIONS WITH FAMILIAL AND NON-FAMILIAL CARDIOVASCULAR RISK FACTORS

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This thesis is presented in partial fulfilment of the requirements for the degree of Master of Clinical Research of The University of Western Australia

Medical School
Faculty of Health and Medical Sciences

2019
I, Cristian Vargas García, certify that:

The context of this thesis has been undertaken during enrolment for the Masters degree.

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Written patient consent has been received and archived for the research involving patient data reported in this thesis, where relevant.

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Technical assistance was kindly provided by administrative personnel from Calvary Lenah Valley Hospital for arranging clinical records access and contacting participants that are described in Chapters three and six; by Jacqueline Ryan NP for contacting and consenting participants from CAUGHT-CAD Heart Study (Royal Perth Hospital) that is described in Chapter four; and by Dr Jing Pang for extracting data of patients from the Lipid Disorders Clinic at Royal Perth Hospital that is described in Chapters four and five.

This thesis contains work prepared for publication, some of which has been co-authored.

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ABSTRACT

**Background:** Coronary artery disease (CAD) is the leading cause of death worldwide. Early detection of individuals with coronary atherosclerosis is fundamental for effective prevention of CAD. Cardiovascular risk assessment is usually performed using population-based algorithms which employ traditional risk factors. However, these methods of assessment do not adequately identify most of individuals who experience a coronary event.

Familial hypercholesterolaemia (FH), which is not included in standard risk assessment methods, is the most prevalent and serious monogenic disorder of lipid metabolism that leads to premature CAD. In an era of precision medicine, the estimation of coronary artery calcium score (CACS) employing cardiac computerised tomography (CT) scan, has emerged as the most direct and reliable approach to cardiovascular disease (CVD) risk assessment.

**Aims:** This thesis had four principal aims: *first*, to evaluate the association between traditional cardiovascular risk factors, employing risk factor counting and absolute risk score, and the presence of coronary artery calcium (CAC); *second*, to compare CACS between asymptomatic subjects with and without phenotypic FH; *third*, to compare CACS between asymptomatic patients with phenotypic FH with and without a pathogenic mutation affecting the low-density lipoprotein (LDL) receptor pathway; *fourth*, to evaluate the level of understanding and perception of patients who underwent estimation of CACS.

**Methods:** The study designs were of an observational nature: Cross-sectional designs for aims one and four and age-matched case-control designs for aims two and three. Subjects without symptomatic CAD were studied. CACS was estimated as Agatston units (AU), employing non-contrast cardiac CT scanning. The presence of coronary atherosclerosis was defined as CACS >0 AU. Statistical analyses were performed using STATA.
14.1. Continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test. Continuous variables with normal distribution were expressed as means and standard deviations; non-normal variables were reported as medians and interquartile ranges (IQR). Categorical variables were expressed as percentages. Normally distributed continuous variables were compared with Student's t-test; Wilcoxon rank-sum and matched-pairs signed-rank tests were used to compare non-normally distributed variables. The frequencies of categorical variables were compared using Pearson's chi-square or Fisher's exact test. Logistic regression analyses were used to investigate the associations between CACS and cardiovascular risk factors. Significance was defined at the 5% level.

**Results:** First, among 144 asymptomatic patients, increasing number of cardiovascular risk factors was a significant predictor of a CACS >0 AU (OR 2.2, 95% CI 1.4 - 3.5; \( p = 0.001 \)), an association that was independent of age and statin therapy. By contrast, the Australian absolute CVD risk score was not a significant predictor of a CACS >0 AU (OR 0.9, 95% CI 0.9 - 1.0; \( p = 0.792 \)). Second, median CACS was significantly higher in FH patients (\( n = 109 \)) compared with non-FH controls (21.0 AU [IQR 152.2] and 0.0 AU [IQR 13.1], respectively; \( p < 0.0001 \)); this association was independent of family history of premature CAD and other cardiovascular risk factors with exception of pre-statin plasma LDL-C concentrations. Third, among patients with a phenotypic diagnosis of FH, median CACS was significantly higher in mutation-positive patients (\( n = 99 \)) compared with mutation-negative controls (26.0 AU [IQR 115.0] and 0.5 AU [IQR 41.0], respectively; \( p = 0.029 \)); this association was independent of statin therapy, treated plasma lipid and lipoprotein concentrations and other cardiovascular risk factors. Fourth, 91 patients completed a survey. Over 96% were informed and understood the nature of the scan results and 85% considered that the test was important to their health. Patients with CACS of zero AU more frequently considered that the result did not influence their cholesterol treatment compared with those with a CACS >0 AU (32.1% and 14.3%, respectively; \( p = 0.048 \)).
Conclusions: The following general conclusions may be drawn from the foregoing studies. *First*, in middle-aged patients, a risk factor counting method may be a better predictor of coronary atherosclerosis than absolute risk assessment based on an Australian risk score. *Second*, among patients with FH, the presence of coronary atherosclerosis is determined primarily by the level of LDL-C, which is in turn dependent on the presence of a pathogenic mutation affecting the LDL receptor pathway. *Third*, there is a potentially high level of understanding and favourable perception of the estimation of CACS among patients in whom this test is employed to assess their risk of CAD. Further research is required to extend the above findings in relation to other sample populations, the evaluation of other cardiovascular risk assessment tools and other cardiovascular imaging methods, such as CTCA.
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<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>American College of Cardiology</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>APOB</td>
<td>gene encoding apolipoprotein B-100</td>
</tr>
<tr>
<td>ASCVD</td>
<td>atherosclerotic cardiovascular disease</td>
</tr>
<tr>
<td>AU</td>
<td>Agatston units</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CAC</td>
<td>coronary artery calcium</td>
</tr>
<tr>
<td>CACS</td>
<td>coronary artery calcium score (Agatston score)</td>
</tr>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>CAUGHT-CAD</td>
<td>coronary artery calcium score: use to guide management of hereditary coronary artery disease</td>
</tr>
<tr>
<td>CKD</td>
<td>chronic kidney disease</td>
</tr>
<tr>
<td>CSANZ</td>
<td>Cardiac Society of Australia and New Zealand</td>
</tr>
<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>CTCA</td>
<td>computed tomography coronary angiography</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>DLCN</td>
<td>Dutch Lipid Clinic Network</td>
</tr>
<tr>
<td>DLCNS</td>
<td>Dutch Lipid Clinic Network score</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>FH</td>
<td>familial hypercholesterolaemia</td>
</tr>
<tr>
<td>HDL-C</td>
<td>high-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HeFH</td>
<td>heterozygous familial hypercholesterolaemia</td>
</tr>
<tr>
<td>HoFH</td>
<td>homozygous familial hypercholesterolaemia</td>
</tr>
<tr>
<td>HREC</td>
<td>Human Research Ethics Committee</td>
</tr>
<tr>
<td>IDL</td>
<td>intermediate-density lipoprotein</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>LDL-C</td>
<td>low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>LDLR</td>
<td>gene encoding low-density lipoprotein receptor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
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<td>------------</td>
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<tr>
<td>Lp(a)</td>
<td>lipoprotein(a)</td>
</tr>
<tr>
<td>MEDPED</td>
<td>make early diagnosis to prevent early death</td>
</tr>
<tr>
<td>MESA</td>
<td>Multi-Ethnic Study of Atherosclerosis</td>
</tr>
<tr>
<td>MLPA</td>
<td>multiplex ligation-dependent probe amplification</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
</tr>
<tr>
<td>NVDPA</td>
<td>National Vascular Disease Prevention Alliance</td>
</tr>
<tr>
<td>OMIM</td>
<td>online Mendelian Inheritance in man</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PCSK9</td>
<td>gene encoding proprotein convertase subtilisin/kexin type 9</td>
</tr>
<tr>
<td>ROC</td>
<td>receiver operating characteristic</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SIFT</td>
<td>scale-invariant feature transform</td>
</tr>
<tr>
<td>TC</td>
<td>total cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>triglyceride</td>
</tr>
<tr>
<td>UCL</td>
<td>University College London</td>
</tr>
<tr>
<td>UWA</td>
<td>University of Western Australia</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low-density lipoprotein</td>
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ABSTRACTS AND PRESENTATIONS ARISING FROM THIS THESIS

Published Abstracts


Presentations at Scientific Meetings

Predictors of a coronary artery calcium score of zero in patients with familial hypercholesterolaemia. C. Vargas-García, J. Pang, GF. Watts. 67th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, 8 - 11 August 2019, Adelaide, Australia.

Precision medicine for assessing coronary artery disease risk in asymptomatic women with familial hypercholesterolaemia. C. Vargas-García, J. Pang, GF. Watts. 66th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, 2 - 5 August 2018, Brisbane, Australia.


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AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains work that has been prepared for publication.

Details of the work: A cross-sectional study including consecutive asymptomatic patients referred to a private coronary risk clinic in Hobart, Tasmania, in whom a cardiac CT scan for CACS was performed as part of the investigations. The aim was to test if the traditional cardiovascular risk factor counting determined the presence of CAC

Location in thesis: Chapter three

Student contribution to work: Literature review, study design, ethics application including UWA recognition, development of the database, data collection from clinical records in site, telephone contact with General Practitioners when data were inaccurate or incomplete, data cleaning, statistical analyses, results interpretation, chapter writing, and presentation of the project in an international conference

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Details of the work: A case-control study of subjects with phenotypic FH (cases) from the Lipid Disorders Clinic at Royal Perth Hospital and subjects with a family history of CAD without FH (controls) from the CAUGHT-CAD study. The aim was to compare the CACS between aged-matched asymptomatic subjects with and without phenotypic FH

Location in thesis: Chapter four

Student contribution to work: Literature review, study design, ethics applications, development of the database, manual data collection from CAUGHT-CAD baseline physical registries, data collection from Royal Perth Hospital clinical records (most of the data from Lipid Disorders Clinic were already available), data combination into a single database, data cleaning, statistical analyses, results interpretation, and chapter writing

Co-author signatures and dates:

Jacqueline Ryan NP
01/05/2019
<table>
<thead>
<tr>
<th>Details of the work: A case-control study of unrelated patients with phenotypic FH with (cases) and without (controls) a pathogenic mutation affecting the LDL receptor pathway from the Lipid Disorders Clinic at Royal Perth Hospital. The aim was to determine if the CACS was greater in those with a mutation compared to those without a mutation</th>
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<td>Location in thesis: Chapter five</td>
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<tr>
<td>Student contribution to work: Literature review, study design, ethics applications, development of the database, data collection from Royal Perth Hospital clinical records (most of the data from Lipid Disorders Clinic were already available), data migration into the database, data cleaning, statistical analyses, results interpretation, and chapter writing</td>
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<tr>
<th>Details of the work: A cross-sectional study including asymptomatic patients referred to a private coronary risk clinic in Hobart, Tasmania, in whom a survey questionnaire was applied following a cardiac CT scan for CACS. The aim was to determine if the patients had a favourable perception of the investigation and test results</th>
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<td>Location in thesis: Chapter six</td>
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<td>Student contribution to work: Literature review, study design, ethics application including UWA recognition, development of the database, survey development, data collection from physical questionnaires, data cleaning, statistical analyses, results interpretation, and chapter writing</td>
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<td>Doctor Jing Pang 01/05/2019</td>
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</table>
I, Winthrop Professor Gerald F. Watts, certify that the student statements regarding their contribution to each of the works listed above are correct.

Coordinating supervisor signature:

Date: 01/05/2019
STATEMENT OF PERSONAL CONTRIBUTION

My direct involvement in the studies presented in this thesis is as below:

Obtaining ethics approval from Calvary Lenah Valley Hospital, Royal Perth Hospital and The University of Western Australia
Review of the literature
Development of databases
Data collection
Monitoring and entering of data into databases
Data cleaning
Statistical analyses
Interpretation of results
Writing of the text of the thesis
Chapter One: Literature Review
1.1 Introduction

Atherosclerosis is a chronic inflammatory disease of the arteries and is the main underlying cause of CVD. ASCVD, resulting in CAD, stroke and peripheral arterial disease, is the leading cause of death and disability worldwide (1). For the past 25 years, fatalities due to CAD increased by 41.7% (2). This disease has long been Australia's leading cause of death. In 2017, it was the principal cause of decease, accounting for 12% of all deaths (3). The progressive development of atherosclerosis, the fundamental pathology of CAD, begins early in life (4,5), in some cases before birth (6), with a prolonged latency period before the initial manifestation (7). However, 50% of the sudden deaths in men and 64% in women occur in persons without prior CAD manifestations (8). With this in mind, early identification of individuals at increased risk of CAD is fundamental to effective prevention of the disease.

In spite of significant technological advances, the use of methods for assessment of subclinical coronary atherosclerosis is not yet routinely incorporated in current guidelines or clinical practice. Cardiovascular risk assessment in usual practice is based on the use of population-based cardiovascular risk tools, such as the Australian absolute CVD risk calculator (9) and the Framingham risk score (10). A major limitation of current absolute CVD risk assessment tools, based on traditional cardiovascular risk factors, is that they do not adequately identify approximately 75% of asymptomatic subjects who experience a coronary event (11).

Familial hypercholesterolaemia is the most prevalent and serious monogenic disorder of lipid metabolism that substantially and independently increases the risk of premature CAD (12–14). This disorder affects more than 30 million people worldwide and most cases remain undetected or undertreated (15–17), leading to a high burden of subclinical atherosclerosis from a very early age. However, in spite of the higher risk compared with subjects without FH, the clinical progression of ASCVD in patients with FH is variable, with higher plasma LDL-C concentrations and other risk factors aggregating risk (15,18).
Although most of untreated patients with FH will have CAD events and early death, some will have those events very late or will not develop CAD (19,20). Therefore, inherited lipid disorders are highly suitable for the application of non-invasive imaging, assessing the carotid or the coronary arteries, in order to determine the presence and extent of subclinical atherosclerosis.

In an era of precision medicine, the most promising and relatively accessible technique, relevant to cardiovascular prevention, is cardiac CT scanning for CACS. The quantification of CACS is considered a useful and reliable technique that allows recognition of premature CAD, principally in asymptomatic subjects, compared with population-based CVD risk calculators (21). Epidemiological data demonstrate CACS as a more significant predictor of CAD events than traditional cardiovascular risk factors (22,23). There is limited information of the value of the non-invasive estimation of CACS for the assessment and management of patients referred to coronary prevention clinics, including those with FH. Furthermore, in spite of the wide recognition of the importance of patient-centred care, there is limited evidence regarding the level of understanding and perception of patients following a cardiac CT scan (24,25).

To address the above issues, the aim of this project is to evaluate the role of cardiac CT scanning for CACS in the assessment of subclinical coronary atherosclerosis burden in asymptomatic subjects with familial and non-familial cardiovascular risk factors. The findings of this investigation could improve risk stratification of patients considering that known environmental and genetic factors can only explain a small part of the variability in ASCVD risk (26). This may also be employed as a method for early identification and prompt treatment initiation, to personalise and guide the intensity of treatment, increase the adherence to therapy, and triage further investigations.
1.2 Atherosclerosis

1.2.1 Definition

Atherosclerosis is a systemic inflammatory process that produces disease in the arterial wall of the coronary, cerebral and peripheral arteries as well as the aorta. It has the potential of causing acute cardiovascular events triggered by atherosclerotic plaque rupture, which predominantly manifests as stroke and myocardial infarction. It is well accepted that atherosclerosis causes increased morbidity and mortality worldwide (27,28).

Atherosclerotic plaques, are irregular focal thickenings of the intima, the innermost tunica of an artery or vein (29). This process, results in lipid accumulation, extracellular matrix protein deposition and calcification of the large to medium size arteries, leading to decreased arterial elasticity (30,31). The development of atherosclerotic plaques involves inflammatory and immune processes, vascular endothelial and smooth muscle cells, and lipids, connective tissue and debris (32).

1.2.2 Epidemiology

Atherosclerosis can commence in childhood with the development of fatty streaks, a collection of lipid-laden cells under the endothelium. Plaques are prevalent in young subjects, rarely causing symptoms and may disappear or progress to atheroma (33). Several studies illustrate the frequency of atherosclerotic plaques in western populations and its evolution with age (32,34,35). In a necropsy study of 2876 individuals aged 15 to 34 years who died of non-cardiac causes, all of them had aortic fatty streaks (36). In another necropsy study of 760 individuals (25 to 34 years of age) who died from violent causes, advanced coronary atherosclerotic plaques were found in 2% and 0% of men and women between 15 and 19 years of age, and 20% and 8% of males and females aged 30 to 34 years (35). A study including 260 peri-renal aortic patches collected during organ transplantation, found that in the first three decades of life atherosclerotic plaques were characterised by intimal thickening and lipid accumulation. In the fourth, fifth
and sixth decades of life the atherosclerotic plaques were characterised by pathologic intimal thickening, fibroatheromas, ruptured lesions, healed ruptures, and fibrotic calcific plaques (37).

Globally, ASCVD claims 17.9 million lives each year, 1/3 prematurely under 70 years (1), representing 37% of death that are attributable to non-communicable diseases in this group of individuals (38). Fatalities due to CAD increased by 41.7% from 1990 to 2013 (2). In 2013, ASCVD deaths represented 31% of all global deaths (39); this number is steadily increasing and is expected to be around 24 million by 2030 (1). Approximately, 80% of all ASCVD deaths are from myocardial infarctions and strokes and 75% occur in low and middle-income countries (1). By contrast, age-adjusted CAD mortality has declined since the 1980s, principally in high-income nations (40).

These diseases also represent an important cause of fatality in Oceania. On average, one Australian dies as a result of ASCVD every 12 minutes and in 2017, the principal cause was CAD with 118,590 deaths (11.6% of all deaths) (3). Notably, 50% of the sudden deaths in men and 64% in women occur in persons without prior CAD manifestations, and 18% of myocardial infarctions in men and 24% in women present as sudden death (8). CAD has long been Australia’s leading cause of death, but death rates have been steadily declining over the past decade. The death rate from CAD has decreased by more than a third from 99.4 deaths per 100,000 people in 2008 to 59.3 per 100,000 in 2017, and the number of deaths has decreased by 21.9% (3).

Between 2014 and 2015, 4.2 million Australian adults (18.3%) reported having CVD, including 1.2 million people with stroke and CAD (3). Since not all coronary atherosclerosis progress to haemodynamically significant CAD, millions more Australian adults have subclinical CAD and are at risk of developing future coronary events.
By 2030, the total global cost of ASCVD is set to rise from approximately US$863 billion in 2010 to an astonishing US$1044 billion. ASCVD is responsible for 151,377 million DALYs, of which 62,587 million are due to CAD and 46,591 million to cerebrovascular disease (41,42), representing 10% of the global disease burden.

1.2.3 Histopathology

A comprehensive and graphic representation of the histopathology of human coronary atherosclerosis progression is depicted in Figures 1 and 2.

The first lesion, known as fatty streaks, is a focal thickening of the intima with a collection of foam cells, a type of macrophage containing free and esterified cholesterol derived from oxidised LDL-C (43). Furthermore, endothelial and vascular smooth muscle cells can also become foam cells (44). Some T cells can also be found in the fatty streaks (33). Smooth muscle cells, some of them arising from haematopoietic stem cells, can occupy the intima and proliferate (45). As the fatty streaks grow, more smooth muscle cells and extracellular lipid accumulate in the intima without the presence of necrosis, a stage known as pathological intimal thickening (46). At these initial stages, there are no calcific components (Figure 1A, B and C).

The next stage is fibroatheroma, considered to be the first advanced form of atherosclerosis (32,47). This lesion is characterised by plaques with a well-defined necrotic core constituted by dead foam cells (48). This core is encapsulated by a layer of fibrous connective tissue, thicker than the normal intima, and may be relatively acellular or rich in smooth muscle cells (49). As the plaque develops, the free to esterified cholesterol ratio increases (50,51). A high ratio of free cholesterol to phospholipids in cellular membrane is toxic to cells and induce cytotoxicity which contributes to foam cell necrosis (52). The covering of the core may eventually become thinned and micro-calculifications can be found at this stage (47) (Figure 1D and E).

There are small blood vessels that supply or drain the walls of larger arteries and veins known as vasa vasorum. Their functions are delivering nutrients
and oxygen as well as removing “waste” products (53,54). The vasa vasorum form a network of micro-vessels that originates mainly from the adventitial layer of large arteries. With the development and growth of atherosclerotic plaques, they acquire their own vasa vasorum, initiating from the adventitia through the media and into the thickened intima (55). These tiny blood vessels usually have an impaired basement membrane, are poorly stabilised by adjacent pericytes and have open endothelial junctions (56). This characteristic makes them prone to rupture, causing haemorrhage within the plaque (57–60). Intraplaque haemorrhage is an important causative aspect for the growth of the necrotic core, leading to accelerated plaque progression (61–64) and instability (65–67), leading to potential ischaemic vascular events (68–71).

Atherosclerotic plaques progress to advanced lesions that have a necrotic lipid-rich core and eventually to macrocalcifications (32) that can be identified on a non-contrast cardiac CT scan. An increase in plaque size leads to luminal loss. In general, the blood flow is severely compromised when the luminal narrowing is 70% or greater. A reduction in diameter of 80% or greater is enough to affect resting flow (72).

The absence of luminal loss in early plaques has been associated with compensatory enlargement of blood vessels, known as arterial remodelling (73). Early plaque development in human coronary arteries with compensatory local enlargement of vessel size known as positive remodelling (Glagov phenomenon). Consequently, luminal size is initially not affected by plaque growth. By contrast, negative remodelling implicates a local reduction of vessel size at the plaque site, contributing to luminal stenosis (74,75).

Advanced lesions may rupture, allowing contact of the contents of the necrotic core, causing a luminal thrombosis (Figures 1F and 2). The presence of eruptive calcific nodules represents a rare form of coronary thrombus. Acute rupture may progress to healing with resolution of the luminal occlusion (Figure 2). Acute cardiovascular events are typically
caused by erosion or rupture of plaques with the development of thrombosis as a consequence, even when the luminal stenosis is less than 50% (76–79). Plaque rupture and erosion can also be an asymptomatic process. Recurrent unnoticed ruptures and thrombosis, followed by lesion healing, may facilitate the progression of plaques, due to a greater atherosclerosis burden, stenosis and negative remodelling (80).
Figure 1. Histopathology of human coronary plaque progression, part 1 (47)

A) Intimal thickening is normal in all age groups and is characterised by smooth muscle cell accumulation within the intima. B) Fatty streak corresponds to accumulation of predominantly macrophages within the intima. C) Pathologic intimal thickening marks the first of the progressive lesions and denotes the accumulation of extracellular lipid with no necrosis. D) Fibroatheroma indicates an encapsulated necrotic core. E) The core may eventually become thinned (thin-cap fibroatheroma). F) This lesion may rupture, allowing contact of the contents of the necrotic core, causing a luminal thrombosis. EL indicates extracellular lipid; NC, necrotic core; FC, fibrous cap; and Th, thrombus.
Figure 2. Histopathology of human coronary plaque progression, part 2 (47)

The thrombus of a plaque erosion occurs in the absence of rupture and may overlie a substrate of pathologic intimal thickening (top left) or fibroatheroma (top right). Eruptive calcific nodules represent a rare form of coronary thrombus. Acute rupture may progress to healing (healed plaque rupture) with resolution of the luminal occlusion. Ca\(^{2+}\) indicates calcification; NC, necrotic core; FC, fibrous cap; and Th, thrombus.
1.2.4 Pathobiology and risk factors

The development of atherosclerotic lesions is a complex, multistep process that usually takes place over many years. The vascular endothelium is a monolayer of cells between the vessel lumen and the vascular smooth muscle cells. It is considered one of the largest organs that interact with virtually every system in the human body (81,82). Nitric oxide is a soluble gas synthetised from the amino acid L-arginine in endothelial cells. This substance has several biological properties that preserve haemostasis including maintenance of adequate vascular tone and organ blood perfusion (controlling vasoconstriction and vasodilation). It also regulates cell growth and inflammation, controls angiogenesis and preserves optimum vascular permeability. On top of this, it protects vessel from injuries from platelets and cells in blood, playing a critical role in the normal endothelial function (83).

Several factors, including those commonly associated as risk factors for atherosclerosis, reduce the release of nitric oxide into the arterial wall, contributing to the pathogenesis of atherosclerosis. These factors include dyslipidaemia, hypertension, smoking, DM, CKD, increasing age, male sex, physical inactivity, inflammation, genetic, and haemodynamic factors. The principal manifestation of endothelial dysfunction is a blunting in the vasodilator function, which is an early event in atherosclerosis linked to a loss of nitric oxide (84).

Dyslipidaemia: Lipid disorders are fundamental to the pathogenesis of atherosclerosis (85–90). An elevated concentration of cholesterol carried by circulating apolipoprotein B-containing lipoproteins (non-HDL-C and LDL-C, named atherogenic cholesterol) is a significant risk factor for atherosclerosis (86,87). Endothelial dysfunction is induced by oxidised LDL (91). Oxidised LDL facilitates foam cells uptake (92,93) and cholesterol accumulates in the foam cells and in the lipid core of plaques (94). This might initially be an adaptive response to prevent LDL-related endothelial dysfunction (95). Nevertheless, LDL-C accumulation leads to mitochondrial dysfunction, apoptosis and necrosis, with a consequential liberation of cytokines and
prothrombotic molecules (95). Epidemiologic studies have indicated an increasing incidence of atherosclerosis when plasma cholesterol concentrations were above 3.9 mmol/L (150 mg/dL) (96). Lowering plasma LDL-C by diet or using statins (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor), increases nitric oxide availability (97,98) and reduces cardiovascular events (99). The lower concentrations of plasma LDL-C are associated with lower rates of cardiovascular events (100).

Chylomicron remnants, the lipoproteins that carry dietary lipids in the blood, also induce macrophage foam cell formation and inflammation in the artery wall. Their effects may be modulated by the type of dietary fat in the particles (101). By contrast, with LDL, oxidation of chylomicron remnants inhibits their uptake and induction of lipid accumulation (102). Lp(a), which is a LDL covalently linked to apolipoprotein (a), promotes atherosclerosis (103–105). Lp(a) binding to macrophages can lead to foam cell development in atherosclerotic plaques (106). Elevated Lp(a) has been clinically associated with increased residual CAD events in studies such as JUPITER (107) and AIM-HIGH (108), suggesting that Lp(a) is an additional risk factor in patients receiving aggressive LDL-C lowering therapies.

By contrast, HDL-C has potentially atheroprotective properties (86) such as reverse cholesterol transport, maintenance of endothelial function and protection against thrombosis. Values >1.9 mmol/L (75 mg/dL) are related to a longevity syndrome and values >1.5 mmol/L (60 mg/dL), are considered a negative risk factor in the Framingham risk score (10). Nevertheless, there is no evidence of a reduction in cardiovascular events due to increased HDL-C concentrations (109–111). Elevated HDL-C concentrations through some genetic mechanisms are not associated with lower risk of CAD events (112).

**Hypertension:** High blood pressure is a major risk factor for atherosclerosis, principally in cerebral and coronary arteries (113–115) and is a manifestation and a cause of endothelial dysfunction (116). Hypertension and dyslipidaemia exert many similar effects on the arterial wall. The increase in oxidative stress, a mechanism common to both conditions, may activate
genes involved in generating an inflammatory response that, in the presence of high LDL-C, leads to the formation of atherosclerotic plaques (117). Hypertension increases arterial wall tension, leading to disturbed restoration processes and possible subsequent aneurysm development (31). Hypertensive arteries are thickened, and there may be increased smooth muscle cell mass and number and a larger deposition in connective tissue (118).

**Smoking:** Tobacco smoke is another major risk factor (113–115), having an impact in all phases of atherosclerosis development from endothelial dysfunction to acute clinical events, principally of thrombotic aetiology (119). Tobacco smoke harms endothelium dependent vasodilation, decreasing nitric oxide bioavailability (120,121). It is also associated with higher levels of inflammatory markers, including C-reactive protein, IL-6 and tumour necrosis factor alpha (122–125). Tobacco smoking reduces platelet sensitivity to nitric oxide (126,127), increases fibrinogen (128,129), reduces fibrinolysis (130), and favours LDL oxidation (131,132).

**Diabetes mellitus:** Individuals with hyperinsulinemia have a higher incidence of CAD events (133–139). DM and atherosclerosis share some pathological mechanisms, including elevation of cytokines, contributing to primary inflammation in both cases (140,141). Clinically, patients with type 2 DM have a 2- to 3-fold increased risk of CAD events (142,143). The increased risk conferred by DM is driven by accelerated development of pre-existing atherosclerotic plaques due to oxidative modification of LDL and glycation of matrix and artery wall proteins (144), accelerating endothelial injury.

**Chronic kidney disease:** CKD is associated with a higher risk of CVD due to traditional risk factors (defined in the Framingham study) and CKD related factors (145). Hence, patients with CKD, are among those in the highest CAD risk category. CVD mortality is 5-fold higher in patients undergoing dialysis compared with the general population (146). However, the early stages of the disease are also associated with higher risk of CAD events (147,148). Therefore, patients with symptomatic CAD should be screened for kidney
disease (147,149). A pro-inflammatory state and oxidative stress may be the primary mediators that explains the acceleration of atherosclerosis and the enormous burden of CVD in patients with CKD (150,151). The high prevalence of hypertension and endothelial dysfunction (152) could also explain the higher risk of developing CAD in this population. A reduction in kidney function is also strongly associated with a higher incidence of coronary artery calcification compared with the general population (153).

**Inflammation:** The presence of inflammation in atherosclerotic plaques has been described from the earliest histologic studies and is fundamental to the pathogenesis of atherosclerosis (154–157). Macrophages with oxidised LDL content liberate different inflammatory substances, cytokines and growth factors (85,158). Among the several molecules involved are: monocyte chemotactic protein 1 (159,160), intercellular adhesion molecule 1 (159), macrophage and granulocyte-macrophage colony stimulating factors (161,162), CD40 ligand, IL-1, IL-3, IL-6, IL-8, IL-18 (163–165), and tumour necrosis factor alpha (166–168). Targeting the above molecules has already shown therapeutic benefits. The recent CANTOS trial (169) using anti-inflammatory therapy with canakinumab, a human IL-1-beta neutralising monoclonal antibody, showed a significantly lower rate of recurrent cardiovascular events when compared to placebo, independent of cholesterol level lowering.

**Infection:** Long-lasting infections with Chlamyphila Pneumoniae (170), Cytomegalovirus (171–178), Coxsackie B virus (179), and Helicobacter Pylori (180–182), have been found to contribute to the development of atherosclerosis. There is also evidence indicating that Human Immunodeficiency Virus infection and the following inflammatory processes accelerate atherosclerosis development (183). Not only the type of pathogen is relevant, also the number of microorganisms to which subjects have been exposed, are reported to be a risk factor (184–187).

**Genetic factors:** Although CAD has long been recognised to be heritable (188), only recently the genetic influence on the development of
Atherosclerosis has aroused the interest of the scientific community (189). The first large-scale prospective study of twins to confirm an increased risk of early-onset CAD among highly related individuals, estimated a heritability of 50% for fatal CAD (190,191). A more recent study, quantified heritability using genome-wide methods similarly estimated the heritability of CAD between 40% and 50% (192). In the Framingham study, a family history of CVD in a first-degree relative was a strong predictor of CVD events (193).

It is unlikely that the multiple pathophysiological processes that occur in the development of atherosclerosis are derived from one or a small group of genes. In order to have a better understanding of the molecular mechanisms of atherosclerosis, two main genetic study methodologies, with the potential to find new genes, have been carried out. First, the candidate gene approach, where genes known to be involved in the atherosclerosis pathways are tested for their contribution in atherosclerotic progress (194–196). Second, genome-wide linkage studies, conducted to look for atherogenesis regulating quantitative trait loci (197). Detailed family-based studies including individuals with a predisposition to early onset CAD, typically executed using linkage analysis, have also contributed in the understanding atherosclerosis (189).

**Haemodynamic factors:** Atherosclerotic plaques are found more frequently at sites of bifurcations, bends and branches of coronary arteries (198), suggesting that low shear stress and turbulent blood flow play a role in the pathogenesis of atherosclerosis. Turbulent blood flow affects endothelial cell function (199), leading to alterations in their atheroprotective capacity (158). These alterations are related to the depletion of protective nitric oxide from the endothelial cells (117,200).

Having reviewed the pathogenesis of atherosclerosis, the following sections will address atherosclerosis in the context of the material in this thesis such as CAD comprising coronary artery calcification and cardiovascular disease risk factors including family history of premature CAD and FH.
1.3 Atherosclerotic cardiovascular disease

The morphology and pathogenesis of ASCVD were described earlier. Atherosclerosis manifests clinically in several ways such as myocardial infarction, stable or unstable angina, stroke, transient ischaemic attack, or peripheral arterial disease. The epidemiology of ASCVD was also reviewed earlier but it is important to highlight that CAD has long been Australia’s leading cause of death and disability (3).

1.3.1 Coronary artery disease

Coronary artery disease refers to “the pathological process of atherosclerosis affecting the coronary arteries. CAD involves a spectrum of diagnoses including angina pectoris, myocardial infarction, silent myocardial ischemia, and sudden death of cardiac origin” (201). Myocardial infarction occurs when the atheromatous process stops blood flow leading to death of myocardium. According to the Framingham study (202), the lifetime risk of CAD events for individuals aged 40 years is 49% for males and 32% for females. At the age of 70 years, the lifetime risk is 35% in men and 25% in women. The incidence increases with age, with women lagging behind men by 10 years. Although de novo presentation of CAD usually involves new symptoms, more than 50% of initial presentations are with a sudden catastrophic event such as infraction or sudden death (203). This characteristic has driven efforts to decrease the risk of subjects with asymptomatic atherosclerosis.

1.3.1.1 Coronary artery calcification

There is evidence of vascular calcification in primitive humans (204). Vascular calcifications have been traditionally accepted as an inevitable result of ageing and its development was considered a passive, degenerative and quiescent phenomenon, as a consequence of mechanisms similar to bone development (205,206).

Calcification does not occur in normal artery walls and is now understood to be an active, tightly regulated pathogenic process that is avoidable (207),
stimulated by inflammatory pathways, typical of the systemic inflammation of DM and metabolic syndrome (208). As atherosclerotic plaque progresses, it may form lipid collections, fibrous tissue and calcium at later stages (32). CAC, defined as the pathological deposition of mineral in the coronary artery wall, predominantly in the intima, is a highly specific feature of coronary atherosclerosis (207). In the initial phases of CAC development, inflammatory cytokines have been shown to activate osteogenic differentiation and mineralisation of vascular cells, leading to ectopic bone production, a common feature of atherosclerosis and the basis for CAC (209). Advanced calcification is associated with increased mineralisation and a reduction in macrophage presence (210).

1.3.1.1.1 Factors associated with coronary artery calcification

High plasma glucose concentrations can directly stimulate calcification in vascular cells (211). By contrast, insulin may be able to inhibit it (212). Adipose derived factors also influence mineralisation, including leptin which is a stimulant (213) and adiponectin which is an inhibitor (214) of blood vessel walls calcification. Calcium intake from diet and supplements also plays a role in this process. A high total calcium intake has been associated with a decreased risk of incidental coronary atherosclerosis over 10-year follow-up, particularly among non-supplement users. Nevertheless, calcium supplement use has been independently associated with incident CAC, whether or not it is adjusted for total calcium intake (215). Clinically, traditional cardiovascular risk factors have been associated with CAC, particularly age, plasma TC concentrations and smoking (216). Elevated Lp(a) concentrations are also associated with higher CAC in asymptomatic individuals with a family history of premature CAD (217). The number of cardiovascular risk factors has also been associated with increased coronary calcification (218).

1.3.1.1.2 Clinical implications of coronary artery calcification

The calcification of coronary arteries has been highly associated with atherosclerotic plaque burden in a large study including histological assessment of 723 coronary artery segments (219). The clinical implication
of the above observation has been evaluated in previous studies and have shown a higher prevalence of CAC in subjects with established forms of CAD (220,221), with a greater resistance to regression with anti-atherosclerotic therapeutics (222). Nevertheless, a more recent study (223) suggested that not all coronary artery calcifications denote a corresponding cardiovascular risk, indicating that the extent and morphology of calcific atherosclerotic plaques should be considered. Calcific dominant plaques might be more stable. Mixed plaques composed of calcific and non-calcific components may be more vulnerable to rupture.

Statin therapy is recommended for patients at high risk of or with established clinical CAD owing to its ability to reduce circulating LDL-C concentrations and future cardiovascular events (224). Previous studies including serial intracoronary imaging for assessing the effect of statin use on coronary artery plaques, have shown favourable morphological modifications involving substantial regression of total plaque volume and lipid composition (225–230). Statin therapy also has an impact on CAC. A meta-analysis including four studies (231–234) revealed a significant increase in the calcific plaque volume in patients on statin therapy (12.0%) compared with controls (3.5%). Two additional studies (232,233) showed no significant difference in calcific volume plaque progression in statin users versus controls. Another meta-analysis of four studies (231,233,235,236) evaluating modifications in calcium density signal after statin use, indicated a significant increase by 22 HU (a quantitative scale for describing radiodensity). Coronary artery calcifications, as assessed by cardiac CT scanning, are also accelerated by statin therapy (237–240). Consequently, the progression of CAC should be interpreted prudently, particularly in individuals who are receiving statin therapy.
1.4 Cardiovascular disease risk factors

In 1961, the term “risk factor” was conceived from the recognition of a set of predictors of the development of CVD in the Framingham study (241). The concept of CVD risk factors is a fundamental part of modern healthcare. In landmark work, Kannel et al. determined that hypercholesterolaemia, hypertension, smoking, and ECG abnormalities were associated with a greater risk of CAD events over a six-year follow-up period. After nearly 60 years of the publication, those findings are still valid, though several additional modifiable (DM and obesity) and non-modifiable cardiovascular risk factors (age [males ≥45 years or females ≥55 years], male sex, ethnic group, and family history of premature CAD) have been recognised (242).

There are recent discussions regarding the definition of a normal CVD risk factor, particularly among those that are continuous, owing to the high potential for misunderstanding. Usually, the most common expression of a risk factor in the community is defined as normal. However, the “new normal” has been proposed as optimal CVD risk factor, defined according to the lowest risk and greatest potential for healthy longevity (243). This is in line with the concept of ideal cardiovascular health defined by the AHA that notably takes into account health related behaviours. This is defined as the absence of clinical CVD and the presence of four favourable health behaviours (no smoking, normal BMI, physical activity at goal levels, and healthy diet) and three favourable health factors (TC <5.2 mmol/L, blood pressure <120/80 mmHg and fasting blood glucose <5.5 mmol/L) (244).

Long recognised to “run in families”, CAD has been linked to approximately 60 genetic loci through common variant associations studies (189). Previous studies including linkage analysis of families with a predisposition to premature CAD, provided the first opportunity to gain insight into monogenic drivers of CAD. A inherited pattern of elevated plasma LDL-C concentrations and premature CAD, now known as FH, was first described among six patients with xanthoma in 1938 (245) and will be described in detail in the 1.4.2 section. Large-scale and functional studies have enabled a better
understanding of causal risk factors, elucidated fundamental biology and the development of new therapeutics (189).

Using a genome-wide association study, the first genetic risk variant was identified at chromosome 9p21. The 9p21 variant was the first risk factor recognised since 1964 (246). The variant is present in 75% of the population except for African Americans and is associated with a 25% increased risk of coronary events with 1 copy and a 50% increased risk with 2 copies. Notably, 9p21 is independent of all known risk factors, indicating there are unknown factors contributing to the pathogenesis of CAD. 9p21 also increases the risk by 2-fold in individuals with premature CAD, similar to that of smoking and cholesterol (247–249). The future may see genetic testing allow precision medicine methods by identifying patients at greater risk of CAD or those in whom a therapeutic or preventive method would be most beneficial.

1.4.1 Family history of premature coronary artery disease

Previous epidemiological studies (193,250) have shown that the lifetime risk of CAD is increased in relatives of patients with premature CAD. In the Framingham study (193), the risk of developing CAD was doubled in individuals with a family history of premature CAD. The MESA cohort (250) included asymptomatic subjects with a mean age of 62 years and showed that the odds ratio for CACS >0 versus without a family history of premature CAD was 1.94 after adjusting for age, sex and ethnic group.

In spite of the evidence, family history has not been incorporated in the majority of the absolute CVD risk calculators (251), with some exceptions such as the Reynolds risk score (252). There are some reasons for not including family history. First, scores have focussed on mid-term risk (five to 10 years) and family history mainly has an impact on lifetime risk (253). Second, the emphasis of algorithm developers has been on conventional cardiovascular risk factors such as hypercholesterolaemia, DM, hypertension, and tobacco use, found in 80% of patients after a CAD event (254). Third, in a population setting, the reclassification of risk including
family history has been shown to be marginal (255), with environmental risk factors compensating the weight of genetics in the models (114). Finally, the information about families is not often available and may be inaccurate. Nevertheless, the most recent ACC/AHA guideline on the assessment of cardiovascular risk (256), recommends family history as a helpful extra risk marker, suggesting that in subjects classified as low-risk by population-based risk calculators, the independent effect of the family history could be most relevant (193).

1.4.2 Familial hypercholesterolaemia

1.4.2.1 Introduction

Familial hypercholesterolaemia is one of the most prevalent inherited disorders in humans and the most common and serious form of inherited hypercholesterolaemia (257). This disorder is present from birth and is characterised by severely elevated plasma LDL-C concentrations, typically >95th percentile adjusted for age and sex (258). Patients may additionally have xanthomas, which progressively grow typically in Achilles and extensor tendons of the hand or at other body locations (259,260). The lifetime exposure to high LDL-C concentrations accelerates all forms of ASCVD (259,261,262), particularly CAD, by one to four decades (257,263,264). The untreated disorder is characterised by a high burden of subclinical atherosclerosis from an early age (265,266). Therefore, FH is highly suitable for the application of non-invasive imaging, either to assess the carotids or the coronary arteries, in order to determine the presence and extent of subclinical atherosclerosis (267).

1.4.2.2 History

The first documented observation associated to hypercholesterolaemia was made in the 19th century. The first description of the relationship between high plasma cholesterol concentrations and tendinous xanthomas was made by Schmidt (268). In 1938, observations regarding the presence of xanthomas, hypercholesterolaemia and premature CAD in six family
members, were published by Thannhauser (245) and Muller (269). In the 1960s, the dominant pattern of inheritance of FH was recognised (270). During the next decade, Goldstein and Brown (Nobel Prize winners) developed extensive research that resulted in the discovery of the LDL receptor and the evidence that FH was caused by a genetic mutation in a gene encoding a protein of this receptor, leading to reduced LDL-C catabolism and hypercholesterolaemia (271,272). A deletion in the gene encoding the LDL receptor, was identified in a patient with FH and in his mother in 1985. This was the first family-based study to recognise a discrete mutation in a single gene predisposing to premature CAD (273). This was due to substantially increased plasma cholesterol concentrations secondary to a decrease in the hepatic uptake of LDL-C. Other family-based studies also recognised a familial defective mutation in the APOB gene as well as a gain of function mutation in the PCSK9 gene as additional causes of FH. The first mutation prevents the binding of LDL-C to LDL receptor for uptake and the latter promotes LDL receptor catabolism (274,275).

1.4.2.3 Diagnostic definitions

Numerous clinical definitions of FH had been proposed in the literature. Some involve genetic testing while others demand very high concentrations of LDL-C with or without other clinical characteristics. FH is a genetic disease that is present in all racial and ethnic groups. This disorder is recognised by the family and personal history, physical features and biochemical analyses in the majority of subjects (15,18,262). There are two forms of FH: HeFH and HoFH (Table 1). HeFH is the most common FH phenotype and is inherited with an autosomal dominant pattern and is less severe than the homozygous phenotype. HoFH, is rare and is caused by either two identical mutations in each allele (true homozygous), two different mutations in the same gene (compound heterozygotes) or two mutations in two different genes (double heterozygous). HoFH also includes the rare autosomal recessive type. This results in a severely reduced ability of the liver to capture and internalise plasma LDL-C particles (276). It is important to consider that other mutations may also be present.
When genetic testing is not available, FH is defined based on clinical criteria. The phenotype is characterised by very elevated serum concentrations of LDL-C which untreated might accumulate in large and medium sized arteries conferring a marked increased risk of premature CAD (15,277), family history of hypercholesterolaemia or premature CVD and the presence of tendinous xanthomas or arcus cornealis.
## Table 1. Familial hypercholesterolaemia diagnostic categories

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical criteria</th>
<th>With genetic testing performed</th>
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<tbody>
<tr>
<td><strong>HeFH</strong></td>
<td>LDL-C ≥160 mg/dL (4 mmol/L) for children and ≥190 mg/dL (5 mmol/L) for adults and with one first-degree relative similarly affected or with premature CAD or with positive genetic testing for an LDL-C-raising gene defect (LDLR, APOB, or PCSK9)</td>
<td>Presence of one abnormal LDL-C-raising (LDLR, APOB or PCSK9) gene defect Diagnosed as HeFH if LDL-C-raising defect positive and LDL-C &lt;160 mg/dL (4 mmol/L) Occasionally, heterozygotes will have LDL-C &gt;400 mg/dL (10 mmol/L); they should be treated similarly to homozygotes Presence of both abnormal LDL-C-raising (LDLR, APOB or PCSK9) gene defect(s) and LDL-C lowering gene variant(s) with LDL-C &lt;160 mg/dL (4 mmol/L)</td>
</tr>
<tr>
<td><strong>HoFH</strong></td>
<td>LDL-C ≥400 mg/dL (10 mmol/L) and one or both parents having clinically diagnosed FH, positive genetic testing for an LDL-C-raising (LDLR, APOB, or PCSK9) gene defect, or autosomal-recessive FH If LDL-C &gt;560 mg/dL (14 mmol/L) or LDL-C &gt;400 mg/dL (10 mmol/L) with aortic valve disease or xanthoma at &lt;20 years of age, homozygous FH highly likely</td>
<td>Presence of two identical mutations in each allele (true homozygous), two different mutations in the same gene (compound heterozygous) or two mutations in two different genes (double heterozygous); includes the rare autosomal recessive type Occasionally, homozygotes will have LDL-C &lt;400 mg/dL (10 mmol/L)</td>
</tr>
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Adapted from Gidding et al. (277).

HeFH indicates heterozygous familial hypercholesterolaemia; HoFH, homozygous familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; LDLR, gene encoding low-density lipoprotein receptor; APOB, gene encoding apolipoprotein B, and PCSK9, gene encoding proprotein convertase subtilisin/kexin type 9.
1.4.2.3.1 Diagnostic criteria

The systematic detection of index cases is of high relevance in the integral care of FH (259,278,279). An “index case” is the first person diagnosed with FH in a family. Their identification is important because it is the first step for family tracing, better known as “cascade screening”, through which FH cases are most efficiently detected (263,280–286). Although FH is a genetic disease, there are several diagnostic tools for clinical application. Consistent application of one of those tools improved documentation of index cases in the Netherlands, where it resulted in the identification of 71% of projected cases (15). However, there is no international consensus for the phenotypic diagnosis of FH.

There are three major diagnostic criteria that predict a pathogenic mutation affecting the LDL receptor pathway with high sensitivity and specificity (287):

1. Dutch Lipid Clinic Network (264) (Table 2).
2. Simon Broome Register (288) (Table 3).
3. United States make early diagnosis to prevent early death (289) (Table 4).

In the Netherlands, the DLCN criteria were developed as part of a public health strategy to genetically identify individuals with FH, initiate early treatment and prevent CAD (283). Those criteria include similar features to the Simon Broome criteria, but add the calculation of a numeric score. This has been widely used in recent studies owing to their simplicity for clinical use and the numerically integrated scoring system (259,290). A diagnosis is considered “definite” if the score is >8, “probable” when it is between 6 and 8 points, “possible” if the score is between 3 and 5 points, and when the score is <3 points, the diagnosis is “unlikely”.

The principal difference between the Simon Broome and the DLCN criteria is the requirement of the first for tendinous xanthoma to be present for a “definite” diagnosis to be made (when a mutation has not been identified or DNA testing is not available). Dutch researchers have established that 83%
of the patients with a DLCNS ≥8 have a pathogenic mutation affecting the LDL receptor pathway. The remaining 17% have a clinical diagnosis, but in all of them, the presence of another mutation is highly probable (291,292).
Table 2. Dutch Lipid Clinic Network criteria for the diagnosis of familial hypercholesterolaemia

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family history (maximum 2 points)</strong></td>
<td></td>
</tr>
<tr>
<td>First-degree relative with known premature coronary and/or vascular disease (men aged &lt;55 years, women aged &lt;60 years) OR First-degree relative with known LDL-C &gt;95th percentile for age and gender</td>
<td>1</td>
</tr>
<tr>
<td>First-degree relative with tendinous xanthoma and/or arcus cornealis OR Children aged &lt;18 years with LDL-C &gt;95th percentile for age and gender</td>
<td>2</td>
</tr>
<tr>
<td><strong>Clinical history (maximum 2 points)</strong></td>
<td></td>
</tr>
<tr>
<td>Patients with premature coronary artery disease (men aged &lt;55 years, women aged &lt;60 years)</td>
<td>2</td>
</tr>
<tr>
<td>Patients with premature cerebral or peripheral vascular disease (men aged &lt;55 years, women aged &lt;60 years)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Physical examination (maximum 6 points)</strong></td>
<td></td>
</tr>
<tr>
<td>Tendinous xanthoma</td>
<td>6</td>
</tr>
<tr>
<td>Arcus cornealis before 45 years of age</td>
<td>4</td>
</tr>
<tr>
<td><strong>Laboratory analysis</strong></td>
<td></td>
</tr>
<tr>
<td>Untreated plasma LDL-C (mmol/L)</td>
<td></td>
</tr>
<tr>
<td>LDL-C ≥8.5</td>
<td>8</td>
</tr>
<tr>
<td>LDL-C 6.5 - 8.4</td>
<td>5</td>
</tr>
<tr>
<td>LDL-C 5.0 - 6.4</td>
<td>3</td>
</tr>
<tr>
<td>LDL-C 4.0 - 4.9</td>
<td>1</td>
</tr>
<tr>
<td><strong>DNA testing</strong></td>
<td></td>
</tr>
<tr>
<td>Functional mutation in the LDLR, APOB or PCSK9 gene</td>
<td>8</td>
</tr>
<tr>
<td><strong>Stratification</strong></td>
<td><strong>Total score</strong></td>
</tr>
<tr>
<td>Definite</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Probable</td>
<td>6 - 8</td>
</tr>
<tr>
<td>Possible</td>
<td>3 - 5</td>
</tr>
<tr>
<td>Unlikely</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

Adapted from Watts et al. (259).

LDL-C indicates low-density lipoprotein; DNA, deoxyribonucleic acid, LDLR, gene encoding low-density lipoprotein receptor, APOB, gene encoding apolipoprotein B-100, and PCSK9, gene encoding proprotein convertase subtilisin/kexin type 9.
The Simon Broome familial hyperlipidaemia register began in 1980 aiming to detect all FH patients in the United Kingdom, and resulting in the creation of the eponymous diagnostic criteria for FH (288,293). These criteria consider the differences in TC and LDL-C concentrations among adults and children. The criteria also take into account the dominant transmission and the age of onset of CAD in the family members. Using this approach, cases are categorised as “definite” and “possible”. In 1994, the evidence of an \textit{LDLR} mutation or familial defective \textit{APOB} was included as sufficient for “definite” diagnosis.

The existence of tendinous xanthomas (described later in the 1.4.2.3.2 section) is the main feature in the “definite” diagnosis of FH according to the Simon Broome criteria. In routine clinical practice in a United Kingdom specialist hospital lipid clinic, the mutation detection rate was 73% in patients classified as “definite” FH according to Simon Broome criteria, whilst for the patients classified as “possible” FH the rate was 27% (294). According to the above, the DLCN criteria predict a pathogenic mutation affecting the LDL receptor pathway with higher sensitivity compared to the Simon Broome criteria.

The United States MEDPED criteria were developed based on the clinical characteristics of families from the western state of Utah (289). The MEDPED tool, based solely on plasma TC and LDL-C concentrations, is less sensitive than the above criteria in predicting a pathogenic mutation. For this reason, a precise application demands that cholesterol concentrations be extensively known in family members (18,289).

In parallel, AHA has also proposed criteria for the clinical diagnosis of FH (295): LDL-C >190 mg/dL (>4.9 mmol/L) and either a first-degree relative with LDL-C >190 mg/dL or with known premature CAD (<55 years men and <60 years women). There is also a recent simplified Canadian diagnostic criteria of FH (296): the major criteria for a definite diagnosis are a genetic mutation affecting the LDL receptor pathway, the presence of tendinous xanthoma or a plasma LDL-C concentration ≥8.5 mmol/L.
Table 3. Simon Broome Registry criteria for the diagnosis of familial hypercholesterolaemia (260)

A. Definite diagnosis of FH requires

(a) TC >7.5 mmol/L (290 mg/dL) in adults or a TC >6.7 mmol/L (260 mg/dL) for children under 16 years of age

OR

LDL-C >4.9 mmol/L (190 mg/dL) in adults (4.0 mmol/L in children) (either pre-treatment or highest on treatment)

PLUS

(b) Tendinous xanthomas in patient or relative (parent, child, sibling, grandparent, aunt or uncle)

OR

(c) DNA-based evidence of an LDLR mutation or familial defective APOB

B. Possible FH is defined as (a) above plus one of (d) or (e)

(d) Family history of myocardial infarction before age 50 in grandparent, aunt, uncle or before age 60 in parent, sibling or child

(e) Family history of raised cholesterol in parent sibling or child, or level above 7.5 mmol/L (290 mg/dL) in grandparent, aunt or uncle

FH indicates familial hypercholesterolaemia; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol, DNA, deoxyribonucleic acid, LDLR, gene encoding low-density lipoprotein receptor, and APOB, gene encoding apolipoprotein B-100.

Table 4. MEDPED criteria for the diagnosis of familial hypercholesterolaemia (289)

<table>
<thead>
<tr>
<th>Age, y</th>
<th>TC and LDL-C (mmol/L) criteria for diagnosis probable heterozygous FH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st degree relative with FH TC (LDL-C)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>5.7 (4.0)</td>
</tr>
<tr>
<td>20 - 29</td>
<td>6.2 (4.4)</td>
</tr>
<tr>
<td>30 - 39</td>
<td>7.0 (4.9)</td>
</tr>
<tr>
<td>≥40</td>
<td>7.5 (5.3)</td>
</tr>
</tbody>
</table>

MEDPED indicates make early diagnosis to prevent early death; TC, total cholesterol; FH, familial hypercholesterolaemia; and LDL-C, low-density lipoprotein cholesterol.
1.4.2.3.2 Clinical features

Typically, the presence of tendinous xanthomas is considered pathognomonic for FH, but they can also occur in cerebrotendinous xanthomatosis, familial betasitosterolemia and in type III dysbetalipoproteinemia (297). The absence does not exclude the FH diagnosis, particularly in younger individuals. Xanthomas are commonly caused by a lipoprotein metabolism disorder and they are basically cholesterol deposits in tendons (298). Histopathologically, they are characterised by vacuolated macrophages in dermis filled with lipid droplets that are dissolved and removed during tissue processing. The lesions appear as slowly enlarging papules or subcutaneous nodules attached to tendons, ligaments, fascia, and periosteum. They are firm, painless and reddish-yellow in colour (Figure 3 B and C). Xanthomas are typically present in the extensor tendons of the hands and the Achilles tendons by age 45 in more than 70% of patients with FH (259,260). Inflammation of the tendons can also be present as Achilles tenosynovitis.

Arcus cornealis is a grey-white or yellowish opacification, 1 - 1.5 mm wide, located near the periphery of the cornea but separated from the limbic margin by a clear corneal zone 0.3 - 1 mm wide (299) called the lucid interval of Vogt (Figure 3 A). It is formed by a deposition of esterified cholesterol, the predominant lipid in LDL particles. The term “corneal ring” is not used because the lipid accumulation firstly forms arcs at the inferior, then superior poles of the cornea. Arcus cornealis is a sensitive clinical sign of FH, especially in patients younger than 50 years of age (300).

1.4.2.4 Epidemiology and cardiovascular risk

The prevalence of HeFH is estimated to be as high as 1 in every 250 individuals (15,301). The prevalence of compound HeFH and HoFH is between one in a million and 1 in 300,000 (302). The prevalence is higher in first-degree relatives of index cases compared with the general population. There is also a higher prevalence in populations known as having a “founder gene effect”, which includes Afrikaners (1:72 - 100), Ashkenazi Jews (1:67), Christian Lebanese (1:85), and Québécois (1:270) (257).
Figure 3. Typical physical signs of familial hypercholesterolaemia (259)
Patients with FH have an 8-fold greater risk of premature CAD compared with non-FH individuals (12–14). In some cases, particularly among those with a pathogenic mutation affecting the LDL receptor pathway, they can have even a 20-fold increased risk of premature CAD (303). Nevertheless, early diagnosis and treatment with cholesterol-lowering drugs reduces the risk of CAD to rates similar to the general population (304–307).

The concept of a cumulative LDL-C burden or cholesterol life-year illustrates the importance of early treatment. The cumulative LDL-C burden of a 55-year-old person without FH is typically 4.1 mmol/L (160 mg/dL), a burden sufficient for CAD to develop (308,309). For an individual with HeFH, this LDL-C burden is reached by age 35 if untreated, by age 48 if treatment is commenced at age 18 and by age 53 if treated by age 10. An untreated subject with HoFH will reach this level at age 12.5. Those patients, develop aggressive CAD at early ages and without treatment, they will generally pass away of acute coronary syndrome before age 30 (310).

FH is relatively common among patients with premature acute coronary syndrome (311,312). Under-diagnosis of FH is a global problem, with estimates of as many as 30 million people affected with FH. Less than one percent of cases are diagnosed in most countries (15,313,314) and suboptimal treatment is common worldwide (15,305,315). Early identification and therapy initiation in patients with FH can decrease the cumulative dose of plasma LDL-C necessary for developing symptomatic CAD (316,317).

Although it is mainly plasma LDL-C concentrations that drive ASCVD risk in patients with FH (318), there is a variation in risk of developing atherosclerosis and ASCVD (319,320). This variation is partially explained by the interactions among the many cholesterol raising and lowering genes that have implications for individuals and their offspring (321). The patients' underlying comorbidities and lifestyle factors also influence LDL-C concentrations and ASCVD risk (319). Stratification of patients with FH according to their individual cardiovascular risk factor burden may be helpful.
in identifying those who would benefit from more intense cholesterol-lowering therapy (322).

More recently, due to this variation in risk, the role of cardiac CT scanning for the assessment of coronary atherosclerosis has been evaluated in patients with FH. The principal studies to date are shown in Table 5. Compared with the general population, the subclinical coronary atherosclerotic burden by cardiac CT scanning is greater in FH patients (323,324). In middle-aged asymptomatic FH patients, the prevalence of CAC has been previously estimated between 48% and 55% (324–326). In spite of the intensive use of cholesterol-lowering therapy, CACS is significantly higher in FH patients compared with general populations (323,324,327). Coronary atherosclerosis is significant, on average, at age 23 and 34 years in male and female patients with HeFH, respectively (328).

There is also accelerated development of CAD in patients with FH despite intensive statin treatment (324,327–329). Moreover, the subclinical coronary atherosclerotic burden has been associated with future CAD events in Japanese FH patients (328) and has been independently associated with cardiovascular risk in a Spanish cohort (326).

1.4.2.5 Pathophysiology and genetics

Cholesterol is a lipid that is an essential structural component of all animal cell membranes and is the precursor of steroid hormones, bile acids and vitamin D. Lipids are transported in the plasma in the form of lipoproteins, which can be classified according to different properties including composition, size and density. Lipoprotein types, in order of increasing density are chylomicrons, chylomicron remnants, VLDL, IDL, LDL, and HDL. An additional lipoprotein called Lp(a), is a genetically determined atherogenic lipoprotein composed of an LDL particle containing apolipoprotein B-100 with a disulphide-linked apo(a) moiety (330–332).
Table 5. Studies including cardiac CT scanning in patients with familial hypercholesterolaemia

<table>
<thead>
<tr>
<th>First author (ref)</th>
<th>Year</th>
<th>n</th>
<th>Mean age</th>
<th>Women</th>
<th>CACS</th>
<th>CS</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miname (323)</td>
<td>2010</td>
<td>102</td>
<td>45 y</td>
<td>65%</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neefjes (329)</td>
<td>2011</td>
<td>101</td>
<td>53 y</td>
<td>36%</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Neefjes (327)</td>
<td>2011</td>
<td>140</td>
<td>52 y</td>
<td>39%</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ten Kate (333)</td>
<td>2013</td>
<td>145</td>
<td>52 y</td>
<td>36%</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Viladés Mendel (324)</td>
<td>2013</td>
<td>50</td>
<td>49 y</td>
<td>56%</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Clarke (334)</td>
<td>2013</td>
<td>204</td>
<td>55 y</td>
<td>53%</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tada (328)</td>
<td>2015</td>
<td>101</td>
<td>52 y</td>
<td>49%</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Galaska (335)</td>
<td>2016</td>
<td>89</td>
<td>50 y</td>
<td>37%</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gallo (325)</td>
<td>2017</td>
<td>112</td>
<td>45 y</td>
<td>50%</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pérez de Isla (326)</td>
<td>2018</td>
<td>404</td>
<td>46 y</td>
<td>52%</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Miname (336)</td>
<td>2018</td>
<td>206</td>
<td>45 y</td>
<td>63%</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CT indicates computed tomography; ref, bibliographic reference; CACS, coronary artery calcium score; CS, coronary stenosis assessment; and PC, plaque characteristics assessment.
The genetics of FH is depicted in Figure 4. FH is caused by dominant mutations of genes predominantly affecting the function of the LDL receptor in the hepatocytes that clears LDL-C particles from the plasma, with a consequent marked elevation in the concentrations of TC and LDL-C (259,262,337–339). The genetic basis for FH lies in multiple variants in at least three causative genes that have been identified. Most patients with FH carry a functional mutation of one of three genes: LDL receptor (LDLR, OMIM # 143890), apolipoprotein B-100 (APOB, OMIM #107730), and gain of function mutations of the proprotein convertase subtilisin/kexin type 9 gene (PCSK9, OMIM # 603776) (12,257,266,271,302,337,338,340–342). These three mutations are present in approximately 93%, 5% and 2% of cases, respectively (257). Patients who have a pathogenic mutation affecting the LDL receptor pathway have a 50% chance of inheriting the mutation to each of their descendants (262,343). Rarely, FH occurs in the absence of mutations from these genes. Although genetic sequencing identifies an FH mutation in only a small proportion of subjects with severe hypercholesterolaemia, for any given observed LDL-C concentration, risk for CAD is substantially higher in FH mutation carriers than in non-carriers. This is likely secondary to a higher lifelong exposure to atherogenic LDL particles (12).

Several protective variants and their therapeutic potential have also been identified. For example, genetic evidence suggesting that carriers of inactivating mutations in PCSK9 have decreased concentrations of plasma LDL-C and lower risk of CAD events led to the development of PCSK9 inhibitors (344). In the same way, oligonucleotides intended to mimic the protective mutations found in APOC3 or LPA genes that had been tested in early phase studies, showed a 70% reduction in TG concentrations and 80% reduction in circulating Lp(a) concentrations, respectively (345,346).
Figure 4. Genetics of familial hypercholesterolaemia (343)

A) Familial inheritance of heterozygous familial hypercholesterolaemia. Squares and circles represent male and female individuals, respectively. Genotypic inheritance of FH-causing mutations and their cosegregation with the HeFH phenotype are shown below each pedigree symbol. 50% of children of such a mating will have HeFH, which is usually fully expressed early in life. 50%, 25% and 12.5% of first-, second- and third-degree relatives of an affected individual will also have HeFH.

B) Main genes causing FH. Chromosomal location of the main genes causing dominant HeFH and their chromosomal location: LDLR encoding the LDL receptor, APOB encoding apolipoprotein B, PCSK9 encoding proprotein convertase subtilisin/kexin 9, and some very rare genes are not shown. Chr indicates chromosome; and HeFH, heterozygous familial hypercholesterolaemia.
1.4.2 Treatment

FH is characterised by an extremely elevated lifelong exposure to LDL-C and their atherogenic effects (347,348). Identification of young individuals with FH, in order to initiate permanent and aggressive management, is of key importance (18,258,262,325,343,347). All patients with a HoFH diagnosis should be referred to a lipid specialist with expertise in their management. Referral must be considered for those with HeFH who have not achieved their plasma LDL-C goal despite statin therapy and lifestyle modifications.

1.4.2.6.1 LDL-C reduction and targets

The primary goal of therapy in patients with FH is to significantly lower plasma LDL-C concentrations. This intense LDL-C lowering reduces progression of angiographically determined CAD (349) and decreases CAD events (304), CAD mortality (307) and all-cause mortality (304,306,307,349).

Owing to ethical reasons, all studies of the impact of cholesterol-lowering therapy on mortality in patients with FH have been observational. However, the findings are consistent with the results of randomised trials that included subjects without FH. Owing to this lack of evidence from clinical trials (350,351), there are no universal treatment targets for patients with FH (18,259,290,352–354). However, most of the international guidelines recommend that targets must be lower with increased CVD risk (259,290,343,353,355). The subsequent targets from various guidelines (18,259,358,290,343,352–357) are recommended in the integrated guidance from the International FH Foundation (262). In patients with HeFH (18,259,352,356), the initial treatment goal is a reduction in plasma LDL-C concentration of at least 50%, followed by a LDL-C <2.5 mmol/L (97 mg/dL) in those without CAD or other major cardiovascular risk factors, and <1.8 mmol/L (70 mg/dL) in patients with CAD or other risk factors (353,354). The LDL-C life-year exposure is recommended to be considered for guiding the initiation and intensity of treatment (359). It is important to know that reaching goals is challenging in a large proportion of patients (17,259,283). Hence, one method is to achieve the greatest possible reduction of LCL-C concentrations according to the tolerance to treatment, especially in those
with additional risk factors or with subclinical CAD demonstrated by non-invasive methods such as cardiac CT scanning for CACS. In patients with HoFH (259,276,310), the plasma LCL-C concentrations should be lowered as much as can be achieved.

### 1.4.2.6.2 Lifestyle modifications

All patients with FH should be counselled in lifestyle changes that may decrease LDL-C concentrations and other cardiovascular risk factors (18,259,262,271,290,343,352–354). A complete CVD risk assessment including measurement of blood pressure, BMI, smoking, glucose, and Lp(a) should be carried out (259,262,343).

Lowering the intake of cholesterol, saturated fat and trans-unsaturated fat contributes to improving the plasma lipid profile (360–362). Routine consumption of vegetables and fruits, whole grains, tree nuts, beans, low-fat dairy products, fish, and lean meats must be promoted (251). Alcohol intake should be moderated and any psychological stress should be managed (259,363).

Smoking must be completely avoided (354), including its passive form (364,365). It is recommended to identify smokers and provide repeated advice on quitting. If necessary, patients should be offered help with nicotine replacement products or drugs that modulate the intensity of nicotine withdrawal (366–368). Hypertension and diabetes should be managed according to guidelines (369,370). Regular aerobic exercise should be recommended. In those with insulin resistance and/or obesity, advice on an aerobic exercise routine and weight loss must be provided (259,360–362,371).

### 1.4.2.6.3 Pharmacologic therapy

Statins are the first-line option to treat hypercholesterolaemia in FH. Guidelines recommend that adults should be treated with the maximal tolerated dose of a high potency statin within six months of first consultation (18,259,262,352,355,356,358). In patients with FH, statin therapy decreases
the progression of coronary atherosclerosis (372), the risk of CAD events (265,304,306,307,373,374), and all-cause mortality (265). Adjuvant treatment with bile acid sequestrants, ezetimibe, plant sterols and stanols, and niacin can also be prescribed (375–378). In most cases, patients may require three or more drugs to achieve the recommended plasma LCL-C reduction (17,259,349). When there is also hypertriglyceridaemia, fenofibrate or omega-3 fish oils can be added to therapy (290,355,379).

Following the discovery of mutations in the PCSK9 gene that regulates LDL-C concentrations (316,340,341,344,380–382), two fully human monoclonal antibodies that inhibit PCSK9 have been recently developed and are currently being used to reduce LDL-C concentrations (383). Evolocumab, the first of this type of drug, reduces LDL-C concentrations by 60% (384–388). In the FOURIER trial (100), the inhibition of PCSK9 with evolocumab in patients on statin therapy, reduced the risk of cardiovascular events, suggesting that patients with ASCVD benefit from lowering plasma LDL-C concentrations below current targets. In patients with HeFH, there is a reported reduction in LDL-C concentration between 43% and 60% compared with placebo, independent of the type of mutation identified (388,389).

1.4.2.6.4 Lipoprotein apheresis

Lipoprotein apheresis is a safe and effective non-surgical technique that consists in the physical removal of lipoproteins from the blood. It requires the initial separation of plasma from blood cells with a cell separator. The plasma is then subject to affinity columns containing anti-apolipoprotein B antibodies or dextran sulphate, or their precipitation at low pH by heparin to remove apolipoprotein B-containing lipoproteins (390). This method used weekly or biweekly, decreases LDL and Lp(a) concentrations by approximately 60 - 70%. LDL apheresis should be contemplated in patients with HoFH or compound HeFH. It should also be considered in patients with HeFH and CAD who are refractory to or cannot tolerate cholesterol-lowering medications (15,259,391,392). When used concomitantly with statins and other cholesterol-lowering drugs, LDL apheresis may also induce the regression of coronary atherosclerotic plaques in FH patients (393).
1.5 Assessment of cardiovascular risk

Risk assessment aims to identify individuals likely to develop ASCVD and ultimately to reduce their risk of cardiovascular events. This prevention is defined as “a coordinated set of actions, at the population level or targeted at an individual, that are aimed at eliminating or minimising the impact of CVDs and their related disabilities” (394). Risk assessment initiates with a meticulous history and physical examination for the evaluation of traditional and non-traditional cardiovascular risk factors. As ASCVD is still the principal leading cause of morbidity and mortality worldwide, the prevention and identification of asymptomatic people at risk of presenting cardiovascular events is one of the most challenging concerns in preventive cardiology.

The use of a population-based cardiovascular risk scoring tools, such as the Australian absolute CVD risk calculator (9) and the Framingham risk score (10), should be employed to determine both absolute and lifetime CVD risk (256). Based on this estimation, evidence-based clinical practice guidelines can be used to define individuals who would most likely benefit from cholesterol-lowering and antiplatelet therapy and appropriate antihypertensive therapy. After determination of cardiovascular risk, a clinician-patient dialogue should take place in which risk data are reviewed, clinical practice guidelines are contemplated and potential side effects to therapy are discussed as part of a shared decision-making approach to care (395). For subjects in whom risk remains intermediate or uncertain, selective use of biomarkers, non-traditional cardiovascular risk factors, social determinants of health and non-invasive measures of subclinical atherosclerosis can be applied to further inform treatment decisions (396).

There is ample evidence that reducing cholesterol, quitting smoking, controlling hypertension, and in certain situations, treating DM, is related to a decreased occurrence of CAD events. As CAD is silent for years and even decades, this prevention period can last for a long time. In recent time, additional CVD risk factors such as a CACS ≥300 AU or ≥75th percentile for sex, age group and race, can be considered (397).
1.5.1 Absolute risk assessment

Traditional risk factors for CVD, have led to the development of risk prediction models and to major improvements in therapy. Nevertheless, 20% of patients with CAD have no traditional risk factors and 40% have only one (398). Non-traditional risk factors such as inherited hypercholesterolaemia, which includes FH and elevated Lp(a), are not included in standard risk assessment tools. As a consequence, individual CVD risk assessment is still inaccurate. The clinical risk scores do not provide confidence intervals, and consequently, may not reflect the within-patient uncertainty in risk (399). Even the most robust risk prediction models achieve a C-statistic (a measure of discrimination ability of a model also known as the area under the ROC curve) of 0.80. Hence, there is a 20% probability that the model is not capable of identifying patients likely to develop CAD (400).

In this context, it is essential to differentiate cardiovascular risk prediction at the individual level from that at the population level (401). The primary care professionals usually treat individual patients and not populations, therefore, to improve individual risk prediction, novel risk factors and more accurate tools may be required. Ultimately, improved prediction and clinical care of the individual patient, will eventually improve the health of the population.

Periodic risk assessment using CVD risk prediction models offers an initial point for office-based discussion and initiation of primordial and primary prevention strategies. Numerous multivariable risk models have advanced through several iterations. The original Framingham risk score was established in 1998 as a means to calculate CAD risk. This algorithm was refined by the third adult treatment panel in 2002 with a focus on hard CAD endpoints, death and non-fatal myocardial infraction (353). The 2008 Framingham risk score integrated the additional endpoints of stroke, heart failure and peripheral arterial disease (402).
Ideally, a CVD risk calculator for Australian population should be developed based on a large Australian cohort study including cardiovascular risk factors and the necessary number of cardiovascular events (403). When this data is not available, an algorithm based on other data is applied following recalibration, a method that adapts algorithms to the differences in the risk of the population target. Before the recalibration, the performance of CVD risk tools varies extensively. However, calibration nearly equalises the performance of the algorithms and improves targeting of preventing actions in clinical practice (404).

The Australian absolute CVD risk calculator (9), the instrument of the NVDPA national guidelines for the management of absolute CVD risk (405), is a recalibration of the Framingham algorithm (402). This algorithm consists of sex, age, systolic blood pressure, smoking status, TC, HDL-C, DM, and left ventricular hypertrophy (optional). The calculation results in the risk, expressed as a percentage, of developing CVD (heart, stroke or blood vessel disease) in the next five years in adults between 35 and 74 years of age.

Other multivariable risk models have been established; the QRISK calculator, the prospective cardiovascular Münster model (PROCAM), the European systematic coronary risk evaluation algorithm (SCORE), and the Reynolds risk score (RRS) are among the risk stratification algorithms included in international clinical practice guidelines (252,359,406–408). More modern recommendations use the 2013 ACC/AHA pooled cohort ASCVD risk score, resulting from numerous cohort studies: The Framingham cohorts, the coronary artery risk development in young adults (CARDIA), the cardiovascular health study (CHS), and the atherosclerosis risk in communities (ARIC) study (241,256,409–412). These algorithms diverge in the endpoints estimated (e.g. SCORE predicts only CV mortality) and predictor variables (e.g. SCORE does not have DM nor HDL-C as factors). Hence, the risk assessments using these other calculators are regularly different from the Framingham risk score.
Current guidelines based on typical CVD risk evaluation instruments, widely used in the present, stratify patients into low or high risk, with a group falling between the 2 categories (256). Nevertheless, subjects stratified between the low and high-risk groups, can have a highly variable CVD risk. This lack of clarity may cause difficulty for clinicians deciding between a conservative or more aggressive therapeutic approach (413).

The current ACC/AHA guidelines (256) recommend considering lifestyle and pharmacological interventions in patients with an estimated 10-year risk of CAD event greater than 7.5%. At that risk level, 92.5% of patients on treatment may not have a CAD event over the next decade, even if the treatment was suspended, probably suggesting that those patients may not necessarily benefit from treatment. Additionally, a systematic review has shown that 67 primary prevention patients with a five-year CAD risk of 5 to 10% need to be treated with statins to prevent one CAD event (414). Owing to these kinds of imprecisions, some experts have claimed the relocation of more funds to public health programs (415).

There are barriers of the typical CVD risk evaluation instruments. First, their incapacity to recognise 75% of asymptomatic subjects who will experience CAD episodes (11). Second, risk stratification by population-based CVD risk assessment tools underestimates future cardiac events. This supports the use of subclinical biomarkers, social determinants of health and non-invasive measures of subclinical atherosclerosis to extend the risk assessment for CAD, particularly among asymptomatic individuals (396).

The existing model for the treatment of CAD is to implement general prevention by concentrating on the modifiable CVD risk factors (hypertension, DM, smoking, obesity, and hypercholesterolaemia) and improving patients' lifestyle by recommending healthy nutrition, maintaining a normal body weight and to participate in routine aerobic exercise (416).
1.5.2 Risk factor counting

The use of a simple algorithm of counting cumulative cardiovascular risk factors is a predictor of clinical CAD events. In the Framingham study (417), participants were stratified according to their number of risk factors at age 50. Average lifetime risks were 51.7% for males and 39.2% for females. When risk factors were considered individually, they discreetly discriminated higher and lower lifetime risk for cardiovascular events. However, when considering the cumulative number of risk factors, there was a marked difference in remaining lifetime risk for CVD. Males and females with no risk factors at age 50 had a residual lifetime risk for CVD of 5% and 8%, respectively. In summary, the absence of cardiovascular risk factors at 50 years of age was associated with very low lifetime risk for CVD events and longer survival. By contrast, with an increase in the number of risk factors, the risk was considerably higher. For middle-aged men and women with two or more major traditional cardiovascular risk factors, the residual lifetime risks were significant, at 69% and 50%, respectively.

Notably, risk factor counting at 50 years of age stratified more than just residual lifetime risk for CAD. Cumulative risk factor counting was also associated with survival. Overall median survival at age 50 were 30 years for men and 36 years for women. However, median survival was >39 years for men and women with no risk factors, compared with only 28 years in men and 31 years in women with risk factors (417). These differences underline the importance of cumulative risk factor counting in middle-aged patients as a determinant of morbidity (e.g. CAD events) and longevity. Using the above, a hypothetical 50-year-old non-smoker, non-diabetic male with a TC of 6.2 mmol/L, HDL-C of 0.9 mmol/L and an untreated systolic blood pressure of 135 mmHg, would have a mean lifetime risk for CAD events of approximately 70% and a survival more than 11 years shorter compared to a 50-year-old man with no risk factors (417). The lifetime risk concept may encourage him to better improve risk factor prevention than solely informing him that over the next 10 years his projected risk of a CAD event is 8%, as predicted by the
Framingham risk score (10) and that over the next five years his risk is 7%, as predicted by the Australian absolute CVD risk calculator (9).

A large pooling study confirmed the above findings using the same risk factor counting approach among more than 45,000 subjects from 16 different US cohort studies and followed for 650,000 person-years for different endpoints, including death of cardiac origin, non-fatal myocardial infarction and stroke (418). Similarly, a study including more than 46,000 Chinese adults (419) showed that following adjustment for sex and age, the odds ratio of CVD gradually increased with the number of traditional cardiovascular risk factors, when compared with subjects with no risk factors.

A greater number of risk factors in middle-aged individuals has been associated with lower scores at older ages on appraisal of social performance, mental wellbeing, walking and health perception (420). Similarly, a greater number of risk factors in middle-aged subjects were related with a higher average annual total and CVD-related costs (421). There is also evidence that physician decision-making is more consistent with the management of individual risk factors than an absolute risk approach. To understand physicians’ use of individual risk factors versus absolute risk in CVD risk management decision-making, a randomised experiment including 144 Australian General Practitioners was performed (422). Absolute risk score, systolic blood pressure, cholesterol ratio (TC/HDL-C) and age were systematically varied in hypothetical cases and physicians indicated whether they would prescribe cholesterol and/or blood pressure lowering medication. Results showed that medical treatment of lower risk patients with mildly elevated blood pressure or cholesterol concentrations is likely to occur even when an absolute risk assessment is specifically provided.

The risk factor counting method is currently recommended for assessing risk of ASCVD by the National Lipid Association recommendations for patient-centred management of dyslipidaemia (423). This guideline defines five major CVD risk factors to be used in the risk classification: age, family history
of premature CAD, current smoking, hypertension, and low HDL-C. Patients with none or one major risk factor are categorised as low-risk, those with two risk factors as moderate risk, those with three or more risk factors as high risk, and those with ASCVD as very high risk. In this thesis, the risk factor counting method was based on the principles of these recommendations by the National Lipid Association.

1.5.3 Cardiac computed tomography and other imaging modalities

The progression of atherosclerosis in all its stages, assessed by different imaging techniques, is depicted in Figure 5. Imaging modalities for these stages should be selected based on the physiological changes expected at each stage. The following sections will address cardiac CT scanning including CACS measurement.

Cardiac imaging is rapidly evolving, with main developments in the diagnostic capabilities of non-invasive cardiac assessment (424). Cardiac CT scanning is one of the most used imaging techniques for cardiovascular risk assessment in asymptomatic individuals. In recent years, the use of cardiac CT scanning has increased owing to the diminution of ionising radiation dose, new technological developments, greater availability, and increased treatment possibilities. This technique provides a feasible approach for determining CAD risk. It is also a better risk prediction tool of CAD events than traditional cardiovascular risk factors (425–428).

Subjects classified as low-risk using absolute CVD risk score, frequently have subclinical coronary atherosclerosis, as assessed by cardiac CT scan (429). Notably, health decision-making based on absolute risk scores that do not include cardiac imaging could result in an inferior impact of approaches for reducing cholesterol (430).
Figure 5. The progression of atherosclerosis as assessed by different imaging modalities. (431)

Imaging modalities for these stages should be selected based on the physiological changes expected at each stage. CAC indicates coronary artery calcium; FDG-PET, 18-fluorodeoxyglucose positron emission tomography; FMD, flow-mediated dilatation; NaF, sodium fluoride; IVUS, intravascular ultrasound; PAT, peripheral arterial tonometry, small arteries; and PWV, pulse wave velocity, large arteries.
Computed tomography coronary angiography, quantified on ECG-gated CT scan, is currently used as a complementary technique for the analysis of coronary atherosclerosis allowing a less invasive (use of intravenous contrast) evaluation of atheromatous lesion morphology and characteristics. Numerous intravascular and histology-based imaging techniques studies have indicated that CTCA permits precise assessment of the lumen and coronary wall, evaluation of atherosclerotic plaque burden and remodelling pattern, as well as the classification of the components of the lesion (49,432–438).

Some studies have established that CTCA permits recognition of calcific atheromatous plaques with a restricted accuracy in distinguishing fat from fibrotic material (49,432,433,437,439). However, recent histological reports have revealed that CTCA, even with its limitations in resolution, permits a precise enough plaque description to detect vulnerable and high risk lesions (described as the napkin-ring radiological sign) with a low sensitivity but high specificity (440,441). CTCA has limited precision for assessing the structure and conformation of atheromatous lesions. There is strong evidence that this technique detects early plaque formations that are expected to transform and produce CAD events (442–445).

Notably, CTCA has a negative predictive value approaching 100% (446), meaning that the absence of coronary plaque or stenosis truthfully correlates to absence of disease on invasive angiography. A negative CTCA is associated with a very low event rate (<1%) over five years and increasing levels of disease detected on CTCA are associated with increasing risk of CAD events and death (447).

1.5.3.1 Coronary artery calcium scoring

Coronary artery calcium scoring is a technique of measuring calcium in the coronary arteries using ECG-gated non-contrast cardiac CT scan (448). The scan acquisition time is less than 10 seconds, does not require special preparation and has a low ionising radiation exposure of approximately 1 mSv (449). On average, Australians are exposed to 1.5 mSv each year from
natural sources (inhalation, external terrestrial, ingestion, and cosmic radiation). Importantly, there is no direct evidence of human health effects on a radiation dose up to 10 mSv (450).

A semi-automated tool is used to calculate a score based in the extent and density of the CAC, which is often routinely performed in patients undergoing cardiac CT scanning. Calcium looks white on the CT image (Figure 6) and the intensity of the signal is assigned a value varying from 1 to 4, with 4 being the most dense. The density is measured in HU, and score of 1 for 130 - 199 HU, 2 for 200 - 299 HU, 3 for 300 - 399 HU, and 4 for ≥400 HU. The area of each plaque is measured (in square millimetres) and multiplied by the intensity index. The resulting number is summed for each coronary artery plaque, generating a CACS in AU (451).

According to the CSANZ CAC scoring position statement (448,452), CACS can be interpreted as follow:

CACS = 0: very low 5-year risk of death (<1%).
CACS = 1 - 100: low risk (<10%).
CACS = 101 - 400: intermediate risk (10 - 20%).
CACS = 101 - 400 and >75th centile: moderate high risk (15 - 20%).
CACS >400: high risk (>20%).
Figure 6. Sample of images from a non-contrast cardiac computed tomography scan for estimating CACS

A) normal appearance. B) calcific lesions in coronary arteries (arrowheads). Image courtesy of Dr Warrick Bishop, Cardiac Centre, Calvary Lenah Valley Hospital (Hobart, TAS).
Screening for calcific coronary atherosclerotic plaques has appeared as a relatively low-cost imaging technique that is usually available to asymptomatic people at risk. The estimation of CAC without using intravenous contrast material, is currently the most robust predictor of CAD events in asymptomatic subjects, even over several traditional cardiovascular risk factors (22,23), particularly in those with an intermediate risk of developing CAD (21). The predictive value of CACS is superior to the Framingham risk score (453), the 2013 ACC/AHA pooled cohort score (251) and the European Society of Cardiology score (454). CACS is also a stronger predictor of ASCVD events compared with other imaging modalities such as carotid intima-media thickness and carotid plaque burden assessment (256,267,455). The extent of CAC is an accurate predictor of all-cause mortality rate at 15 years in asymptomatic patients, as follows: CACS of zero AU: 3%, 1 - 100 AU: 6 - 9%, 101 - 399 AU: 14%, 400 - 999 AU: 21%, and ≥1000 AU: 28% (456).

For the above reasons, the cardiac CT scan for CACS may be considered as a valuable tool for the assessment of CAD as it permits the non-invasive and direct measurement of the atherosclerotic plaque burden (457). This could provide a more personalised estimation of the risk of developing CAD events. Different studies have shown the significance of CACS for the reclassification and stratification of the patients most likely to have benefit from appropriate clinical management (427,458,459).

In a study derived from the MESA cohort aimed to evaluate the associations of cardiovascular risk factor with CAC volume and CAC density, (460) most risk factors were associated with higher CAC volume scores and many risk factors were associated with lower CAC density scores. Importantly, a higher CAC density score was found to be associated with a lower risk of CVD events when adjusted for the CAC volume score (461). This is consistent with previous studies that have shown that sparsely calcific plaques are associated with higher risk of coronary events compared with heavily calcific plaques (462–464).
Although it is well known that men have a higher risk of CAD than women (202), sex differences in calcific plaque and long-term cardiovascular mortality are poorly understood. A recent study from the CAC Consortium (465) including more than 60,000 asymptomatic women and men with a median follow-up of 12.6 years, found that despite women having fewer calcific lesions and vessels involved than men, cardiovascular mortality was higher among women with more extensive, numerous or larger CAC lesions. Women with larger sized and more numerous calcific plaques had a 2.2-fold higher CVD mortality than men. These findings suggest that assessments beyond the CACS alone provide important data about sex differences in coronary atherosclerosis burden which has the potential of improving risk estimations.

The progression of CAC, as assessed by cardiac CT scanning, was associated with a slightly higher risk of CAD events in the Heinz Nixdorf Recall study (466). The most important elements for risk prediction were the most recent CACS and risk factor assessment. However, statin therapy is also associated with CAC progression that could represent plaque repair and stabilisation rather than an increase in the coronary atherosclerotic burden (237–240). Age, male sex, white race, hypertension, BMI, DM, glucose, and family history of CAD had been generally associated with both CAC incidence and progression, over 2.4 years; LDL-C and HDL-C were related only to incident CAC risk (467). CAC progression over 10 years (mean 2.5 scans), has demonstrated a strong association with traditional cardiovascular risk factors including age, male sex, hypertension, and DM which were significant predictors of long term CAC progression (468).

Currently, there are several major guidelines and statements that recommend the use of CACS for risk assessment in asymptomatic patients. The summary of the recommendations is shown in Table 6.
Table 6. Summary of major guidelines and statements on use of coronary artery calcium for risk assessment in asymptomatic patients

<table>
<thead>
<tr>
<th>Guideline/Statement</th>
<th>Summary of recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013  American College of Cardiology/American Heart Association risk assessment guideline</td>
<td>If, after quantitative risk assessment using traditional risk factors, a risk-based treatment decision is uncertain, CACS may be considered to inform treatment decision-making (256)</td>
</tr>
<tr>
<td>2016 European guidelines on cardiovascular disease prevention</td>
<td>CAC scoring may be considered as a risk modifier in cardiovascular risk assessment (354)</td>
</tr>
<tr>
<td>2016 Canadian Cardiovascular Society guidelines for the management of dyslipidaemia for the prevention of cardiovascular disease in the adult</td>
<td>For asymptomatic, middle-aged adults (Framingham risk score 10 - 20%) for whom treatment decisions are uncertain and for low-risk middle-aged individuals with family history of premature CAD. CAC screening not to be undertaken for high-risk individuals; patients receiving statin treatment; or most asymptomatic, low-risk adults (469)</td>
</tr>
<tr>
<td>2017 Expert consensus from the Society of Cardiovascular Tomography</td>
<td>It is appropriate to perform CAC testing in the context of shared decision-making for asymptomatic individuals without clinical ASCVD who are 40 - 75 years of age in the 5 - 20% 10-year ASCVD risk group and selectively in the &lt;5% ASCVD risk group, such as those with a family history of premature CAD (470)</td>
</tr>
<tr>
<td>2017 Cardiac Society of Australia and New Zealand coronary artery calcium scoring position statement</td>
<td>Should be performed in asymptomatic patients without CAD, aged 45 - 75 years and with absolute five-year intermediate cardiovascular risk (10-20%). May be considered for low and medium risk patients (&lt;10%) particularly in those with a strong family history of premature CVD, diabetic aged 40 - 60 years old and indigenous patients &gt;40 years old (448,452)</td>
</tr>
<tr>
<td>2018 Risk assessment for CVD with non-traditional risk factors: US preventive services task force recommendation statement</td>
<td>In asymptomatic adults, the current evidence is insufficient to assess the balance of benefits and harms of adding CACS to traditional risk assessment for CVD prevention (471)</td>
</tr>
<tr>
<td>2018 American College of Cardiology/American Heart Association guideline on the management of blood cholesterol</td>
<td>In intermediate-risk or selected borderline-risk adults, if the decision about statin use remains uncertain, it is reasonable to use a CACS in the decision to withhold, postpone or initiate statin therapy (355)</td>
</tr>
</tbody>
</table>

Adapted from Greenland et al. (472).  
CACS indicates coronary artery calcium score; CAD, coronary artery disease; ASCVD, atherosclerotic cardiovascular disease; and CVD, cardiovascular disease.
1.5.3.1.1 Coronary artery calcium measurement in reclassification of cardiovascular risk

Knowing the imprecision of CVD risk predictions calculators (400), additional non-invasive testing for assessing the presence of subclinical atherosclerotic burden is reasonable. The Society of Cardiovascular Computed Tomography recommends performing CAC testing for asymptomatic individuals without clinical ASCVD who are 40 - 75 years of age with 10-year ASCVD risk between 5 - 20%; additionally, for individuals with 10-year risk <5% and a family history of premature CAD (267). This recommendation aims to re-stratify the risk and select the treatment based on the MESA coronary heart disease risk score (458).

Reclassification of marginal or intermediate-risk individuals with a CACS ≥100 AU or ≥75th percentile for age, sex and race according to MESA cohort (458), detects individuals with a 10-year event rate ≥7.5%, who would expect to have greater benefit from statin therapy, up-risking them (427,455,458). By contrast, reclassification of intermediate-risk individuals with a CACS of zero detects those with low calculated 10-year event rates that fall below the range where statins provide net benefit, down-risking them (455,458,473,474).

1.5.3.1.2 The power of a CACS of zero

The presence and extent of coronary atherosclerosis is a strong predictor of CAD events (470). Evidence supports that CACS has an exceptional assessment role in its power of zero in asymptomatic subjects, even in those who have multiple risk factors (475–477) or who meet criteria for statin therapy initiation (459,478). Previous studies have shown that no recognisable calcific disease is associated with a very low risk of a CAD event over the next 10 to 15 years (428,473,479–481), even among the elderly (473,474,481–483). In addition, a CACS of zero has also been identified as the best predictor of overall survival out to 15 years (425). This has been called the “warranty period” by some researchers (479,480). In a study derived from the CAC Consortium (465), for those with a CACS of
zero, cardiovascular mortality was very low and virtually equal between men and women (0.4% over the mean follow-up period).

Based on the above evidence, the European Atherosclerosis Society guideline (15) recommend a cardiac CT scan for CACS to assess the presence and extent of subclinical atherosclerosis in patients with FH who may benefit from more aggressive cholesterol-lowering therapy. Although patients with FH are at high risk of developing CAD, their prognosis is very variable (262). A study included 206 middle-aged patients with molecularly defined HeFH who were followed for an average of 3.7 years (336). Importantly, none of the 101 (49%) patients with a CACS of zero experienced an event. Conversely, those with CACS of 1 to 100 and >100 AU had a yearly event rate of 26% and 44%, respectively. In other studies (323,329), the absence of CAC also ruled out obstructive disease in this higher risk population group. By contrast, 69% of patients with a CACS >400 AU presented obstructive atherosclerotic plaques (329).

1.5.3.2 Patients’ perceptions of cardiac CT scanning

Patients have traditionally delegated decision-making to physicians. Nevertheless, over the last decades, patients have been encouraged to become more involved in their medical care and health decisions. Patient-centred care is the practice of caring for patients (and their families) in ways that are meaningful and valuable to the individual; it includes listening to, informing and involving patients in their care (484).

The Institute of Medicine defines patient-centred care as “providing care that is respectful of, and responsive to, individual patient preferences, needs and values, and ensuring that patient values guide all clinical decisions” (485). Unfortunately, evidence has shown that patients often are misinformed about the risks and benefits associated with tests and therapies and have little participation in the decision-making process (486,487). Accordingly, one of the biggest challenges of increasing patient engagement is ensuring that patients make informed, evidence-based decisions that are aligned with their preferences and values.
Shared decision-making has several benefit that include (488,489):

- Enhances patient engagement and involvement in healthcare.
- Evidence of better clinical outcomes for patients who share decision-making compared with those who are passive recipients of care.
- Each participant (physician and patient) has a better understanding of the relevant factors and shares responsibility in the decision about how to proceed.
- Makes it easier to implement evidence-based decisions regarding treatment.

The use of screening images for cardiovascular risk assessment have previously shown positive consequences for patient understanding and reassurance (490). A cardiac CT scan for CACS influences a positive health behaviour change, including adherence to cardiac medication (491) and smoking cessation (492,493), particularly when a CACS >0 is identified (494). Following the positive health behaviour change with the presence of more advanced disease on cardiac CT scan, a subsequent reduction of estimated risk has been established (326).

Other studies including pre-test and follow-up surveys, have detected psychological effects following cardiac CT scanning. A study conducted in New Zealand (495), included 45 non-acute cardiac patients who were referred for diagnostic CTCA. Patients were surveyed prior to testing and after the receipt of test results, at which point illness perceptions and intentions to take cardiac medications, as well as diet and exercise intentions were measured (exercise and dietary behaviours were measured at follow-up 6 weeks later). Compared to positive testing patients, those with normal test results reported significant changes toward more positive illness perceptions, with improvements in emotional effect of illness, illness concern, consequences, and personal control of illness. Health behaviour intentions (cardiac medication and exercise) and physical activity at follow-up increased for positive testing patients only.
Another study (496) surveyed 351 adult patients without diagnosed CAD who underwent initial evaluation for ischaemic heart disease with stress testing with imaging or CTCA. Initial test results were positive in 11% of patients. 28% of participants did not feel their initial test was very important for their health, 18% did not have an accurate understanding of their results and 38% did not have strong preferences for completing recommended follow-up. Subsequent tests or procedures were performed in 12% of patients. In adjusted analyses, patients who had a precise understanding of their initial test result were less likely to undergo follow-up test or procedures when the initial test was positive. A systematic review including 15 publications (25), also showed that CAC investigations enhanced medication utilisation and adherence and could impact other important domains such as beneficial behavioural or lifestyle changes to improve CAD.

The patient-centred imaging concept involves the decisions about radiation exposure and how to provide patients with language that clearly explains and appropriately contextualises the risk of exposure to ionising radiation (497). The goal of radiological protection is the safeguarding of subjects from potentially harmful effects of ionising radiation, while ensuring the benefits related to its use. However, there are concerns regarding the extent to which current medical practice is aligned with patient-centred imaging quality, particularly those related to radiation safety principles of justification and optimisation. A study showed that most patients undergoing cardiac CT imaging or single-photon emission CT imaging were unaware that these procedures expose them to ionising radiation or were insufficiently informed of the potential radiation exposure risk (498).

There are also concerns of the potential for detecting incidental findings of undefined clinical significance (e.g. lung nodules). Incidental findings may be present in approximately 10% of asymptomatic individuals undergoing cardiac CT imaging (449). Most of these findings correspond to benign lesions but follow-up imaging to assess their potential health impact is often required (499,500). The monetary expenses and anxiety that may be derived from these findings can be diminished by limiting the window of imaging or
reading to only the cardiac region. Implementing recommendations that have been published for the management of these findings based on size, neoplasm risk categories and other variables, may be useful (501,502).
1.6 Knowledge gaps addressed in the thesis

A) Risk assessment methods as predictors of coronary artery calcification: There are two ways of assessing cardiovascular risk, absolute risk scores and risk factor counting. Currently, most of the clinical decisions in Australia are made based on the Australian absolute CVD risk calculator. Existing guidelines recommend the non-invasive estimation of CAC in individuals with a borderline or intermediate five-year risk of developing CAD according to absolute risk assessment scores. Nevertheless, even the most robust risk prediction models achieve a C-statistic of 0.80. Consequently, there is a 20% probability that the prediction model is not capable of discriminating between patients likely and not likely to develop CAD. Furthermore, absolute risk scores do not provide confidence intervals and so do not reflect the estimation in the uncertainty of risk assessment. Additionally, decision-making in primary care entails the management of individual risk factors rather than absolute risk.

The above methods of assessing cardiovascular risk have never been compared in terms of CACS on cardiac CT scanning with the view to evaluating which is the better predictor of the presence of coronary atherosclerosis. The chapter 3 study will compare cardiovascular risk factor counting and the Australian absolute CVD risk score in order to determine which is the better predictor of the presence of CAC in asymptomatic patients.

B) Differences in coronary artery calcium between patients with and without FH and the contribution of family history of CAD: Previous studies in non-FH populations have indicated that an increased CACS is an independent predictor of CAD events. There is also evidence indicating that the clinical course of CAD in patients with FH is variable in its onset and severity. Other studies have shown that some FH patients present clinical manifestations earlier than others despite the markedly elevated plasma LDL-C concentrations from an early age. Therefore, the use of imaging
techniques for quantifying subclinical coronary atherosclerosis has been proposed to improve cardiovascular risk assessment in FH patients. However, the contribution of family history of CAD towards the development of subclinical coronary atherosclerosis, independent of hypercholesterolaemia, has never been assessed in this context. Additionally, only a few studies of FH patients have included a non-FH control group.

The chapter 4 study will compare the burden of coronary atherosclerosis, as assessed by CACS, between asymptomatic subjects who have and do not have a phenotypic diagnosis of FH; the impact of the family history of CAD on the CAC will be also evaluated.

C) Differences in coronary artery calcium between patients with and without a genetic diagnosis of FH: Compared with the general population, patients with FH have a greater subclinical coronary atherosclerotic burden as assessed by cardiac CT scan. Those patients also present an accelerated development of CAD despite the intensive statin therapy. However, the relationship between the presence of a mutation and the existence of subclinical coronary atherosclerosis has not been adequately investigated. Furthermore, there have been no Australian studies that have reported this association.

The chapter 5 study will independently assess whether the presence of a pathogenic mutation among asymptomatic patients with a phenotypic diagnosis of FH is associated with a greater presence and extent of CAC.

D) Understanding and perception of patients regarding the estimation of coronary artery calcium score: There is an increasing number of guidelines recommending CACS measurement for cardiovascular risk assessment in asymptomatic individuals. At the present time, medical care is becoming more patient-centred. This means that patients are involved in the decision-making about their investigations, treatments, management or support packages, based on clinical evidence and their informed
preferences. In order to adequately involve patients, it is important to know what their level of understanding and perceptions are. However, there have been few studies of a patient-centred approach to the assessment of CAC with regard how they perceive the test and results. Furthermore, no studies in Australia have been conducted to investigate patients' perceptions and understanding following the estimation of CACS.

The chapter 6 study will evaluate whether asymptomatic patients who had a cardiac CT scan for CACS have a high level of understanding and a favourable perception of the investigation and test results.
Chapter Two: Hypotheses, Aims and Study Designs
2.1 Overview of the philosophy of the thesis

The present chapter describes the hypotheses, aims and design of the studies included in the thesis. From the literature review and the knowledge gaps, an overall hypothesis was generated from which four sub-hypothesis were derived. The aims of the thesis were to employ observational studies to test each of the sub-hypotheses and hence to make an inductive inference about the overall hypothesis. This approach is consistent with the hypothetico-deductive method described by Karl Popper (503).

2.2 Overall hypothesis and aim

In asymptomatic subjects without a history of CAD, familial and non-familial cardiovascular risk factors determine the presence of coronary atherosclerosis as assessed by CACS.

The aim of the thesis was to address the sub-hypotheses in order to confirm the overall hypothesis.

2.3 Sub-hypotheses and specific aims

Hypothesis 1
In asymptomatic patients, the number of traditional cardiovascular risk factors determines the presence of CAC.

Aim and study design 1
The aim was to address the above hypothesis by undertaking a cross-sectional study of consecutive asymptomatic adults referred to a coronary risk clinic, in whom a cardiac CT scan for CACS was performed to assess the presence of subclinical coronary atherosclerosis.
Hypothesis 2
In asymptomatic subjects, CACS is higher in those with a phenotypic diagnosis of FH than in those without FH.

Aim and study design 2
The aim was to test the above hypothesis by conducting a case-control study comparing CACS between asymptomatic subjects with and without a phenotypic diagnosis of FH.

Hypothesis 3
In asymptomatic patients with a phenotypic diagnosis of FH, CACS is higher in those with a pathogenic mutation affecting the LDL receptor pathway than in those without a pathogenic mutation.

Aim and study design 3
The aim was to address the above hypothesis by undertaking a case-control study comparing CACS between asymptomatic patients with phenotypic FH with and without a pathogenic mutation affecting the LDL receptor pathway.

Hypothesis 4
Asymptomatic patients who undergo a cardiac CT scan for CACS have a high level of understanding of the test result and a favourable perception of the investigation.

Aim and study design 4
The aim was to test the above hypothesis by conducting a cross-sectional study of asymptomatic adults referred to a coronary risk clinic who underwent a cardiac CT scan for CACS and who were invited to complete a questionnaire survey concerning their understanding and perception of the investigation.
Chapter Three: Cardiovascular Risk Factor Counting as a Predictor of Coronary Artery Calcium in Asymptomatic Patients
ABSTRACT

Background: A simple algorithm of counting cumulative traditional cardiovascular risk factors can stratify the residual lifetime risk for CAD events. Moreover, physicians’ decision-making is more consistent with the management of individual risk factors compared with an absolute risk assessment. Nevertheless, there is limited evidence about the role of risk factor counting in the context of the non-invasive assessment of subclinical CAC.

Aim: The aim was to assess whether the number of traditional cardiovascular risk factors can predict the presence of subclinical CAC quantified as Agatston score on cardiac CT scanning in asymptomatic patients.

Methods: This was a single centre cross-sectional study to evaluate the association between traditional cardiovascular risk factors, employing risk factor counting and absolute risk score, and the presence of subclinical CAC. The CACS on cardiac CT scanning was quantified as part of a coronary risk assessment in a private clinic at the Calvary Lenah Valley Hospital in Hobart, Tasmania. Univariate and multivariable logistic regression analyses were performed to assess whether the number of risk factors and the Australian absolute CVD risk score predicted the presence of CAC.

Results: One hundred and forty-four consecutive patients were studied, 61% were male, the overall mean age was 58 ± 9 years and women were significantly older than men. 65% of patients had a CACS >0 AU, the overall median CACS was 8.0 AU (IQR 71.0), with a significant difference between males and females (15.5 AU [IQR 85.3] and 5.0 AU [IQR 34.5], respectively; p = 0.039). The increasing number of traditional cardiovascular risk factors was a significant predictor of a CACS >0 AU (OR 2.2, 95% CI 1.4 - 3.5; p = 0.001). By contrast, the Australian absolute CVD risk score was not a significant predictor of a CACS >0 AU (OR 0.9, 95% CI 0.9 - 1.1; p = 0.792).
**Conclusion:** In middle-aged asymptomatic patients, the number of traditional cardiovascular risk factors was a significant predictor of the presence of coronary atherosclerosis as assessed by the estimation of CACS employing cardiac CT scanning. By contrast, the Australian absolute CVD risk score was not a significant predictor of the presence of coronary atherosclerosis. It appears, therefore, that a risk factor counting method may be a better predictor of the presence of subclinical coronary atherosclerosis than the absolute risk score.
3.1 Subjects and Methods

3.1.1 Study design

This was a single centre analytical cross-sectional study aimed to evaluate the association between the number of traditional cardiovascular risk factors and the presence of subclinical coronary artery calcification, quantified as CACS on cardiac CT scanning.

3.1.2 Study population

The study population consisted of consecutive adult outpatients with no history of symptomatic CAD, referred to a coronary risk assessment at the Calvary Lenah Valley Hospital (Hobart, Tasmania), a Catholic not-for-profit private organisation. Under the specialist care of Dr Warrick Bishop (WB), patients were receiving best standard of care. Cardiac CT scans for CACS were performed in addition to standard care and were privately funded by patients.

3.1.3 Study variables

Data were collected on the demographic and clinical characteristics of the patients included date of birth, sex, history of CAD, hypertension, DM, smoking status, family history of premature CAD in first-degree relatives, and cholesterol-lowering, antiplatelet and antihypertensive medications.

Laboratory data comprised fasting blood glucose and pre-statin plasma TC, TG, HDL-C and LDL-C concentrations; non-HDL-C was calculated by subtracting HDL-C from TC. From the cardiac CT scan reports, date and CACS in AU were collected.

3.1.4 Inclusion criteria

- Patients who were aged ≥18 years.
- Have undergone a cardiac CT scan including CACS.
3.1.5 Exclusion criteria

- History of symptomatic CAD (defined as previous myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery).
- Inability to give informed consent for cardiac CT scanning.

3.1.6 Data sources

The primary source for obtaining the data was the electronic clinical records of WB which are stored at the Calvary Lenah Valley Hospital. When data were not obtainable from clinical records, telephone contact was made with Hobart Pathology, a medical testing laboratory based in Hobart. If the information was either inaccurate or incomplete, telephone contact was made with their General Practitioner.

3.1.7 Development of database

Before creating the database, the design of the structure of the data elements was performed by creating a coding manual. Each of the variables in the dataset was coded and the data validation tool was used to prevent input errors. Once created, structure and integrity were tested through data entry tests.

Data were collected and grouped manually from primary and secondary sources in a unique password-protected Excel 2016 file (Microsoft Corp., Redmond, WA, USA) at the Cardiac Centre of the Calvary Lenah Valley Hospital.

3.1.8 Data acquisition process

Data of patients who met the specific eligibility criteria were collected retrospectively, covering a 4-year period (2014 - 2017). Patients' names and Medicare numbers were initially recorded to match records from primary and secondary data sources.

Following data collection, the completed dataset in identifiable form was kept on a password-protected Excel 2016 file on the personal work computer of
WB, Consultant Cardiologist from the Calvary Lenah Valley Hospital. Thereafter, names and other identifying information were removed from the database prior to encryption using 7-Zip 16.00 [64-bits] file archiver (Igor Pavlov, Russia) which was also password-protected. The dataset was sent via email to the Royal Perth Hospital and the passwords were sent via text message.

Dataset was regularly profiled to check for missing and spurious data and against duplicate measurements. Information quality assurance measures were applied to regularly profile the data to discover inconsistencies. After database closure, data cleaning was performed. The definite dataset was stored as a password-protected Excel file on a University of Western Australia networked drive at the Medical Research Foundation, Royal Perth Hospital.

Clinical measurements and risk assessment
From key traditional cardiovascular risk factors identified by the Framingham study (241), the following were recorded:

1) Hypertension: defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or on antihypertensive medications (369).
2) DM: defined as a fasting blood glucose ≥7 mmol/L (on two separate occasions) or haemoglobin A1c ≥6.5% (48 mmol/mol) (on two separate occasions) (504).
3) Current smoker or ex-smoker who quit within last year.
4) Hypercholesterolaemia: defined as an LDL-C >4 mmol/L or on cholesterol-lowering medication.
5) Family history of premature CAD in first-degree relative: defined as age of onset <55 years for men and <60 years for women.
6) Sex.

The cumulative number of the above risk factors was assigned to each patient. Stratification according to their number of risk factors, as done in previous studies (417–419) was performed. The risk of developing cardiovascular disease was estimated using the Australian absolute CVD risk
calculator (9). This algorithm is part of the NVDPA national guidelines for the management of absolute CVD risk (405) and the equation includes the following predictors: sex, age, systolic blood pressure, smoking status, TC, HDL-C, and DM. This calculation results in the risk, expressed as a percentage, of developing CVD (heart, stroke or blood vessel disease) in the next five years for adults between 35 and 74 years of age. According to the NVDPA national guidelines for the management of absolute CVD risk (405), a systolic blood pressure value ≥180 mmHg, TC >7.5 mmol/L or DM diagnosis in a person aged ≥60 years old indicates an increased absolute risk of CVD (>15%) and numerical calculation of absolute risk is not computed by the calculator.

**Biochemical analyses**
All lipid profiles analyses were performed in the same medical testing laboratory, Hobart Pathology (2 - 4 Kirksway Place, Hobart TAS 7000). Venous blood was collected after 8 - 15 hours fast by a phlebotomist into EDTA or lithium heparin tubes. Plasma TC, TG and HDL-C concentrations were measured by standard enzymatic methods using the Cobas C701 platform (Roche Diagnostics, Risch-Rotkreuz, ZG, Switzerland). LDL-C concentrations were calculated using the Friedewald Equation (505), but in patients with plasma TG ≥4.5 mmol/L, a direct LDL-C assay was used. Plasma glucose was measured by standard enzymatic methods using the Modular P Chemistry Analyzer (Roche Diagnostics, Risch-Rotkreuz, ZG, Switzerland).

**Cardiac imaging**
The cardiac CT scans were carried out on a GE Revolution 128-slide scanner (GE Healthcare, Chicago, IL, USA) in the facilities of Regional Imaging at Calvary Lenah Valley Hospital (49 Augusta Road, Lenah Valley TAS 7008). Non-contrast-enhanced CT images were acquired using a prospective electrocardiographic triggering on the volumetric mode, and images were reconstructed with 3.0 mm axial slices. Acquisition parameters included a gantry rotation time of 0.35 sec, collimation of 20 mm x 280 mm,
tube voltage of 120 kV, and tube current of 200 mA, with a 25 cm displayed field of view.

The images were performed at a temporal resolution of 330 ms and spatial resolution 0.24 mm. The estimated radiation dose was approximately 1 mSv (dose length product 90 mGycm). The 3.0 mm images were loaded into the GE Advantage workstation and calcific plaques in coronary arteries were quantified using the SmartScore 4.0 package (GE Healthcare, Chicago, IL, USA). Agatston scores were calculated according to the method described by Agatston et al. (451).

3.1.9 Statistical methods

Participants that met the eligibility criteria were consecutively selected from the electronic clinical records of WB during the established period. Statistical analyses were performed with the use of STATA software, version 14.1 (StataCorp LLC., College Station, TX, USA).

The prevalence of cardiovascular risk factors was calculated as the presence of the risk factors divided by the total number of the cohort and expressed as a percentage. The number of cardiovascular risk factors were calculated for each patient and subsequently, stratified according to the counting. The Australian absolute CVD risk score (9) was also estimated. A Pearson correlation coefficient was computed to assess the relationship between the number of cardiovascular risk factors and the Australian absolute CVD risk score.

Continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test. Continuous variables with normal distribution were expressed as means and standard deviations; non-normal variables were reported as medians and interquartile ranges. Categorical variables were expressed as percentages. Normally distributed continuous variables were compared with Student’s t-test; Wilcoxon rank-sum test was used to compare non-normally distributed variables. The frequencies of categorical variables were compared using Pearson's chi-square or Fisher's exact test.
As CACS was a skewed variable and given the high proportion of patients with zero AU, transformation of the data was required. To transform the skewed CACS to approximately conform to normality, different types of data transformations (logarithm, cubic, identity and square root) were attempted without reaching normality (Appendix 1). Univariate and multivariable logistic regression analyses were used to investigate the associations between CACS and the number of cardiovascular risk factors (including and excluding family history of premature CAD) and the absolute risk scores. Fasting blood glucose was used as independent variable owing to all patients with a history of DM having CACS >0 AU. Given the relatively small sample size (50 patients with CACS >0 AU, 28 patients with >100 AU, and 19 patients with >200 AU), the selection of variables was limited to a maximum of four for the first multivariable regression model, to a maximum of three for the second and to a maximum of two for the third model. In the multivariable logistic regression analyses, there was restricted selection of variables to only those with a p value <0.1 in univariate analysis or for which there was good a priori reason (e.g. age). Bootstrapping was employed to ensure p values were robust due to concerns with overfitting. Area under the ROC curve was calculated to describe the discriminant ability of the multivariable models. Significance was defined at the 5% level.

3.1.10 Ethical considerations

A low-risk clinical audit application form was submitted to the Tasmanian Health and Medical HREC. An approval letter with reference number H0016382 (Ethics 1) and an amendment (Ethics 2) were received, constituting ethical clearance by the Health and Medical HREC. The recognition of an ethics approval from a non-UWA Research Ethics Committee was awarded by the UWA Human Ethics Office (Ethics 3) with file reference RA/4/1/9238. All participants signed an informed consent for the use of their de-identified data.
3.2 Results

3.2.1 Patients characteristics

A total of 144 consecutive asymptomatic adults were studied, 61% male and an overall mean age of 58 years. The demographic, clinical and biochemical characteristics of the patients in relation to sex are shown in Table 7. Approximately half the cohort had a history of hypercholesterolaemia, a third part had a history of hypertension, a quarter had a family history of premature CAD; 85% of the patients had never smoked, 5% were current smokers and 6.3% had a history of type 2 DM. Approximately 20% of patients were on antiplatelet and about the same proportion on statins. The median length of statin treatment was 19.5 months (IQR 93.5) and the most commonly used molecule was rosuvastatin (53.6%).

Women were significantly older and had a higher pre-treated TC and HDL-C concentrations than men. Males had a significantly higher TG concentration than women, however, there were no significant differences in the LDL-C and non-HDL-C concentrations between genders. With respect to the cardiovascular risk factors, there were no significant differences between genders in the prevalence of family history of premature CAD, DM, hypertension, smoking and hypercholesterolaemia. In spite of the absence of difference in the prevalence of DM, fasting blood glucose concentrations were significantly higher in men than in women.
Table 7. Demographic, clinical and biochemical characteristics of the patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>144</td>
<td>88</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58.3 ± 9.4</td>
<td>56.1 ± 9.9</td>
<td>61.8 ± 7.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking*, n (%)</td>
<td>7 (4.9)</td>
<td>5 (5.7)</td>
<td>2 (3.6)</td>
<td>0.566</td>
</tr>
<tr>
<td>Hypertension*, n (%)</td>
<td>52 (36.1)</td>
<td>30 (34.1)</td>
<td>20 (39.3)</td>
<td>0.527</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus*, n (%)</td>
<td>9 (6.3)</td>
<td>6 (6.8)</td>
<td>3 (5.3)</td>
<td>0.724</td>
</tr>
<tr>
<td>Hypercholesterolaemia*, n (%)</td>
<td>79 (55.6)</td>
<td>45 (51.1)</td>
<td>34 (60.7)</td>
<td>0.325</td>
</tr>
<tr>
<td>Family history premature CAD, n (%)</td>
<td>39 (27.1)</td>
<td>24 (27.3)</td>
<td>15 (26.8)</td>
<td>0.949</td>
</tr>
<tr>
<td>Pre-statin TC, mmol/L</td>
<td>6.3 ± 1.3</td>
<td>6.1 ± 1.4</td>
<td>6.6 ± 1.1</td>
<td>0.037</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>4.0 ± 1.2</td>
<td>3.9 ± 1.2</td>
<td>4.1 ± 1</td>
<td>0.375</td>
</tr>
<tr>
<td>Pre-statin triglyceride, mmol/L</td>
<td>1.3 (1.0)</td>
<td>1.4 (1.0)</td>
<td>1.2 (1.0)</td>
<td>0.049</td>
</tr>
<tr>
<td>Pre-statin HDL-C, mmol/L</td>
<td>1.5 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>1.8 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-statin non-HDL-C</td>
<td>4.8 ± 0.1</td>
<td>4.8 ± 1.5</td>
<td>4.8 ± 1.2</td>
<td>0.825</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.3 ± 1.2</td>
<td>5.4 ± 1.3</td>
<td>5.2 ± 1.1</td>
<td>0.021</td>
</tr>
<tr>
<td>On antiplatelet, n (%)</td>
<td>30 (20.8)</td>
<td>20 (22.7)</td>
<td>10 (39.3)</td>
<td>0.483</td>
</tr>
<tr>
<td>On antihypertensive, n (%)</td>
<td>46 (31.9)</td>
<td>24 (27.3)</td>
<td>22 (39.3)</td>
<td>0.132</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>28 (19.4)</td>
<td>17 (19.3)</td>
<td>11 (19.7)</td>
<td>0.962</td>
</tr>
<tr>
<td>Australian absolute CVD risk score</td>
<td>6.0 (5.0)</td>
<td>8.0 (6.0)</td>
<td>4.0 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Agatston score</td>
<td>8.0 (71.0)</td>
<td>15.5 (85.3)</td>
<td>5.0 (34.5)</td>
<td>0.039</td>
</tr>
<tr>
<td>Agatston score &gt;0, n (%)</td>
<td>94 (65.3)</td>
<td>64 (72.7)</td>
<td>30 (53.5)</td>
<td>0.019</td>
</tr>
<tr>
<td>Agatston score &gt;100, n (%)</td>
<td>28 (19.4)</td>
<td>20 (22.3)</td>
<td>8 (14.3)</td>
<td>0.212</td>
</tr>
<tr>
<td>Agatston score &gt;200, n (%)</td>
<td>19 (13.2)</td>
<td>12 (13.6)</td>
<td>7 (12.5)</td>
<td>0.844</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range).
CAD indicates coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.
* Modifiable risk factor. p value for male versus female.
The Australian absolute CVD risk score was not calculated in two patients owing to their age (25 and 31 years old); for the remaining 142 patients, the overall median risk of developing CVD in the next five years was 6.0% (IQR 5.0), with most of the patients classified as low-risk (62.9%), followed by high- (21.4%) and medium-risk (15.7%). The low-, medium- and high-risk categories had a male proportion of 54.5%, 81.8% and 60.6% and a mean age of 57.9 ± 8.5, 62.6 ± 7.0 and 59.0 ± 9.7 years, respectively. Compared by sex, men had a higher median absolute risk score than women (8.0% [IQR 6.0] and 4.0% [IQR 3.0], respectively; p <0.001). Overall, an increase in the number of cardiovascular risk factors was positively correlated with the Australian absolute CVD risk score (r = 0.305, p = 0.001).

According to the CSANZ CAC scoring position statement (448,452), 60 patients (41.6%) were suitable for CAC. The median CACS in the cohort was 8 AU (IQR 71.0). Fifty (34.7%) patients were classified as with very low risk of CV event in the next 10 years, 65 (45.1%) as low, 15 (10.4%) as intermediate, and 14 (9.7%) as high risk. Males had a significantly higher CACS than females (15.5 AU [IQR 85.3] and 5.0 AU [IQR 34.5], respectively; p = 0.039).

The proportion of patients with subclinical coronary artery calcification according to age is depicted in Figure 7. No CAC was identified in patients in the third and fourth decade of life. By contrast, patients in the seventh decade of life, had the greater proportion of the presence of CACS >0 AU (74.5%), followed by patients in the eighth (73.3%), sixth (66%) and fifth (52.6%) decade of life. There was a significant difference in the proportion of patients with CACS >0 AU along the age groups (p = 0.020).
Figure 7. Proportion of patients with CACS >0 according to age
3.2.2 Number of risk factors and Australian absolute CVD risk score as predictors of coronary artery calcification

The proportion of patients with subclinical coronary artery calcification tended to increase with the number of cardiovascular risk factors (Figure 8). The proportion of patients with CACS >0 AU and 2 or more cardiovascular risk factors was significantly higher than those with no risk factors ($p = 0.014$ and $p = 0.042$, respectively).

Notably, 47.4% of patients with no cardiovascular risk factors had coronary artery calcifications; these patients were mainly men and older than the overall population (78% males and mean age of 62.7 ± 4.9 years). By contrast, 11% of patients with three or more cardiovascular risk factors did not have subclinical coronary artery calcifications; these patients were all men and younger than the overall population (mean age 52.2 ± 12 years). Furthermore, the proportion of patients with coronary artery calcification increased with the number of modifiable cardiovascular risk factors (Figure 9). The proportion of patients with a CACS >0 AU with one or more modifiable cardiovascular risk factors was significantly higher than those with no risk factors ($p <0.001$ for all).

In univariate logistic regression analysis, the increasing number of cardiovascular risk factors by the risk factor counting method was a significant predictor of a CACS >0 AU (OR 1.77, 95% CI 1.16 - 2.70; $p = 0.008$). In multivariable logistic regression analysis, the association remained significant after adjusting by age (OR 2.20, 95% CI 1.38 - 3.51; $p = 0.001$), area under ROC curve 0.8076 (95% CI 0.734 - 0.881), indicating a good discriminant ability of the model (Appendix 2). When family history of premature CAD was removed from the risk factor counting, the increasing number of cardiovascular risk factors remained as a significant predictor of a CACS >0 AU in the multivariable logistic regression analysis after adjusting by age (OR 2.94, 95% CI 1.72 - 5.03; $p <0.001$), area under ROC curve 0.7568 (95% CI 0.677 - 0.836).
On the other hand, in univariate logistic regression analysis, the Australian absolute CVD risk score was not a significant predictor of a CACS >0 AU (OR 0.98, 95% CI 0.90 - 1.07; $p = 0.792$).
Figure 8. Proportion of patients with CACS >0 according to the number of cardiovascular risk factors (male sex, hypertension, DM, hypercholesterolaemia, smoking, and family history of premature CAD)
Figure 9. Proportion of patients with CACS >0 according to the number of modifiable cardiovascular risk factors (hypertension, DM, hypercholesterolaemia, and smoking)
3.2.3 Predictors of coronary artery calcification

Tables 8 and 9 shows the univariate logistic regression analyses in search of predictors of the presence of subclinical CAC in asymptomatic patients, using two different cut-offs: CACS >0 AU and >100 AU, respectively. No significant predictor variables were found when CACS >200 AU was used as the cut point (Appendix 3).

In univariate logistic regression analyses, age and hypertension were consistent significant predictors of a CACS >0 AU and >100 AU. Fasting blood glucose concentration and male sex were significant predictors of a CACS >0 AU but not of a CACS >100 AU. Smoking, hypercholesterolaemia, family history of premature CAD, and statin therapy were not significant predictors of a CACS >0 AU nor >100 AU.

The multivariable logistic regression analyses models are shown in Table 10. Age, male sex and hypertension remained independent predictors of both, a CACS >0 AU (model 1) and >100 AU (model 2). Fasting blood glucose concentration also persisted as an independent predictor of a CACS >0 AU. Appendices 4 and 5 depicts the area under the ROC curve for model 1 0.8003 (95% CI 0.726 - 0.873) and model 2 0.8079 (95% CI 0.678 - 0.855), respectively, indicating a good discriminant ability of both multivariable logistic regression models.
**Table 8. Predictors of coronary artery calcification (defined as CACS >0) in univariate logistic regression**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.07</td>
<td>1.02 - 1.11</td>
<td>0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.31</td>
<td>1.14 - 4.67</td>
<td>0.020</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.23</td>
<td>1.44 - 7.21</td>
<td>0.004</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.55</td>
<td>1.20 - 5.39</td>
<td>0.014</td>
</tr>
<tr>
<td>Smoking</td>
<td>3.34</td>
<td>0.39 - 28.55</td>
<td>0.271</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>0.74</td>
<td>0.38 - 1.59</td>
<td>0.413</td>
</tr>
<tr>
<td>Family history of premature CAD</td>
<td>0.68</td>
<td>0.36 - 1.50</td>
<td>0.334</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>2.24</td>
<td>0.84 - 5.95</td>
<td>0.106</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; OR, odds ratio; CI, confidence interval; and CAD, coronary artery disease. 

*p value for univariate logistic regression analyses.*

**Table 9. Predictors of coronary artery calcification (defined as CACS >100) in univariate logistic regression**

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.07</td>
<td>1.01 - 1.13</td>
<td>0.010</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.76</td>
<td>0.71 - 4.33</td>
<td>0.216</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.31</td>
<td>2.18 - 12.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.20</td>
<td>0.89 - 1.60</td>
<td>0.228</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.71</td>
<td>0.31 - 9.30</td>
<td>0.536</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>0.90</td>
<td>0.39 - 2.06</td>
<td>0.806</td>
</tr>
<tr>
<td>Family history of premature CAD</td>
<td>1.09</td>
<td>0.44 - 2.74</td>
<td>0.844</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>1.16</td>
<td>0.42 - 3.21</td>
<td>0.768</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; OR, odds ratio; CI, confidence interval; and CAD, coronary artery disease. 

*p value for univariate logistic regression analyses.*
Table 10. Multivariable logistic regression models for prediction of coronary artery calcification

<table>
<thead>
<tr>
<th>Model 1 (CACS &gt;0)</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.11</td>
<td>1.04 - 1.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>4.80</td>
<td>1.73 - 13.29</td>
<td>0.003</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.19</td>
<td>1.05 - 4.55</td>
<td>0.035</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3.75</td>
<td>1.44 - 9.76</td>
<td>0.007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model 2 (CACS &gt;100)</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.10</td>
<td>1.03 - 1.17</td>
<td>0.004</td>
</tr>
<tr>
<td>Male sex</td>
<td>3.26</td>
<td>1.11 - 9.53</td>
<td>0.031</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5.98</td>
<td>2.00 - 17.83</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; OR, odds ratio; and CI, confidence interval. p value for multivariable logistic regression analyses.
3.5 Discussion

**New and main findings**

The principal finding was that the number of traditional cardiovascular risk factors was a significant predictor of the presence of subclinical coronary artery calcification as assessed by cardiac CT scanning; this association remained significant after excluding the family history of premature CAD from the risk factor counting. By contrast, the Australian absolute CVD risk score was not a significant predictor of the presence of CAC. This is the first study in Australia that has compared the risk factor counting method and absolute risk assessment in predicting the presence of CAC as assessed by cardiac CT scanning.

Although the Australian absolute CVD risk score and the number of cardiovascular risk factors were positively correlated, only the latter was a significant predictor of the presence of subclinical CAC. Several factors may explain this finding. First, the Australian absolute CVD risk equation (9), derived from population-based studies, is a recalibration of a Framingham algorithm (402) which has a different coefficient (weighting) to each variable (cardiovascular risk factor). This could diminish the ability to detect CAC in a clinic population, whilst in the risk factor counting method, the weighting to each variable is the same, without assumptions, and calibration is not necessary. Second, the Australian absolute CVD risk calculator is designed to predict cardiovascular events in the short term (in the next five years) and not the burden of subclinical coronary atherosclerosis represented as CAC, a process that usually takes decades. Third, only individuals between 35 and 74 years of age can be assessed with the absolute risk score. In the present study, 5% of the patients were outside this age range and 16% were younger than 50 years of age, a group of patients in whom the probability of having CAC is lower. Fourth, the inclusion of a highly selected group of patients who afforded to attend a private clinic clearly does not represent the Australian general population.
Among the cardiovascular risk factors, age, male sex and hypertension were independent predictors of both a CACS >0 AU and >100 AU. Fasting blood glucose concentration was an independent predictor of a CACS >0 AU. Hypercholesterolaemia did not predict the presence of CAC, possibly because some of these patients were on cholesterol-lowering therapy. Notably, statin therapy was not a determinant of the presence of subclinical CAC, therefore, it was not likely a confounder factor of the results. No significant predictor variables for a CACS >200 AU were found, this is probably due to the low statistical power related to the sample size.

**Previous studies**

The present study supports the hypothesis that the number of cardiovascular risk factors predicts the presence of subclinical CAC in middle-aged patients. This finding is consistent with the results of the CARDIA study (506) which aimed to determine whether early adult levels of modifiable risk factors predicted subsequent CAC. In 3043 young adult participants, risk factors were measured in six different moments in times and CAC was assessed at year 15. Baseline number of risk factors predicted CAC presence equally as well as the number of risk factors at 15 years, despite the change in risk factor burden along the follow-up. Above findings suggest that earlier risk assessment including risk factor counting and CACS measurement, together with efforts to achieve and maintain optimal risk factors is needed. Similarly, in a study including 410 patients with atypical chest pain and without CAD (218), CACS was measured using cardiac CT scan and multiple analyses employing different thresholds showed that the number of cardiovascular risk factors had a strong positive association with CACS.

Other studies in young adults have evaluated the relationship of cardiovascular risk factors with the presence of subclinical CAC, although the risk factor counting method has not been employed. A cross-sectional study including 384 white young adults ages 20 to 34 years (507), showed that greater systolic blood pressure, BMI, and LDL-C, and lower HDL-C concentrations were associated with subsequent CAC at ages 29 to 37 years, but only diastolic blood pressure, BMI and the ratio of TC to HDL-C
predicted CAC independently of other risk factors. Similarly, the Prospective Army Coronary Calcium Project (508), a study of 630 multiethnic active-duty US Army personnel found that the prevalence of CAC was 17% and that greater concentrations of LDL-C, TG, a higher systolic blood pressure and BMI were associated with CAC, but only LDL-C was independently associated. In this age-homogeneous, low-risk screening cohort, traditional risk factors significantly underestimated the presence of premature, subclinical CAC, however, results cannot be generalised to general population and cannot be compared with the present study.

In the present study, males had a significantly higher CACS than females, even though women were significantly older than men, which is consistent with previous studies (458,507,508). Other studies showed that there is a higher prevalence of CAC in subjects with established forms of CAD (220,221). Notably, epidemiological data have recognised that CACS is an independent predictor of adverse cardiovascular episodes over several traditional cardiovascular risk factors, particularly in asymptomatic patients with an intermediate cardiovascular risk estimation (22,425,426,509–511). The predictive value of CACS is superior to the Framingham risk score (453) and the 2013 ACC/AHA pooled cohort equations (251). Given this, the situation would not be different with the use of the Australian absolute CVD risk score (9), a tool based on Framingham risk score (405), which would support the findings of the present study.

Other non-invasive approaches to assess subclinical atherosclerotic burden can be considered in young and middle-aged patients. However, CACS is also a stronger predictor of ASCVD events compared with other imaging modalities such as carotid intima-media thickness and the ultrasonographic measure of carotid plaque burden (256,267,455).

**Limitations and strengths**

Limitations are mainly related to the design of the study. This was an observational study, therefore, could not prove causality. This study was not performed in a population group; a highly selected population that attended
to a single private clinic with interest in knowing their cardiovascular health status was studied. Other limitation was the low statistical power due to convenience sampling although statistical significance was employed in the analyses. Additionally, due to the survival bias of the cohort, the proportion of women was higher; this is explained for the higher risk of CAD associated with male sex, hence, among asymptomatic patients, a higher proportion of females was expected.

In spite of statin therapy not being a predictor of the presence of CAC in the present study, some of the patients included were on statins at the moment of the cardiac CT scan. The inability to define cholesterol-lowering therapy duration was a potential confounder because it could not be adjusted by cholesterol content and statins could have influenced the progression of CAC in those patients (237–240,512). However, this limitation was addressed by using only the pre-statin lipid profiles and the history of hypercholesterolaemia in the analyses.

Another limitation was that neither the presence, extent nor distribution of coronary atherosclerosis was assessed employing CTCA. Although CACS is associated to the total atherosclerosis burden, it represents only 20% of total plaque content and does not account for the component of non-calcific plaque or the luminal stenosis severity, that are not visible on a non-contrast cardiac CT scan (513–515). This is relevant because CTCA can identify plaques that are expected to transform and produce future CAD events (442–445). Additionally, no other measurements derived from the CACS estimation that could be used for additional analyses were available (number of lesions and vessels affected, lesion size, volume, and plaque density).

Strengths of the present study include the relatively high number of asymptomatic patients, most of them (58%) without an indication of CACS measurement according to the CSANZ CAC scoring position statement (448,452). This showed that CAC is present in whom CTCA is not indicated according to local guidelines and that more patients could benefit from this test in order to improve coronary risk prevention. Another strength was the
similarity in the proportion of the presence of traditional cardiovascular risk factors between gender.

Scope for further work and practical implications

Findings needs to be further study in a larger sample size, future studies ought to be multicentre, with long-term follow-up and including a more diverse defined group of patients not only from private centres. Measurements resulting from the CACS quantification should be incorporated in future studies. Non-calcific coronary atherosclerosis and luminal obstruction should be explored using CTCA. This would imply higher risks and financial costs, therefore, only patients with an indication of CTCA should be included. Other non-invasive imaging methods for assessing atherosclerotic burden, as carotid ultrasound, might be used in young adults.

The number of cardiovascular risk factors was a better predictor of the presence of CAC than absolute risk score. This could mean that the use of risk factor counting in the clinic may be a more useful indicator of the patients' coronary risk, particularly in cases that quantification of CACS is not available. However, the more information clinicians and patients have, the better the management of the individual risk. The assessment of CACS allows premature detection of coronary atherosclerotic burden allowing the management initiation without waiting until symptoms appear.
3.6 Conclusion

The present study demonstrated that the traditional cardiovascular risk factor counting method (including and excluding family history of premature CAD) may be a valid predictor of the presence of subclinical coronary artery calcification in asymptomatic patients with no history of CAD. However, the Australian absolute CVD risk score, a tool widely used by health professionals in Australia, did not predict the presence of CAC. Notably, there were patients with no traditional risk factors and CAC and others with 3 or more risk factors who did not have CAC, suggesting that the risk factor burden is not a perfect predictor, although the age could be an important factor that explains these findings.

Traditional CVD risk factor counting algorithms, which have been derived from multiple different cohorts that are generally similar, provide effective means of predicting future risk for overall mortality and fatal and non-fatal CVD events, as well as the occurrence of non-CVD death. The risk factor counting, applied to middle-aged population, have also been shown to be associated with quality of life and healthcare costs at older ages. Specifically, those who reach middle-age free of risk factors have substantially prolonged longevity, lower lifetime risks of disease, greater disease-free longevity and greater health-related quality of life at older ages, compared with those with one or more risk factors. With this in view, greater efforts are needed to prevent the development of risk factors not just to prevent disease once present, suggesting the clinical relevance of risk factor counting schemes.

In conclusion, evaluation of asymptomatic patients referred for cardiovascular risk assessment should include risk factor counting. However, this approach is still imprecise and in those with recognised cardiovascular risk factors, cardiac imaging for CACS should be employed. This allows a direct and non-invasive estimation of coronary atherosclerosis. As a consequence, clinicians can provide a more personalised coronary risk assessment leading to clearer risk communication and easier implementation of modifiable risk factors...
treatment. These findings should also promote efforts to prevent the development of cardiovascular risk factors in young individuals.
Chapter Four: Coronary Artery Calcium in Asymptomatic Subjects in Relation to the Presence and Absence of a Phenotypic Diagnosis of Familial Hypercholesterolaemia
ABSTRACT

Background and aim: FH is a commonly inherited disorder of LDL-C metabolism that is associated with a higher risk of premature CAD. Early identification of individuals with a higher subclinical coronary atherosclerotic burden may be useful in risk stratifying and focusing therapy. The aim of this study is to compare the burden of coronary atherosclerosis, as assessed by the CACS, between asymptomatic subjects with and without a phenotypic diagnosis of FH.

Methods: An age-matched case-control study was undertaken. Asymptomatic adults with (cases) and without (controls) a phenotypic diagnosis of FH were studied. Agatston scores were compared between groups using the Wilcoxon matched-pairs signed-rank test. Univariate and multivariable logistic regression analyses were used to investigate the associations between CACS and cardiovascular risk factors.

Results: A total of 218 asymptomatic subjects were studied (109 FH patients and 109 controls). The overall mean age was 54 ± 7 years and 41% were male. There were no significant differences between groups in terms of the proportion of males, DM, hypertension, current smokers, obesity, and treated plasma LCL-C concentrations. Compared with FH patients, controls had a significantly higher proportion of family history of premature CAD (p <0.001). However, the family history of premature CAD was not a significant predictor of a CACS >0 (p = 0.128). Median CACS was significantly higher in patients with FH compared with controls (21.0 AU [IQR 152.2] and 0.0 AU [IQR 13.1], respectively; p <0.001). Among patients with FH, median CACS was significantly higher in those with a pathogenic mutation affecting the LDL receptor pathway than in those without a mutation (42.0 AU [IQR 183.5] and 4.0 AU [IQR 136.0], respectively; p = 0.003). Age, male sex and pre-statin plasma LDL-C concentrations were independent predictors of a CACS >0 (p <0.001, p = 0.018 and p = 0.002, respectively); these associations were independent of statin therapy.
**Conclusion:** In asymptomatic subjects with a phenotypic diagnosis of FH, there is a greater extent of coronary atherosclerosis, as assessed by cardiac CT scan for CACS, than in those without FH. This association was independent of statin therapy, family history of premature CAD and other cardiovascular risk factors, with exception of pre-statin plasma LDL-C concentrations. The higher CACS in FH patients was determined primarily by the level of LDL-C. Given this, cholesterol is probably a more powerful predictor of subclinical coronary atherosclerosis than other familial and non-familial cardiovascular risk factors.
4.1 Subjects and methods

4.1.1 Study design

An age-matched case-control study was undertaken to investigate the difference in CACS, as assessed on cardiac CT, in asymptomatic subjects with (cases) and without (controls) a phenotypic diagnosis of FH. Age-matching was carried out in order to ensure equal numbers of cases and controls in each age stratum.

4.1.2 Study population

The study population consisted of adult subjects without symptomatic CAD. Data on patients with phenotypic diagnosis of FH were derived from the Lipid Disorders Clinic at Royal Perth Hospital (Perth, Western Australia). Patients with suspected FH were recruited via referral from general practice, from coronary care or from private specialists. The most common reasons for suspecting FH were an elevated plasma LDL-C concentration with a family history of premature CAD. If the phenotypic diagnosis was at least possible FH (DLCNS >3), they were offered genetic testing with appropriate counselling and informed written consent.

Data on asymptomatic subjects without FH were derived from the baseline of the CAUGHT-CAD study (516) (Australian New Zealand Clinical Trials Registry 12614001294640, https://www.anzctr.org.au/). Only participants screened in the Royal Perth Hospital were invited to participate.

4.1.3 Study variables

The demographic and clinical characteristics of the patients included date of birth, sex, height, weight, presence of tendinous xanthoma (bilateral, subcutaneous nodules on Achilles tendons or at ligamentous insertions), history of CAD, hypertension, DM, smoking status, and family history of premature CAD in a first-degree relative. Cholesterol-lowering and hypertension medications were also recorded.
For patients with FH, laboratory variables comprised pre-statin plasma LDL-C concentrations and the treated lipid and lipoprotein analyses closest to the date of the cardiac CT scan (TC, TG, HDL-C, and LDL-C concentrations); the phenotypic DLCNS was also collected (259,264). For controls, pre-statin plasma TC, TG, HDL-C, and LDL-C concentrations were recorded. From the cardiac CT scan reports, date and CACS in AU were collected for both groups.

For patients with FH, the presence, type and description of a pathogenic mutation affecting the LDL receptor pathway was obtained from the genetic reports. In cases when a gene variant of uncertain (or unknown) significance was reported, the genetic diagnosis was assumed as mutation negative (517).

4.1.4 Inclusion criteria

Patient with FH:
1) Patients who were aged 35 - 70 years.
2) Had phenotypic diagnosis of FH (DLCNS >5).
3) Had undergone a cardiac CT scan including CAC scoring.
4) Were genetically tested for a mutation affecting the LDL receptor pathway.

Controls:
1) Subjects aged 35 - 70 years old who are not already on statins.
2) Had a family history of CAD involving first-degree relatives <60 years old or second-degree relatives <50 years old.
3) Had undergone a cardiac CT scan including CAC scoring.

4.1.5 Exclusion criteria

Patient with FH:
1) HoFH, compound and double HeFH.
2) History of symptomatic CAD (defined as previous myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery).
3) Inability to provide informed consent.
Controls:
1) History of symptomatic CAD (defined as previous myocardial infraction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery).
2) Inability to provide informed consent.

4.1.6 Data sources
Patient with FH: The primary source for obtaining the data was the FHWA Access database (Microsoft Corp., Redmond, WA, USA) from the Lipid Disorders Clinic at Royal Perth Hospital. When data were not obtainable from the FHWA database, clinical records were accessed.

Controls: The primary source for acquiring data was the CAUGHT-CAD study baseline physical records.

4.1.7 Development of database
Before creating the database, the design of the structure of the data elements was performed by creating a coding manual. Each of the variables in the dataset was coded and the data validation tool was used to prevent input errors. Once created, structure and integrity were tested through data entry tests.

Patient with FH: Data from the FHWA database were extracted in non-identifiable format by the FHWA Project Coordinator in a password-protected Excel 2016 file (Microsoft Corp., Redmond, WA, USA). The password was sent by a separate text message.

Controls: Data were collected manually in a unique password-protected Excel 2016 file at the Medical Research Foundation, Royal Perth Hospital (Perth, Western Australia).

4.1.8 Data acquisition process
Patient with FH: Data from the FHWA cohort were collected prospectively by the FHWA Project Coordinator from the clinical records and stored in the
FHWA database at the Medical Research Foundation. Data of patients who met the specific eligibility criteria were collected retrospectively, covering a 10-year period (2008 - 2018).

Controls: Mrs Jacqueline Ryan, the CAUGHT-CAD study Coordinator, sent an email (Ethics 4) to participants explaining the aim of the study and querying whether they authorise the use of their de-identified baseline clinical data. Among those who accepted, a participant information sheet and consent form (Ethics 5) was sent via email in order to be read, sign, scan, and return. Under direct supervision of the study Coordinator, data were collected in a non-identifiable format and grouped manually from the study baseline physical records, in a password-protected Excel file.

Dataset was regularly profiled to check for missing and spurious data and against duplicate measurements. Information quality assurance measures were applied to regularly profile the data to discover inconsistencies. After database closure, data cleaning was performed. At the Medical Research Foundation, Royal Perth Hospital, data were combined into a single password-protected Excel file and stored on a University of Western Australia networked drive.

Clinical measurements and risk assessment

From key cardiovascular risk factors identified by the Framingham study (241), the following were recorded:

1) Hypertension: defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or on antihypertensive medications (369).
2) DM: defined as a fasting blood glucose ≥7 mmol/L (on two separate occasions) or haemoglobin A1c ≥6.5% (48 mmol/mol) (on two separate occasions) (504).
3) Current smoker or ex-smoker who quit within last year.
4) Hypercholesterolaemia: defined as an LDL-C >4 mmol/L or on cholesterol-lowering medication.
5) Family history of premature CAD in first-degree relative: defined as age of onset <55 years for men and <60 years for women.
6) Obesity: defined as a BMI >30 kg/m^2 (518).
7) Sex.

**Biochemical analyses**

For both groups, plasma lipid and lipoprotein analyses were performed in PathWest Laboratory Medicine WA (196 Goderich Street, Perth WA 6000 - Barry Marshall Parade, Murdoch WA 6150). Venous blood was collected after 12 hours fast by a phlebotomist into EDTA or lithium heparin tubes. Plasma TC, TG and HDL-C concentrations were measured by standard enzymatic methods using the ARCHITECT c16,000 platform (Abbott Laboratories, Abbott Park, IL, USA). LDL-C concentrations were calculated using the Friedewald Equation (505), but in patients with plasma TG ≥4.5 mmol/L, a direct LDL-C assay was employed. Non-HDL-C was calculated by subtracting HDL-C from TC.

**Genetic analyses**

In patients with FH, genetic analyses were performed in PathWest Laboratory Medicine WA. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), as previously described (517). The MLPA-LDLR kit P062 (MRC-Holland, The Netherlands) was used according to the manufacturer's instructions. Deletions/duplications were confirmed by a second MLPA reaction. M13-tagged primers for the amplification of the 18 exons of the LDLR plus part of APOB exons 26 and 29 were designed de novo using CLC Main Workbench (CLC Bio, Denmark) with reference to the NCBI and the UCL databases. PCSK9 exon 7 primers have been reported previously (519). Sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems) in the forward and reverse directions and aligned using CLC Main Workbench; mutations were described using HGVS nomenclature and reference sequence NM_000384.2 (APOB), AY114155.1 (LDLR) or NM_174936.3 (PCSK9). Mutations were confirmed by sequencing of a second PCR product. Pathogenicity was assessed by reference to published data and for novel variants by in-silico methods using the online tools.
PolyPhen2 (520), SIFT (521), and MutationTaster (522) and determining whether other mutations had been reported at the same position.

In 10% of patients, genetic testing was based on next-generation sequencing, performed by Ion Torrent sequencing using a TargetSet (Life Technologies, Waltham, MA, USA) custom capture panel of lipid genes and polymorphisms, derived from LipidSeq (523). MLPA was performed in all patients with phenotypic DLCNS probable or definite FH in whom Sanger sequencing or next-generation sequencing did not identify a mutation.

**Cardiac imaging**

For patients with FH, cardiac CT scans were performed at the Imaging Service, Royal Perth Hospital (197 Wellington St, Perth WA 6000) on a GE Revolution ACTs EX 256-slide scanner (GE Healthcare, Chicago, IL, USA). Non-contrast-enhanced CT images were acquired using a prospective electrocardiographic triggering on the volumetric mode, and images were reconstructed with 3.0 mm axial slices. Acquisition parameters included a gantry rotation time of 0.28 sec, collimation of 160 mm, tube voltage of 120 kV, and tube current of 60 mA. The CACS images were performed at a temporal resolution of approximately 140 ms and spatial resolution 0.4 mm. The estimated radiation dose was approximately 1 mSv. The 3.0 mm images were loaded into the GE Advantage workstation and calcific plaques in coronary arteries were quantified using the SmartScore 4.7 package (GE Healthcare, Chicago, IL, USA). Agatston scores were calculated according to the method described by Agatston et al. (451).

For controls, cardiac CT scanning was performed at Imaging Central (345 Stirling Hwy, Claremont WA 6010) on a dual-source 128-slice CT scanner equipped with radiation dose reduction systems and appropriate reconstruction algorithms. Two machines were used, a Philips iCT scanner (Koninklijke Philips N.V., Amsterdam, The Netherlands) and a Siemens Somatom Definition AS+ scanner (Siemens Aktiengesellschaft, Munich, Germany).
Images were performed at a temporal resolution of 330 ms and spatial resolution of 0.24 mm. The estimated effective radiation dose was calculated to be 0.52 mSv. On the volumetric mode, images were reconstructed with 3.0 mm axial slices. The scan parameters were 0.3 sec rotation time, tube voltage of 120 kV, 32x1.2 mm beam width and a scanned sequentially (no pitch) with the scanned region covered the heart. The calcific plaques in coronary arteries were quantified on standard commercial workstations using synge Calcium Scoring package (Siemens Aktiengesellschaft, Munich, Germany) and Heartbeat-CS package (Koninklijke Philips N.V., Amsterdam, The Netherlands). Agatston scores were calculated according to the method described by Agatston et al. (451).

4.1.9 Statistical methods

Subjects that met the eligibility criteria were consecutively selected from the CAUGHT-CAD Study baseline and the FHWA database. No sample size calculation was formally carried out, but the sample size was derived from other studies that have shown differences in the CACS between subjects with and without FH (323,324,327). Statistical analyses were performed with the use of STATA software, version 14.1 (StataCorp LLC., College Station, TX, USA). Patients with FH were initially age-matched to within one year of the controls in a 1:1 ratio employing STATA's “ccmatch” command to derive the two groups employed in the analyses. A matching by family history of premature CAD was also carried out for subanalysis. As CACS was a skewed variable and given the high proportion of patients with zero AU, transformation of the data was required. To transform the skewed CACS to approximately conform to normality, different types of data transformations (logarithm, cubic, identity and square root) were attempted without reaching normality.

The prevalence of traditional cardiovascular risk factors was calculated as the presence of the risk factors divided by the total number of the cohort and expressed as a percentage. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. Continuous variables with normal distribution were expressed as means and standard deviations; non-
normal variables were reported as medians and interquartile ranges. Categorical variables were expressed as percentages. Normally distributed continuous variables were compared with paired sample Student's t-test; Wilcoxon matched-pairs signed-rank test was used to compare non-normally distributed continuous variables. The frequencies of categorical variables were compared using Pearson's chi-squared or Fisher's exact test. Univariate and multivariable logistic regression analyses were used to investigate the associations between CACS and cardiovascular risk factors. Significance was defined at the 5% level.

4.1.10 Ethical considerations

This project has been granted ethical approval by the Royal Perth Hospital HREC with number RGS0000000748 (Ethics 6) and governance authorisation by the East Metropolitan Health Service Executive (Ethics 7). The recognition of an ethics approval from a non-UWA Research Ethics Committee was awarded by the UWA Human Ethics Office with file reference RA/4/20/4848 (Ethics 8). All participants signed an informed consent for the use of their de-identified data.

For FH patients, previous ethics approval relies on the consent form of the BioBank for Inherited Disorders of Lipid Metabolism (Ethics 12) and/or the National FH Registry Information and Consent Form for Participants (Ethics 13). For controls, ethics approval was based on the Participant Information Sheet and Consent Form sent to the CAUGHT-CAD Study participants (Ethics 5).
4.2 Results

4.2.1 Subjects characteristics

A total of 472 individuals (361 FH and 111 non-FH) were initially identified. Following age-matching, 218 asymptomatic adults were studied (109 patients with FH and 109 controls). The demographic, clinical and biochemical characteristics of subjects studied are shown in Table 11. The overall proportion of males was 41.3% and the mean age was 54.5 ± 7.4 years, with no significant differences between patients with FH and controls. None of the subjects had a history of stroke or transient ischaemic attack and one patient with FH had a history of peripheral vascular disease.

There were no significant differences in the prevalence of hypertension, smoking, DM, and obesity between FH patients and controls. Compared with controls, patients with FH had a significantly higher pre-statin plasma LDL-C and treated TG concentrations, as well as a higher prevalence of hypercholesterolaemia and tendinous xanthoma. By contrast, the family history of premature CAD was significantly more frequent in controls than in those with FH. There were no significant differences between groups in the treated plasma TC, LDL-C, HDL-C, and non-HDL-C concentrations.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>FH</th>
<th>Non-FH</th>
<th>p value</th>
</tr>
</thead>
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<tr>
<td>Number of subjects</td>
<td>218</td>
<td>109</td>
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<tr>
<td>Male, n (%)</td>
<td>90 (41.3)</td>
<td>44 (40.4)</td>
<td>46 (42.2)</td>
<td>0.783</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.5 ± 7.4</td>
<td>54.5 ± 7.4</td>
<td>54.5 ± 7.4</td>
<td>1.000</td>
</tr>
<tr>
<td>Family history premature CAD, n (%)</td>
<td>137 (62.8)</td>
<td>45 (41.3)</td>
<td>92 (84.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>7 (3.2)</td>
<td>5 (4.6)</td>
<td>2 (1.8)</td>
<td>0.249</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>38 (17.4)</td>
<td>24 (22.0)</td>
<td>14 (12.8)</td>
<td>0.074</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>9 (4.1)</td>
<td>6 (5.5)</td>
<td>3 (2.7)</td>
<td>0.307</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>46 (21.1)</td>
<td>24 (22.1)</td>
<td>22 (20.2)</td>
<td>0.556</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>123 (56.4)</td>
<td>109 (100)</td>
<td>14 (12.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>4.6 (3.8)</td>
<td>7.0 (1.9)</td>
<td>3.2 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.3 (1.4)</td>
<td>5.2 (2.0)</td>
<td>5.3 (1.0)</td>
<td>0.708</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.2 (1.3)</td>
<td>3.0 (1.9)</td>
<td>3.2 (0.9)</td>
<td>0.330</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.2 (1.0)</td>
<td>1.3 (1.2)</td>
<td>1.1 (0.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.4 (0.5)</td>
<td>1.4 (0.4)</td>
<td>1.4 (0.6)</td>
<td>0.127</td>
</tr>
<tr>
<td>Non-HDL-C, mmol/L</td>
<td>3.8 (1.3)</td>
<td>3.8 (2.0)</td>
<td>3.7 (1.0)</td>
<td>0.410</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>62 (28.4)</td>
<td>62 (56.9)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tendinous xanthoma, n (%)</td>
<td>17 (7.8)</td>
<td>17 (15.6)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range).
FH indicates familial hypercholesterolaemia; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

p value for FH versus non-FH.
4.2.1.1 Characteristics of subjects matched by family history of premature coronary artery disease

Following a matching by family history of premature CAD, 124 subjects were included in this subanalysis (62 patients with FH and 62 controls). Demographic, clinical and biochemical characteristics of subjects matched for family history of premature CAD are shown in Table 12.

The overall proportion of males was 37.9% and the mean age was 55.4 ± 7.8 years, with no significant differences between patients with FH and controls. Compared with controls, patients with FH had a significantly higher frequency of statin use, a higher prevalence of hypertension, hypercholesterolaemia and tendinous xanthoma, as well as a significantly higher pre-statin plasma LDL-C and treated TG concentrations. The median CACS was significantly higher in patients with FH than in controls (40.5 AU [IQR 221.0] and 0.0 AU [IQR 17.0], respectively; p <0.001). By contrast, treated plasma HDL-C concentrations were significantly higher in controls than in FH patients. There were no significant differences between groups in the prevalence of DM, smoking and obesity, as well as in the treated plasma TC, LDL-C and non-HDL-C concentrations.

In univariate logistic regression analyses, male sex, smoking, DM, hypertension, and obesity were not significant predictors of a CACS >0 (p = 0.267, p = 0.372, p = 0.534, p = 0.233, and p = 0.560, respectively). By contrast, age and pre-statin plasma LDL-C concentrations were significant predictors of a CACS >0 (p = 0.015 and p = 0.003, respectively). In multivariable logistic regression analysis, age and pre-statin plasma LDL-C concentrations remained independent predictors of a CACS >0 (OR 2.91, 95% CI 1.17 - 7.23; p = 0.004 and OR 1.30, 95% CI 1.10 - 1.54; p = 0.018, respectively); this association was independent of sex and statin therapy.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>FH</th>
<th>Non-FH</th>
<th>p value</th>
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<td>Number of subjects</td>
<td>124</td>
<td>62</td>
<td>62</td>
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</tr>
<tr>
<td>Male, n (%)</td>
<td>47 (37.9)</td>
<td>22 (35.5)</td>
<td>25 (40.3)</td>
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</tr>
<tr>
<td>Age, y</td>
<td>55.4 ± 7.8</td>
<td>55.4 ± 8.2</td>
<td>55.4 ± 7.6</td>
<td>0.990</td>
</tr>
<tr>
<td>Family history of premature CAD, n (%)</td>
<td>90 (72.6)</td>
<td>45 (72.6)</td>
<td>45 (72.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>4 (3.2)</td>
<td>3 (4.8)</td>
<td>1 (1.6)</td>
<td>0.309</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>21 (16.9)</td>
<td>15 (24.2)</td>
<td>6 (9.7)</td>
<td>0.031</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>5 (4.0)</td>
<td>3 (4.8)</td>
<td>2 (3.2)</td>
<td>0.648</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>28 (22.6)</td>
<td>13 (20.1)</td>
<td>15 (24.2)</td>
<td>0.818</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>68 (54.8)</td>
<td>62 (100)</td>
<td>6 (9.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>4.7 (4.1)</td>
<td>7.3 (2.5)</td>
<td>3.1 (0.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1 (1.1)</td>
<td>5.1 (1.3)</td>
<td>5.2 (0.9)</td>
<td>0.885</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.1 (1.2)</td>
<td>2.9 (1.6)</td>
<td>3.1 (0.7)</td>
<td>0.162</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.1 (1.0)</td>
<td>1.3 (1.2)</td>
<td>1.0 (0.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.5 (0.5)</td>
<td>1.3 (0.5)</td>
<td>1.5 (0.5)</td>
<td>0.021</td>
</tr>
<tr>
<td>Non-HDL-C, mmol/L</td>
<td>3.7 (1.1)</td>
<td>3.8 (1.8)</td>
<td>3.7 (1.0)</td>
<td>0.546</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>40 (32.2)</td>
<td>40 (64.5)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tendinous xanthoma, n (%)</td>
<td>10 (8.0)</td>
<td>10 (16.1)</td>
<td>0 (0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Coronary artery calcium score, AU</td>
<td>6.0 (92.3)</td>
<td>40.5 (221.0)</td>
<td>0.0 (17.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range). FH indicates familial hypercholesterolaemia; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and AU, Agatston units. p value for FH versus non-FH.
4.2.2 Genetic testing

Genetic testing was only performed in patients with a phenotypic diagnosis of FH. A pathogenic mutation affecting the LDL receptor pathway was identified in 48 patients (44%). Among those patients, 37 (77.1%) had a mutation in the LDLR gene (59.5% missense, 21.6% non-sense/frameshift, 13.5% splice, 5.4% large deletion/duplication and zero promoter variants). The remainder had familial defective APOB (n = 9) and gain of function mutation in the PCSK9 gene (n = 1).

4.2.3 Cardiac CT scanning

The CACS of subjects in relation to the presence and absence of a phenotypic diagnosis of FH are shown in Table 13. The overall median CACS was 2.9 AU (IQR 60.25). The median CACS in patients with FH was significantly higher compared with controls (21.0 AU [IQR 152.25] and 0.0 AU [IQR 13.10], respectively; p <0.0001) (Table 13 A). The differences remained significant between groups when CACS categories were compared (CACS >0 AU, >100 AU and >200 AU; p <0.001 for all) (Table 13 B). The proportion of patients with and without FH according to the above described categories, are depicted in Figures 10, 11 and 12, respectively.

Comparison of CACS between patients with FH and controls, according to age groups, are shown in Table 14. The CACS increased with age in both groups. Among subjects in the fifth decade of life, CACS was just significantly higher in FH patients than in controls (p = 0.046). In the 51 to 60 and 61 to 70 years of age groups, the CACS was significantly higher in patients with FH than in controls (p <0.001 for both).

There were no significant differences in the median CACS between subjects with and without a family history of premature CAD (0.5 AU [40.50] and 7.0 AU [82.50], respectively; p = 0.262).
### Table 13. Coronary artery calcium score categories of FH and non-FH subjects

#### (A) All subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>FH</th>
<th>95% CI</th>
<th>Non-FH</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACS (AU)</td>
<td>2.9 (60.2)</td>
<td>21.0 (152.2)</td>
<td>6.0 - 41.5</td>
<td>0.0 (13.1)</td>
<td>0.0 - 0.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

#### (B) Subjects categorised by coronary artery calcium score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>FH</th>
<th>95% CI</th>
<th>Non-FH</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACS &gt;0 n (%)</td>
<td>120 (55.0)</td>
<td>75 (68.8)</td>
<td>59.2 - 77.3</td>
<td>45 (41.2)</td>
<td>31.9 - 51.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CACS &gt;100 n (%)</td>
<td>43 (19.7)</td>
<td>32 (29.3)</td>
<td>21.0 - 38.8</td>
<td>11 (10.1)</td>
<td>5.1 - 17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CACS &gt;200 n (%)</td>
<td>24 (11.0)</td>
<td>21 (19.2)</td>
<td>12.3 - 27.9</td>
<td>3 (2.7)</td>
<td>0.5 - 7.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

FH indicates familial hypercholesterolaemia; CI, confidence interval; AU, Agatston units; and CACS, coronary artery calcium score.

p value for FH versus non-FH.

### Table 14. Coronary artery calcium scores of FH and non-FH subjects according to age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>FH</th>
<th>Non-FH</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CACS</td>
<td>n</td>
</tr>
<tr>
<td>31 - 40 y</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>41 - 50 y</td>
<td>33</td>
<td>5.0 (77.5)</td>
<td>34</td>
</tr>
<tr>
<td>51 - 60 y</td>
<td>49</td>
<td>18.0 (75.4)</td>
<td>48</td>
</tr>
<tr>
<td>61 - 70 y</td>
<td>26</td>
<td>156.5 (265.2)</td>
<td>26</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range).

FH indicates familial hypercholesterolaemia; and CACS, coronary artery calcium score.

p value for FH versus non-FH.
Figure 10. Proportion of FH and non-FH subjects with CACS >0

Error bars refer to 95% confidence intervals.
Figure 11. Proportion of FH and non-FH subjects with CACS >100

Error bars refer to 95% confidence intervals.
Figure 12. Proportion of FH and non-FH subjects with CACS >200

Error bars refer to 95% confidence intervals.
4.2.4 Predictors of coronary artery calcification

Predictors of the presence of coronary artery calcification (defined as a CACS >0) in univariate logistic regression analysis are shown in Table 15. Age, pre-statin plasma LDL-C concentrations, statin therapy, and the presence of tendinous xanthoma were significant predictors of a CACS >0 (p = 0.003, p <0.001, p = 0.019, and p = 0.012, respectively). Male sex and family history of premature CAD were not significant predictors of a CACS >0 (p = 0.339 and p = 0.128, respectively).

The multivariable logistic regression model for prediction of the presence of coronary artery calcification (defined as a CACS >0) is shown in Table 16. Age, male sex and pre-statin plasma LDL-C concentrations remained independent predictors of a CACS >0 (p <0.001, p = 0.018 and p = 0.002, respectively). Statin therapy and the presence of tendinous xanthoma were not independent predictors of the presence of CAC.
Table 15. Predictors of coronary artery calcification (defined as CACS >0) in univariate logistic regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05</td>
<td>1.02 - 1.10</td>
<td>0.003</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.30</td>
<td>0.75 - 2.24</td>
<td>0.339</td>
</tr>
<tr>
<td>Pre-statin LDL-C</td>
<td>1.29</td>
<td>1.14 - 1.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tendinous xanthoma</td>
<td>1.37</td>
<td>1.07 - 1.77</td>
<td>0.012</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>2.10</td>
<td>1.13 - 3.89</td>
<td>0.019</td>
</tr>
<tr>
<td>Family history of premature CAD</td>
<td>0.64</td>
<td>0.37 - 1.13</td>
<td>0.128</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; and CAD, coronary artery disease. p value for univariate logistic regression analyses.

Table 16. Multivariable logistic regression model for prediction of coronary artery calcification (defined as CACS >0)

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.03 - 1.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.18</td>
<td>1.14 - 4.16</td>
<td>0.018</td>
</tr>
<tr>
<td>Pre-statin LDL-C</td>
<td>1.30</td>
<td>1.10 - 1.55</td>
<td>0.002</td>
</tr>
<tr>
<td>Tendinous xanthoma</td>
<td>1.17</td>
<td>0.88 - 1.55</td>
<td>0.273</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>0.81</td>
<td>0.34 - 1.87</td>
<td>0.619</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; OR, odds ratio; CI, confidence interval; LDL-C, low-density lipoprotein cholesterol. p value for multivariable logistic regression analysis.
4.3 Discussion

New and main findings
The major finding of the present study is the significantly higher CACS in patients with FH compared with subjects without FH, even after adjusting for family history of premature CAD and other cardiovascular risk factors. This difference applies to patients with a phenotypic diagnosis of FH independently of the presence of a pathogenic mutation affecting the LDL receptor pathway. The difference remained significant comparing within age groups. Age, male sex and plasma LDL-C concentration were independent predictors of a CACS >0; this association was independent of statin therapy and family history of premature CAD.

This is the first Australian study comparing CACS between asymptomatic patients with FH and controls with a family history of CAD. The novelty aspect of the present study lies in the possibility of assessing the impact of a family history of CAD in the coronary atherosclerotic burden. This is important when comparing with a higher risk population as FH patients because most of them have a very strong family history of CAD which could be a confounder.

Previous studies
The prevalence of the presence of subclinical coronary atherosclerosis in FH patients clearly exceed that reported in the general population (524). As shown by previous studies, patients with a phenotypic diagnosis of FH present with a greater prevalence, extension and severity of subclinical coronary atherosclerosis than general population (323,324,326,329). The present study is in line with previous results from a study by Miname et al. (323). Subclinical coronary atherosclerosis was assessed by cardiac CT scan and then compared between 102 FH patients and 35 controls. It is important to note that controls in this Brazilian study had a lower prevalence of family history of premature CAD compared with controls in the present study (31% versus 84.4%, respectively). The mean CACS was significantly higher in FH patients than controls (55 ± 129 AU and 38 ± 140 AU, respectively; p =
CACS was associated with the presence of stenosis and a CACS of zero AU excluded obstructive disease. The burden of coronary atherosclerosis by CTCA was also significantly higher in patients with FH in that study.

Findings from a study by Neefjes et al. (327) also correspond with the present study. A cardiac CT scan including CACS and CTCA was carried out in 101 asymptomatic patients with FH and 126 controls with non-anginal chest pain. The median CACS was significantly higher in FH patients than in controls (87 AU [IQR 406] and 7.3 AU [IQR 125], respectively; p <0.001). The total CACS was higher in FH patients in all age groups and increased with age, which is in line with the findings in the present study. Moreover, the severity and extent of subclinical coronary atherosclerosis, per patient and per segment level, was significantly higher in patients with FH.

A study by Viladés et al. (324) studied 50 consecutive patients with phenotypic diagnosis of FH and a control group of 70 asymptomatic subjects. The prevalence of CAC was significantly higher in FH patients compared with controls (48% and 33%, respectively). That study also shown a significantly higher CACS in FH patients than in controls (260 AU and 46 AU, respectively; p = 0.002). Compared with the present study, the prevalence of CACS was lower for both study groups. However, the overall mean age was also lower in the present study, which could explain the difference. Additionally, instead of reporting the prevalence of family history of premature CAD, they reported the prevalence of family history of CAD in the control group, which was much lower compared with the present study (1.4% and 100%, respectively).

Among patients with a phenotypic diagnosis of FH, the presence of a pathogenic mutation affecting the LDL receptor pathway was associated with a higher CACS compared with those without a mutation, similarly to previous studies (333,335,525). Among patients without FH, the family history of CAD was most likely driven by a cluster of genes that increased the risk of CAD in families (189).
**Limitations and strengths**

The observational study design and the low statistical power due to the convenience sampling are important limitations of the present study. The control group with no previous use of statins and a family history of CVD was included as an opportunistic sample. Although subjects in the control group did not have a phenotypic diagnosis of FH despite having a family history of CAD, they were not genetically tested for mutations in the LDL receptor pathway. Hence, the genetic basis of these subjects is unknown. Further, these patients were highly selected and will not represent the typical population of Australia.

The higher CACS and the prevalence of a family history of premature CAD in patients with FH were most likely driven by the high plasma LDL-C concentrations as a consequence of a monogenic disorder affecting the LDL receptor pathway from birth. Among controls, the higher prevalence of a family history of premature CAD was most likely driven by several genes (polygenic disorder) that increased the risk of CAD. These genes would include those in relation with Lp(a) metabolism (e.g. LPA), physiological pathways related to TG-rich lipoproteins (e.g. LPL, TRIB1 and APOA5), inflammation (e.g. IL6R and CXCL12), cellular proliferation and vascular remodelling (e.g. COL4A1-COL4A2 and MIA3), and vascular tone and nitric oxide signalling (e.g. GUCY1A3, EDNRA and NOS3) (189). However, it appears that the polygenic disorder that increased the risk of CAD alone is not as powerful as the monogenic disorder that increases cholesterol.

In FH patients, the statin therapy in more than a half of them, could partially lead to an increase in the CACS (237–240,512) which could act as a confounder. Another limitation was that the age of onset of premature CAD in the relatives of subjects studied was not available for further analyses. Additionally, the overall proportion of women was higher due to the survival bias of the cohort. This bias exits because male sex is associated with a higher risk of CAD events. Therefore, it was expected to have more women because only asymptomatic subjects were included. An additional limitation is that only the CACS was used to assess coronary atherosclerosis. This did
not allow the evaluation of non-calcific plaques that are not visible on non-contrast cardiac CT scans, considering that the calcific components represent only 20% of the total atherosclerotic burden (513–515).

Strengths of the present study include the relatively high number of participants, the inclusion of asymptomatic and no related participants with HeFH, the exclusion of HoFH and double heterozygous patients, and the age-matching. The availability of genetic testing for all the FH participants was also a strength. An additional strength was that analyses were adjusted by family history of CAD, suggesting that factors beyond just the family history are causally related to the presence and extent of coronary artery calcification. Another strength was the similarity in sex and in the prevalence of traditional cardiovascular risk factors between groups, with exception of pre-statin plasma LDL-C concentrations.

Scope for further work and practical implications
Further studies ought to be multicentre and population-based including a larger sample size from diverse ethnic groups. Future longitudinal studies are required to assess whether the estimation of CACS might improve the risk stratification in patients with FH, as currently recommended in the general population (355). Non-calcific coronary atherosclerosis and luminal obstruction should be explored using CTCA, particularly in patients with FH. Measurements derived from the CACS estimation such as the number of lesions and vessels affected, lesion size, volume, and plaque density, should also be incorporated in future studies. Investigations by environmental factors, elevated Lp(a), polygenic risk scores and protective genes related to CAD, should also be undertaken.

Future studies should include younger patients with FH to determine the age when a cardiac CT scan should be carried out in this high-risk population. Currently, the ideal age to start coronary artery screening in patients with FH is uncertain. The results from the present and previous studies (326) suggest that it should be approximately in the fourth decade of life, especially in men with a pathogenic mutation affecting the LDL receptor pathway. However,
other non-invasive imaging methods no requiring ionising radiation such as carotid ultrasound, might be used in younger adults with FH to assess the presence of atherosclerosis and carotid intima-media thickness (277).
4.4 Conclusion

Among middle-aged asymptomatic subjects with no history of CAD, those with a phenotypic diagnosis of FH had a significantly greater coronary atherosclerosis, as assessed by CACS on cardiac CT scan, compared with non-FH subjects. This association was independent of age, sex, obesity, DM, hypertension, smoking, treated plasma lipid and lipoprotein concentrations, and family history of premature CAD.

This difference in the burden of coronary atherosclerosis could be attributed to cholesterol life-year in FH patients. This is supported by pre-statin LDL-C concentrations, age and male sex as independent predictors of the presence of CAC. Statin therapy was not a significant predictor of the presence of CAC and was likely not a confounder of the results. Given this, cholesterol is probably a more powerful predictor of subclinical coronary atherosclerosis than other familial and non-familial cardiovascular risk factors. This suggest that coronary risk in patients with FH is not determined by a family history of CAD perse, but by cumulative burden of plasma LDL-C present from an early age before detection and cholesterol-lowering therapy initiation. Therefore, in patients with phenotypic FH, the non-invasive estimation of CAC could be employed as a safe initial screening method for coronary atherosclerosis.
Chapter Five: Coronary Artery Calcium in Asymptomatic Patients with Familial Hypercholesterolaemia with and without a Genetic Mutation
ABSTRACT

Background and aims: Patients with FH have an 8-fold greater risk of premature CAD compared with non-FH individuals. This risk is substantially higher in FH mutation carriers compared with non-carriers. The non-invasive quantification of CACS using cardiac CT scanning has emerged as the most direct and reliable marker that strongly predicts CAD events beyond traditional cardiovascular risk factors. The aim was to compare the atherosclerotic burden, quantified as CACS, between asymptomatic patients with phenotypic FH with and without a pathogenic mutation affecting the LDL receptor pathway.

Methods: An age-matched case-control study was undertaken including unrelated patients with a DLCNS >5 selected from the Lipid Disorders Clinic at Royal Perth Hospital. Cases were patients with a pathogenic mutation affecting the LDL receptor pathway (LDLR, APOB or PCSK9 genes) and controls were those without a mutation. The median CACS was compared between groups using the Wilcoxon matched-pairs signed-rank test. Univariate and multivariable logistic regression analyses were used to investigate the associations between CACS and cardiovascular risk factors.

Results: One hundred and ninety-eight patients were studied (99 cases and 99 controls). The mean age was 50.9 ± 10 years and 39.9% were male. The median CACS in patients with a mutation was significantly higher compared with controls (26.0 AU [IQR 115.0] and 0.5 AU [IQR 41.0], respectively; p = 0.029). There were no significant group differences in the proportion of men and current smokers, as well as in the prevalence of type 2 DM, hypertension, family history of premature CAD, and obesity. Mutation positive patients had a significantly higher frequency of tendinous xanthoma, higher pre-statin LDL-C concentrations and phenotypic DLCNS, compared with controls (p <0.001 for all). The treated plasma TC, LDL-C, HDL-C, non-HDL-C and Lp(a) concentrations were not significantly different between groups. Among mutation positive patients, 80 (80.8%) had a mutation in the LDLR gene, 15 patients (15.1%) in the APOB gene and one patient (1.0%) a gain of
function mutation in the PCSK9 gene. Patients with a mutation in the LDL receptor pathway were more frequently on statin therapy, however, statin use was not a significant predictor of the presence of CAC.

**Conclusion:** Among middle-aged asymptomatic patients with a phenotypic diagnosis of FH, the presence of a pathogenic mutation affecting the LDL receptor pathway was associated with a higher degree of subclinical coronary atherosclerosis, as assessed by CACS. This association was independent of statin therapy, treated plasma lipid and lipoprotein concentrations, and other cardiovascular risk factors. In spite of cholesterol-lowering therapy, the presence of a genetic diagnosis of FH predicts those at greater risk of CAD. It appears, therefore, that the presence of coronary atherosclerosis is determined primarily by the level of LDL-C which is in turn dependent on the presence of a pathogenic mutation affecting the LDL receptor pathway. Given this, a genetic test should be offered to patients with a phenotypic diagnosis of FH to stratify their risk of CAD. Genetic testing may also be employed to select and direct the intensity of LDL-C lowering and to enable more precise cascade testing of family member for FH.
5.1 Subjects and Methods

5.1.1 Study design

A single centre age-matched case-control study was undertaken. Unrelated patients with a phenotypic diagnosis of FH, with (cases) and without (controls) a pathogenic mutation affecting the LDL receptor pathway were studied. CACS, as assessed on cardiac CT scanning, was compared between cases and controls. Age-matching was carried out in order to ensure equal numbers of cases and controls in each age stratum.

5.1.2 Study population

The study population consisted of asymptomatic adult patients with phenotypic diagnosis of FH derived from the Lipid Disorders Clinic at Royal Perth Hospital (Perth, Western Australia) between 2008 and 2018. Patients with suspected FH were recruited via referral from general practice, from coronary care or from private specialists. The most common reasons for suspecting FH were an elevated plasma LDL-C concentration with a family history of premature CAD. If the diagnosis was at least possible FH (DLCNS >3), they were offered genetic testing with appropriate counselling and informed written consent.

5.1.3 Study variables

The demographic and clinical characteristics of the patients included date of birth, sex, height, weight, presence of tendinous xanthoma (bilateral, subcutaneous nodules on Achilles tendons or at ligamentous insertions), arcus cornealis, history of CAD, hypertension, DM, smoking status, and family history of premature CAD in first-degree relatives. Cholesterol-lowering medications were also recorded. Partial statin intolerance was defined as any patient self-reported intolerance even if a lower dose or other statin was tolerated as recorded elsewhere (284,517,526).

Laboratory variables comprised pre-statin plasma LDL-C concentrations and the treated lipid and lipoprotein analyses closest to the date of the cardiac CT scan (TC, TG, HDL-C, LDL-C, and Lp(a) concentrations). From the cardiac
CT scan reports, date and CACS in AU were collected together with the phenotypic DLCNS (259,264).

The presence, type and description of a pathogenic mutation affecting the LDL receptor pathway was obtained from the genetic reports. In cases when a gene variant of uncertain (or unknown) significance was reported, the genetic diagnosis was assumed as mutation negative (517).

5.1.4 Inclusion criteria

1) Patients who were aged ≥18 years.
2) Had phenotypic diagnosis of FH (DLCNS >5).
3) Had undergone a cardiac CT scan for CAC scoring.
4) Were genetically tested for a mutation affecting the LDL receptor pathway.

5.1.5 Exclusion criteria

1) HoFH, compound and double HeFH.
2) History of symptomatic CAD (defined as previous myocardial infraction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery).
3) Inability to provide informed consent.

5.1.6 Data sources

The primary source for obtaining the data was the FHWA Access database (Microsoft Corp., Redmond, WA, USA) from the Lipid Disorders Clinic at Royal Perth Hospital. When data were not obtainable from the FHWA database, hospital clinical records were accessed.

5.1.7 Development of database

Before creating the database, the design of the structure of the data elements was performed by creating a coding manual. Each of the variables in the dataset was coded and the data validation tool was used to prevent input errors. Once created, structure and integrity were tested through data entry tests.
Data from the FHWA database were extracted in non-identifiable format by the FHWA Project Coordinator in a password-protected Excel 2016 file (Microsoft Corp., Redmond, WA, USA); the password was sent by a separate text message.

5.1.8 Data acquisition process

Data from the FHWA cohort were collected retrospectively by the FHWA Project Coordinator from the clinical records and stored in the FHWA database at the Medical Research Foundation. Data of patients who met the specific eligibility criteria were collected retrospectively, covering a 10-year period (2008 - 2018).

Dataset was regularly profiled to check for missing and spurious data and against duplicate measurements. Information quality assurance measures were applied to regularly profile the data to discover inconsistencies. After database closure, data cleaning was performed. The definite dataset was stored as a password-protected Excel file on a University of Western Australia networked drive at the Medical Research Foundation, Royal Perth Hospital.

Clinical measurements and risk assessment

From key cardiovascular risk factors identified by the Framingham study (241), the following were recorded:

1) Hypertension: defined as a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or on antihypertensive medications (369).
2) Type 2 DM: defined as a fasting blood glucose ≥7 mmol/L (on two separate occasions) or haemoglobin A1c ≥6.5% (48 mmol/mol) (on two separate occasions) (504).
3) Current smoker or ex-smoker who quit within last year.
4) Hypercholesterolaemia: defined as an LDL-C >4 mmol/L or on cholesterol-lowering medication.
5) Family history of premature CAD in first-degree relative: defined as age of onset <55 years for men and <60 years for women.
6) Obesity: defined as a BMI >30 kg/m² (518).
7) Sex.

**Biochemical analyses**

Plasma lipid and lipoprotein analyses were performed in PathWest Laboratory Medicine WA (196 Goderich Street, Perth WA 6000 - Barry Marshall Parade, Murdoch WA 6150). Venous blood was collected after 12 hours fast by a phlebotomist into EDTA or lithium heparin tubes. Plasma TC, TG and HDL-C concentrations were measured by standard enzymatic methods. LDL-C concentrations were calculated using the Friedewald Equation (505), but in patients with plasma TG ≥4.5 mmol/L, a direct LDL-C assay was employed. Non-HDL-C was calculated by subtracting HDL-C from TC. Total apolipoprotein B and Lp(a) were measured by a latex-enhanced immunoassay considered to be independent of apoA isoform size (Quantia Lp(a) assay and standard) (527). Assays were run using the ARCHITECT c16,000 platform (Abbott Laboratories, Abbott Park, IL, USA), with resulting inter and intra-assay coefficients of variation of less than 5%.

**Genetic analyses**

Genetic analyses were performed in PathWest Laboratory Medicine WA. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), as previously described (517). The MLPA-LDLR kit P062 (MRC-Holland, The Netherlands) was used according to the manufacturer's instructions. Deletions/duplications were confirmed by a second MLPA reaction. M13-tagged primers for the amplification of the 18 exons of the LDLR plus part of APOB exons 26 and 29 were designed de novo using CLC Main Workbench (CLC Bio, Denmark) with reference to the NCBI and the UCL databases. PCSK9 exon 7 primers have been reported previously (519). Sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems) in the forward and reverse directions and aligned using CLC Main Workbench; mutations were described using HGVS nomenclature and reference sequence NM_000384.2 (APOB), AY114155.1 (LDLR) or NM_174936.3 (PCSK9). Mutations were confirmed by sequencing of a second PCR product. Pathogenicity was assessed by reference to published data and for
novel variants by in-silico methods using the online tools PolyPhen2 (520), SIFT (521), and MutationTaster (522) and determining whether other mutations had been reported at the same position.

In 10% of patients, genetic testing was based on next-generation sequencing, performed by Ion Torrent sequencing using a TargetSet (Life Technologies, Waltham, MA, USA) custom capture panel of lipid genes and polymorphisms, derived from LipidSeq (523). MLPA was performed in all patients with phenotypic DLCNS probable or definite FH in whom Sanger sequencing or next-generation sequencing did not identify a mutation.

**Cardiac imaging**

Cardiac CT scans were performed at the Imaging Service, Royal Perth Hospital (197 Wellington St, Perth WA 6000) on a GE Revolution ACTs EX 256-slide scanner (GE Healthcare, Chicago, IL, USA). Non-contrast-enhanced CT images were acquired using a prospective electrocardiographic triggering on the volumetric mode, and images were reconstructed with 3.0 mm axial slices. Acquisition parameters included a gantry rotation time of 0.28 sec, collimation of 160 mm, tube voltage of 120 kV, and tube current of 60 mA. The CACS images were performed at a temporal resolution of approximately 140 ms and spatial resolution 0.4 mm. The estimated radiation dose was approximately 1 mSv. The 3.0 mm images were loaded into the GE Advantage workstation and calcific plaques in coronary arteries were quantified using the SmartScore 4.7 package (GE Healthcare, Chicago, IL, USA). Agatston scores were calculated according to the method described by Agatston et al. (451).

**5.1.9 Statistical methods**

Patients who met the eligibility criteria were consecutively selected from the FHWA database. No sample size calculation was formally carried out, but the sample size was derived from other studies that have shown differences in the CACS between patients with and without a pathogenic mutation affecting the LDL receptor pathway (326,333,335,525). Statistical analyses were performed with the use of STATA software, version 14.1 (StataCorp LLC.,
College Station, TX, USA). Patients with a pathogenic mutation affecting the LDL receptor pathway were initially age-matched to within one year of the controls in a 1:1 ratio employing STATA's “ccmatch” command to derive the two groups employed in the analyses. As CACS was a skewed variable and given the high proportion of patients with zero AU, transformation of the data was required. To transform the skewed CACS to approximately conform to normality, different types of data transformations (logarithm, cubic, identity and square root) were attempted without reaching normality (Appendix 6).

The prevalence of traditional cardiovascular risk factors was calculated as the presence of the risk factors divided by the total number of the cohort and expressed as a percentage. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test. Continuous variables with normal distribution were expressed as means and standard deviations; non-normal variables were reported as medians and interquartile ranges. Categorical variables were expressed as percentages. Normally distributed continuous variables were compared with paired sample Student's t-test; Wilcoxon matched-pairs signed-rank test was used to compare non-normally distributed continuous variables. The frequencies of categorical variables were compared using Pearson's chi-squared or Fisher's exact test. Univariate and multivariable logistic regression analyses were used to investigate the associations between CACS and cardiovascular risk factors. Significance was defined at the 5% level.

5.1.10 Ethical considerations

The study was approved by the Royal Perth Hospital HREC with reference number EC 2012/063 (Ethics 9 and 10). The previous ethics approval relies on the consent form of the BioBank for Inherited Disorders of Lipid Metabolism (Ethics 12) and/or the National FH Registry Information and Consent Form for Participants (Ethics 13). The recognition of an ethics approval from a non-UWA Research Ethics Committee was awarded by the UWA Human Ethics Office with file reference RA/4/20/4866 (Ethics 11). All participants signed an informed consent for the use of their de-identified data.
5.2 Results

5.2.1 Patients characteristics

A total of 299 patients with phenotypic FH (153 mutation positive and 146 mutation negative) were initially identified. Following age-matching, 198 patients (99 mutation positive and 99 controls) were studied. The demographic, clinical and biochemical characteristics of patients are shown in Table 17.

The mean age was 50.9 ± 10.0 years and the overall proportion of males was 39.9%, with no significant differences between FH mutation positive patients and controls. Compared with controls, statin therapy was significantly more frequent in FH mutation positive patients. The most commonly prescribed statins were atorvastatin (22.2%), rosuvastatin (20.7%) and simvastatin (8.1%). Pre-statin plasma LDL-C concentrations and phenotypic DLCNS, as well as the frequency of tendinous xanthoma, were significantly higher in FH mutation positive patients than controls. By contrast, treated plasma TG concentrations were significantly higher in FH mutation negative patients than in those with a mutation.

There were no significant differences between groups in the prevalence of DM, hypertension, smoking, obesity, and family history of premature CAD, as well as in the proportion of patients with arcus cornealis and partial statin intolerance. There were also no significant differences in treated plasma TC, LDL-C, HDL-C, non-HDL-C, Lp(a), and apolipoprotein B concentrations between FH mutation positive patients and controls.

5.2.2 Genetic testing

Among patients with a pathogenic mutation affecting the LDL receptor pathway, 82 (82.8%) had a mutation in the LDLR gene (60.0% missense, 17.5% non-sense/frameshift, 15.0% splice, 6.2% large deletion/duplication and 1.2% promoter variants). The remainder had familial defective APOB (n = 16) and gain of function mutation of PCSK9 gene (n = 1).
Table 17. Demographic, clinical and biochemical characteristics of patients with familial hypercholesterolaemia in relation to the absence and presence of a pathogenic mutation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Mutation -</th>
<th>Mutation +</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>198</td>
<td>99</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>79 (39.9)</td>
<td>38 (38.4)</td>
<td>41 (41.4)</td>
<td>0.663</td>
</tr>
<tr>
<td>Age, y</td>
<td>50.9 ± 10.0</td>
<td>50.9 ± 10.0</td>
<td>50.9 ± 10.0</td>
<td>1.000</td>
</tr>
<tr>
<td>Family history premature CAD, n (%)</td>
<td>88 (44.4)</td>
<td>45 (45.4)</td>
<td>43 (43.4)</td>
<td>0.775</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>12 (6.0)</td>
<td>6 (6.0)</td>
<td>6 (6.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>39 (19.7)</td>
<td>19 (19.2)</td>
<td>20 (20.2)</td>
<td>0.858</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>14 (7.0)</td>
<td>5 (5.0)</td>
<td>9 (9.0)</td>
<td>0.267</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>38 (19.2)</td>
<td>20 (20.2)</td>
<td>18 (18.2)</td>
<td>0.687</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8 (5.4)</td>
<td>26.7 (5.5)</td>
<td>27.2 (5.6)</td>
<td>0.648</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>103 (52.0)</td>
<td>39 (39.4)</td>
<td>64 (64.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Partial statin intolerance, n (%)</td>
<td>81 (40.9)</td>
<td>46 (46.4)</td>
<td>35 (35.3)</td>
<td>0.112</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>7.0 (1.8)</td>
<td>6.5 (1.7)</td>
<td>7.7 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.8 (2.3)</td>
<td>5.8 (2.8)</td>
<td>5.7 (2.3)</td>
<td>0.974</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.6 (2.3)</td>
<td>3.5 (2.5)</td>
<td>3.7 (2.1)</td>
<td>0.247</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.4 (1.1)</td>
<td>1.7 (1.1)</td>
<td>1.2 (0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.4 (0.6)</td>
<td>1.4 (0.5)</td>
<td>1.4 (0.5)</td>
<td>0.380</td>
</tr>
<tr>
<td>Non-HDL-C, mmol/L</td>
<td>4.4 (2.3)</td>
<td>4.4 (2.6)</td>
<td>4.4 (1.9)</td>
<td>0.765</td>
</tr>
<tr>
<td>Lipoprotein(a), g/L</td>
<td>0.2 (0.7)</td>
<td>0.3 (0.8)</td>
<td>0.2 (0.4)</td>
<td>0.063</td>
</tr>
<tr>
<td>Apolipoprotein B-100, g/L</td>
<td>1.1 (0.6)</td>
<td>1.0 (0.7)</td>
<td>1.1 (0.4)</td>
<td>0.597</td>
</tr>
<tr>
<td>Tendinous xanthoma, n (%)</td>
<td>27 (13.6)</td>
<td>3 (3.0)</td>
<td>24 (24.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arcus cornealis, n (%)</td>
<td>75 (38.3)</td>
<td>34 (34.3)</td>
<td>41 (41.4)</td>
<td>0.305</td>
</tr>
<tr>
<td>Phenotypic DLCN criteria score</td>
<td>8.0 (5.0)</td>
<td>8.0 (3.0)</td>
<td>11.0 (7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Probable FH, n (%)</td>
<td>105 (53.0)</td>
<td>72 (72.7)</td>
<td>33 (33.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Definite FH, n (%)</td>
<td>93 (46.9)</td>
<td>27 (27.2)</td>
<td>66 (66.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range). CAD indicates coronary artery disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; DLCN, Dutch Lipid Clinic Network; and FH, familial hypercholesterolaemia.

p value for FH mutation negative (-) versus FH mutation positive (+).
5.2.3 Cardiac CT scanning

The CACS of patients with FH in relation to the absence and presence of a pathogenic mutation affecting the LDL receptor pathway are shown in Table 18. The overall median CACS was 4.90 AU (IQR 79.50). The median CACS was significantly higher in FH mutation positive patients than controls (26.00 AU [IQR 115.00] and 0.50 AU (IQR 41.00), respectively; \( p = 0.029 \)) (Table 18 A).

Overall, CAC was detected in more than a half of the patients (58%). The prevalence of a CACS >0 AU was significantly higher in FH mutation positive patients than controls (65.6% and 50.5%, respectively; \( p = 0.031 \)). There were no significant differences in the prevalence of CACS >100 AU and >200 AU between groups (Table 18 B). The proportion of FH patients with a CACS >0 AU and >100 AU, according to the absence and presence of a pathogenic mutation affecting the LDL receptor pathway, are depicted in Figures 13 and 14, respectively.

The CACS of FH patients with and without a pathogenic mutation affecting the LDL receptor pathway, according to age groups, is shown in Table 19. FH mutation positive patients between 51 and 70 years of age, had a significantly higher CACS compared with controls (\( p = 0.029 \)). In the 31 to 50 years of age group, CACS tended to be higher in FH mutation positive patients than controls, but the difference just failed to meet statistical significance (\( p = 0.059 \)) (Table 19 A). In the 31 to 50 years of age group, FH mutation positive patients had a significantly higher proportion of a CACS >0 than controls (\( p = 0.049 \)). There were no significant differences between groups in the proportion of patients with a CACS >0 in the 51 to 70 years of age group (Table 19 B).
Table 18. Coronary artery calcium score of patients with familial hypercholesterolaemia in relation to the absence and presence of a pathogenic mutation

(A) All patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Mutation -</th>
<th>95% CI</th>
<th>Mutation +</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACS (AU)</td>
<td>4.9 (79.5)</td>
<td>0.5 (41.0)</td>
<td>0.0-10.6</td>
<td>26.0 (115.0)</td>
<td>4.2-40.7</td>
<td>0.029</td>
</tr>
</tbody>
</table>

(B) Patients categorised by coronary artery calcium score

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Mutation -</th>
<th>95% CI</th>
<th>Mutation +</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CACS &gt;0, n (%)</td>
<td>115 (58.0)</td>
<td>50 (50.5)</td>
<td>40.2-60.7</td>
<td>65 (65.6)</td>
<td>55.4-74.9</td>
<td>0.031</td>
</tr>
<tr>
<td>CACS &gt;100, n (%)</td>
<td>43 (21.7)</td>
<td>17 (17.1)</td>
<td>10.3-26.0</td>
<td>26 (26.2)</td>
<td>17.9-36.0</td>
<td>0.121</td>
</tr>
<tr>
<td>CACS &gt;200, n (%)</td>
<td>28 (14.1)</td>
<td>11 (11.1)</td>
<td>5.6-19.0</td>
<td>17 (17.1)</td>
<td>10.3-26.0</td>
<td>0.221</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range).
CACS indicates coronary artery calcium score; AU, Agatston units; CI, confidence interval; and FH, familial hypercholesterolaemia.

p value for FH mutation negative versus FH mutation positive.

Table 19. Coronary artery calcium scores of patients with familial hypercholesterolaemia according to age groups

(A) All patients

<table>
<thead>
<tr>
<th>Age group</th>
<th>Mutation -</th>
<th>Mutation +</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CACS</td>
<td>n</td>
</tr>
<tr>
<td>31 - 50 y</td>
<td>46</td>
<td>0.0 (15.5)</td>
<td>46</td>
</tr>
<tr>
<td>51 - 70 y</td>
<td>50</td>
<td>9.5 (159.9)</td>
<td>50</td>
</tr>
</tbody>
</table>

(B) Patients with CACS >0

<table>
<thead>
<tr>
<th>Age group</th>
<th>CACS &gt;0 Mutation -</th>
<th>CACS &gt;0 Mutation +</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>31 - 50 y</td>
<td>18</td>
<td>40.0</td>
<td>28</td>
</tr>
<tr>
<td>51 - 70 y</td>
<td>31</td>
<td>62.0</td>
<td>36</td>
</tr>
</tbody>
</table>

Values are expressed as median (interquartile range).
CACS indicates coronary artery calcium score; and FH, familial hypercholesterolaemia.
Figure 13. Proportion of patients with CACS >0 according to the absence and presence of a pathogenic mutation

Error bars refer to 95% confidence intervals.
Figure 14. Proportion of patients with CACS >100 according to the absence and presence of a pathogenic mutation

Error bars refer to 95% confidence intervals.
5.2.4 Predictors of coronary artery calcification

Predictors of coronary artery calcification (defined as CACS >0) in univariate logistic regression are shown in Table 20. Age, pre-statin plasma LDL-C concentration, phenotypic DLCNS, and the presence of tendinous xanthoma and a pathogenic mutation affecting the LDL receptor pathway were significant predictors of a CACS >0 (p <0.001, p = 0.009, p <0.001, p = 0.001, and p = 0.031, respectively). Statin therapy, male sex and a family history of premature CAD were not significant predictors of a CACS >0 (p = 0.229, p = 0.973 and p = 0.541, respectively).

The multivariable logistic regression model for prediction of the presence of coronary artery calcification (defined as CACS >0) is shown in Table 21. Age and pre-statin plasma LDL-C concentration remained independent predictors of a CACS >0 (p <0.001 and p = 0.049, respectively). Male sex, statin therapy and the presence of a pathogenic mutation affecting the LDL receptor pathway were not independent predictors of the presence of coronary artery calcifications.
### Table 20. Predictors of coronary artery calcification (defined as CACS >0) in univariate logistic regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.05 - 1.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.01</td>
<td>0.56 - 1.79</td>
<td>0.973</td>
</tr>
<tr>
<td>Pre-statin LDL-C</td>
<td>1.25</td>
<td>1.05 - 1.48</td>
<td>0.009</td>
</tr>
<tr>
<td>Tendinous xanthoma</td>
<td>1.49</td>
<td>1.17 - 1.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Phenotypic DLCN criteria score</td>
<td>1.18</td>
<td>1.08 - 1.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>1.41</td>
<td>0.80 - 2.49</td>
<td>0.229</td>
</tr>
<tr>
<td>Family history of premature CAD</td>
<td>0.83</td>
<td>0.47 - 1.47</td>
<td>0.541</td>
</tr>
<tr>
<td>Pathogenic mutation LDL receptor pathway</td>
<td>1.87</td>
<td>1.05 - 3.31</td>
<td>0.031</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; LDL-C, low-density lipoprotein cholesterol; DLCN, Dutch Lipid Clinic Network; CI, confidence interval; and, CAD, coronary artery disease.

p value for univariate logistic regression analyses.

### Table 21. Multivariable logistic regression models for prediction of coronary artery calcification (defined as CACS >0)

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.09</td>
<td>1.05 - 1.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.80</td>
<td>0.90 - 3.59</td>
<td>0.093</td>
</tr>
<tr>
<td>Pre-statin LDL-C</td>
<td>1.14</td>
<td>1.01 - 1.29</td>
<td>0.049</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>1.13</td>
<td>0.59 - 2.16</td>
<td>0.706</td>
</tr>
<tr>
<td>Pathogenic mutation LDL receptor pathway</td>
<td>1.66</td>
<td>0.83 - 3.33</td>
<td>0.150</td>
</tr>
</tbody>
</table>

CACS indicates coronary artery calcium score; and, LDL-C, low-density lipoprotein cholesterol.

p value for multivariable logistic regression analysis.
5.3 Discussion

New and main findings
The major finding of the present study was that subclinical coronary atherosclerosis as assessed by CACS using cardiac CT scanning, was significantly higher in asymptomatic patients with an identified pathogenic mutation affecting the LDL receptor pathway than in those without a mutation. This association was independent of age, sex, DM, hypertension, obesity, smoking, family history of premature CAD, treated plasma cholesterol concentrations, and statin therapy.

There was a higher plasma TG concentration in patients without a mutation than in those with a mutation. However, there were no significant differences in the non-HDL-C concentrations between groups. The difference in the plasma TG concentration could be explained by a presence of familial combined hyperlipidaemia in patients without a mutation. The present study is the first Australian comparison of CACS in asymptomatic patients with FH according to the presence and absence of a pathogenic mutation affecting the LDL receptor pathway.

Previous studies
The presence of a pathogenic mutation in patients with FH has been associated with premature and higher risk of CAD events compared with the absence of a mutation (528). However, whether the presence of a mutation contributes to the increased coronary atherosclerotic burden by the non-invasive estimation of CACS, remains to be established in this population. Several studies have assessed the coronary atherosclerotic burden using cardiac CT scan in patients with FH (323–329,333). Nevertheless, few previous studies have examined the effect of a pathogenic mutation affecting the LDL receptor pathway on CACS.

A study by Ten Kate et al. (333) evaluated the influence of a mutation in the \(LDLR\) gene on coronary atherosclerosis in asymptomatic patients with HeFH. The CACS in 86 patients with a mutation in the \(LDLR\) gene were compared
with non-age-matched 59 mutation negative patients. Different from the present study, patients with a mutation were significantly older than those without a mutation. In agreement with the present study, CACS was significantly higher in the mutation carriers than in non-carriers ($p = 0.038$). However, the proportion of patients with a CACS >100 AU was higher in mutation-negative patients than in those with a mutation, whereas in the present study, there were no significant differences between groups. This discrepancy could be explained by a higher pre-statin plasma TC and treated LDL-C concentrations in mutation-negative patients. By contrast, in the present study, the pre-statin plasma TC concentration was higher in the FH mutation-positive patients, as well as the frequency of tendinous xanthoma. In the study by Ten Kate et al, no patients with CACS <100 AU had obstructive lesions on CTCA, supporting that low CACS is highly predictive of the absence of obstructive CAD (323,329); However, this is related to a lower patients age compared with the present study.

A study by Clarke et al. (334) included 112 FH mutation-positive and 92 FH mutation-negative patients diagnosed with Simon Broome criteria. Patients with FH mutation-positive had a higher CACS than those without a mutation but the difference was not significant (280 AU and 55 AU, respectively; $p = 0.16$). Their findings are different and not comparable to the present study that employed DLCN criteria for the phenotypic diagnosis of FH. Another study by Galaska et al. (335) compared 89 FH and 50 non-FH patients who underwent CT with coronary and thoracic aorta calcium scoring. No significant differences were found in the CACS between FH and non-FH patients. However, after adjusting for age, sex, smoking, blood pressure, DM, and plasma LDL-C concentrations, the presence of a pathogenic mutation affecting the LDL receptor pathway was an independent predictor of having non-zero ascending aorta calcium score, high descending aorta calcium score and high CACS. In a study by Chaudhuri et al. (525), CACS was compared between 24 FH mutation positive patients and 24 gender- and age-matched patients without a mutation. In spite of having similar treated TC and LDL-C concentrations between groups, similar to the present study,
CACS was significantly higher in patients with a pathogenic mutation affecting the LDL receptor pathway compared with those without a mutation.

A study by Pérez de Isla et al. (326) included 440 asymptomatic molecularly defined FH patients from the SAFEHEART cohort. Although no comparison between FH patients according to the absence or presence of a mutation was undertaken, the results are comparable with the present study. The prevalence of a CACS >0 was 56%, comparable to 58% in the present study. Remarkably and in the same way that in the above-mentioned study (333), only one patient (0.2%) with a CACS of zero had at least one coronary stenosis ≥50% on CTCA.

Neefjes et al. (329) reported the presence of a CACS >0 in 80% of 140 asymptomatic FH patients, 66% of them genetically diagnosed. This higher prevalence compared with the present study may be explained by the higher proportion of male patients (64% versus 40%), the presence of a mutation in 2/3 of the patients and the slightly older population. In other study by Gallo et al. (325), 112 genetically diagnosed HeFH index patients were studied. The overall mean age was 45 years and 50% were males; the prevalence of a CACS >0 was 58%, similar to the present study.

Patients with a pathogenic mutation affecting the LDL receptor pathway have been reported to have a 3-fold higher risk of premature CAD, as well as earlier onset and greater disease severity than mutation-negative patients (528–530). Tada et al. (531) also demonstrated that FH mutation-positive patients had 3- to 4-fold higher odds of developing CAD than patients without a mutation, although the findings revealed an additive effect of the presence of clinical FH signs as xanthoma and/or family history of CAD. Previous prospective studies also shown that clinical signs of FH were associated with higher risk of CAD events in patients with a genetic diagnosis of FH (532,533). Similarly, in the present study, there was a significantly higher frequency of tendinous xanthoma in patients with a pathogenic mutation compared with those without a mutation. Mangili et al (534) also showed that the presence of Achilles tendinous xanthomas were independently
associated with the existence of subclinical coronary atherosclerosis quantified as CACS in FH patients. Comparably, the present of tendinous xanthoma was a significant predictor of the presence of CAC in the present study. With this in view, the importance of the finding in the present study is that the preclinical coronary atherosclerotic burden, as assessed by CACS on cardiac CT scanning, is consistent with the known higher risk of CAD in FH mutation carriers.

**Limitations and strengths**

The limitations of the present study are mainly related to the observational design. The no inclusion of a non-FH control group and the lack of statistical power due to the convenience sampling are important limitations.

The coronary atherosclerotic burden in patients younger than 45 years of age could not be explored in the present study. Additionally, the proportion of women was higher due to the survival bias of the cohort. This bias exits because male sex is associated with a higher risk of CAD events. Consequently, it was expected to have more women because only asymptomatic patients were included. Another limitation is that only the CACS was used to assess coronary atherosclerosis. The presence, extent, composition, and distribution of coronary atherosclerosis were not assessed employing CTCA. This is important because 80% of the total coronary plaque burden is composed of non-calcific components (513–515).

Strengths include the relatively high number of asymptomatic and no related participants with HeFH, the exclusion of HoFH and double heterozygous patients and the age-matching. An additional strength was the similarity in the prevalence of traditional cardiovascular risk factors and in the plasma Lp(a) and apolipoprotein B-100 concentrations between groups. Moreover, statin therapy was not a significant predictor of the presence of CAC and therefore not a confounder in the present study.

**Scope for further work and practical implications**

Future studies should include a larger sample size and more diverse
population. Further longitudinal studies are required to assess whether the non-invasive estimation of CAC might improve the risk stratification in patients with FH, as currently recommended in the general population (355). Data derived from CTCA should be analysed in order to evaluate the non-calcific components and luminal obstructions, particularly in FH mutation-positive patients. Measurements derived from the CACS estimation should also be integrated in future studies. Investigations by type of mutation, polygenic risk scores and other genetic abnormalities related to CAD, should also been undertaken.

Currently, the ideal age to start coronary artery screening in patients with FH is uncertain, although it is clear that after 45 years of age is not ideal. The results from the present and previous studies (326) suggest that it should be approximately in the fourth decade of life owing to the presence of coronary atherosclerosis was detected in patients older than 30 years of age. However, other non-invasive imaging methods no requiring ionising radiation such as carotid ultrasound, might be used in younger adults with FH to assess the presence of plaques and carotid intima-media thickness (277).

The estimation of CACS allows earlier detection of coronary atherosclerosis and therefore, those who require earlier and more aggressive interventions to reduce coronary risk and all-cause mortality (265,307). It is also beneficial to risk-stratify asymptomatic patients with FH according to severity (320,322,325,535,536). The identification of a pathogenic mutation affecting the LDL receptor pathway could help to predict those at an even greater risk of developing CAD. Hence, in patients with an FH phenotype, there is a need for early genetic assessment. In those with an identified pathogenic mutation, especially in the 31 to 50 years of age group, early intensive treatment and follow-up initiation are paramount to diminish their CAD risk. Additionally, the presence of a zero or low CACS is associated with very low frequency of obstructive lesions in CTCA. Therefore, the non-invasive estimation of CAC could be employed as a safe initial screening method excluding the additional risk and financial cost from the CTCA.
5.4 Conclusion

Among asymptomatic patients with an FH phenotype and no history of CAD, CACS was significantly higher in patients with a pathogenic mutation affecting the LDL receptor pathway compared with those without a mutation. This association was independent of age, sex, obesity, DM, hypertension, smoking, treated plasma lipid and lipoprotein concentrations, and statin therapy.

In conclusion, in spite of cholesterol-lowering therapy, the presence of a genetic diagnosis of FH predicts those at greater risk of CAD according to the estimated CACS as assessed by cardiac CT scanning. Given this, it appears that the presence of coronary atherosclerosis is determined principally by the exposure to LDL-C over time, which is in turn dependent on the presence of a pathogenic mutation affecting the LDL receptor pathway. Consequently, a genetic test should be offered to patients with phenotypic FH to stratify their risk of CAD. The value of genetic testing is in risk stratification of patients as shown in this study, in genetic counselling and also in a therapeutic sense for selection of specific treatments and guiding the intensity of cholesterol-lowering therapy. Genetic testing may also be used to enable more precise cascade testing of family members for FH.
Chapter Six: Patients' Understanding and Perceptions of the Estimation of Coronary Artery Calcium
ABSTRACT

Background and aims: Cardiac imaging is being increasingly employed in cardiovascular risk assessment as part of the incorporation of precision medicine into contemporary clinical practice. Over the last decades, patients have been encouraged to become more involved in their medical care and health decisions. It is therefore important to investigate patients’ understanding and perception of the cardiac imaging. A positive influence of the communication of the outcome of CACS has been established in adherence to cardiac medications and smoking cessation. The aim was to assess whether asymptomatic patients who had a cardiac CT scanning for CACS have a favourable perception and understanding of the investigation and test results.

Methods: This was a single-centre, cross-sectional study including asymptomatic adults referred to a private coronary prevention risk clinic at Calvary Lenah Valley Hospital (Hobart, Tasmania). Patients were invited to complete a questionnaire survey about test understanding and health perception following a cardiac CT scan for the estimation of CACS between 2014 and 2017. Data were described as proportions and group comparisons were made by chi-squared test.

Results: Ninety-one out of 144 patients contacted, completed and returned the questionnaire survey (participation rate: 63.2%). The mean age was 58.6 ± 8 years, 59% were men and a CACS >0 AU was detected in 69.2% of the patients. Over 96% of patients understood the rationale for the CT scan and the nature of the test outcome. 85% of patients considered that the test was very important for their health, 66% considered that it would influence their health and risk of heart disease, and 45% considered that it made a difference to the way they viewed their treatment for cholesterol. Between patients with and without CAC, there were no significant differences in responses regarding the understanding of the test and the perception of the importance of the test and the way they viewed their health. By contrast, patients with CACS of zero more frequently considered that the result did not
influence their perception of cholesterol treatment compared with those with a CACS >0 (32.1% and 14.3%, respectively; \( p = 0.048 \)). The time elapsed between the CT scan and survey was not a predictor of more favourable responses.

**Conclusion:** Asymptomatic adult patients attending a private coronary prevention clinic who had a cardiac CT scan for CACS, had a potentially high level of understanding of the rationale and nature of the investigation, as well as a favourable perception of the test results. Patients with a CACS of zero more frequently considered that the result did not influence their perception of cholesterol treatment. Given these points, a cardiac CT scan for CACS is well accepted and understood by patients and the test could be valuable in risk stratification, motivational care, reinforcing healthy behaviours, and intensifying treatments of cardiovascular risk factors including hypercholesterolaemia. Consequently, precision medicine tools, such as cardiac CT scanning for CACS, could be included routinely as part of coronary risk assessment.
6.1 Subjects and methods

6.1.1 Study design
This was a single centre, cross-sectional study that aimed to retrospectively assess the understanding and health perception of asymptomatic patients in whom the presence of subclinical coronary atherosclerosis was quantified using the measurement of CACS with cardiac CT scanning.

6.1.2 Study population
The study population consisted of adult outpatients without symptomatic CAD, who underwent coronary risk assessment between 2016 and 2017 at Calvary Lenah Valley Hospital (Hobart, Tasmania), a Catholic not-for-profit private institution. Under the specialist care of WB, patients were receiving best standard of care. Cardiac CT scans for CACS were performed in addition to standard care and were privately funded by patients.

WB communicated the outcome of cardiac CT imaging employing a systematic approach that is described elsewhere (537,538). In patients with a CACS of zero, he explained the low risk of a coronary event in the next decade. In those with a CACS >0, he showed the results and CT images, explained the percentile for sex, age and race based on the MESA cohort (458) and discussed the value of further investigations and treatments.

6.1.3 Study variables
The demographic and clinical data included date of birth, sex, history of symptomatic CAD, hypertension, DM, smoking status, family history of first-degree relative with premature CAD, and cholesterol-lowering, antiplatelet and antihypertensive medications.

Laboratory data included pre-statins plasma TC, TG, LDL-C, and HDL-C concentrations; non-HDL-C was calculated by subtracting HDL-C from TC. From the cardiac CT scan reports, date and CACS in AU were collected.
A six-question questionnaire survey was developed (Appendix 7) in association with the Health Psychologist Martin Hagger and adapted from previous publications (496). To assess patients' understanding of the test, three questions were formulated:

1) “Do you know why the heart scan was done?” (yes or no).
2) “Were you informed of the scan results?” (yes or no).
3) “Did you understand the results?” (yes or no).

To evaluate patients' health perception following the test, three additional questions were formulated:

1) “How important for your health do you think the test was?” (nil, slight, somewhat or very).
2) “Did the test results influence the way you viewed your health and risk of heart disease?” (no, slight, somewhat or very).
3) “Did the test results make a difference to the way you viewed your treatment for cholesterol?” (no, slight, somewhat or very).

6.1.4 Inclusion criteria

1) Patients who were aged ≥18 years.
2) Have undergone a cardiac CT scan including CACS.

6.1.5 Exclusion criteria

1) History of symptomatic CAD (defined as previous myocardial infraction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft surgery).
2) Inability to give informed consent for cardiac CT scanning.

6.1.6 Data sources

The primary sources for obtaining the data were the electronic clinical records of WB which are stored at the Calvary Lenah Valley Hospital and the six-question questionnaire survey. When data were not obtainable from clinical records, telephone contact was made with Hobart Pathology, a medical testing laboratory based in Hobart. If the information provided was
either inaccurate or incomplete, telephone contact was made with their General Practitioner.

6.1.7 Development of database

Before creating the database, a coding manual was employed to design the structure of the data elements. Each of the variables in the dataset was coded and the data validation tool was used to prevent input errors. Once created, structure and integrity were tested through data entry tests.

Data were collected and grouped manually from primary and secondary sources in a unique password-protected Excel 2016 file (Microsoft Corp., Redmond, WA, USA) at the Cardiac Centre at Calvary Lenah Valley Hospital.

6.1.8 Data acquisition process

Information of patients who met the specific eligibility criteria were collected retrospectively, covering a four-year period (2014 - 2017). Patients' names and Medicare numbers were initially recorded to match records from primary and secondary data sources.

Following data collection, the completed dataset in identifiable form was kept on a password-protected Excel 2016 file on the personal work computer of WB, Consultant Cardiologist from the Calvary Lenah Valley Hospital. Thereafter, names and other identifying information were removed from the database prior to encryption using 7-Zip 16.00 [64-bits] file archiver (Igor Pavlov, Russia) which was also password-protected. The dataset was sent via email to the Royal Perth Hospital and the passwords were sent via text message.

Dataset was regularly checked for missing and spurious data and against duplicate measurements. Information quality assurance measures were applied to regularly profile the data to discover inconsistencies. After database closure, data cleaning was performed. The definitive Excel spreadsheet was stored on a University of Western Australia networked drive.
**Clinical measurements and risk assessment**

From key traditional cardiovascular risk factors identified by the Framingham study (241), the following were recorded:

1) **Hypertension**: defined as a systolic blood pressure $\geq 140$ mmHg, diastolic blood pressure $\geq 90$ mmHg or on antihypertensive medications (369).

2) **Type 2 DM**: defined as a fasting blood glucose $\geq 7$ mmol/L (on two separate occasions) or haemoglobin A1c $\geq 6.5\%$ (48 mmol/mol) (on two separate occasions) (504).

3) **Current smoker or ex-smoker who quit within last year**.

4) **Hypercholesterolaemia**: defined as an LDL-C $> 4$ mmol/L or on cholesterol-lowering medication.

5) **Family history of premature CAD in first-degree relative**: defined as age of onset $< 55$ years for men and $< 60$ years for women.

6) **Sex**.

The surveys were initially sent by certified mail to the 144 patients included in the Chapter 3 study. In cases where there was no response after one month, telephone contact was made with the non-responders and the questionnaire was applied telephonically to whom accepted.

**Biochemical analyses**

All lipid profiles analyses were performed in Hobart Pathology (2 - 4 Kirksway Place, Hobart TAS 7000). Venous blood was collected after 8 - 15 hours fast by a phlebotomist into EDTA or lithium heparin tubes. Plasma TC, TG and HDL-C concentrations were measured by standard enzymatic methods using the Cobas C701 platform (Roche Diagnostics, Risch-Rotkreuz, ZG, Switzerland). LDL-C concentrations were calculated using the Friedewald Equation (505), but in patients with plasma TG $\geq 4.5$ mmol/L, a direct LDL-C assay was employed.

**Cardiac imaging**

The cardiac CT scans were carried out on a GE Revolution 128-slide scanner (GE Healthcare, Chicago, IL, USA) in the facilities of Regional
Imaging at Calvary Lenah Valley Hospital (49 Augusta Road, Lenah Valley TAS 7008). Non-contrast-enhanced CT images were acquired using a prospective electrocardiographic triggering on the volumetric mode, and images were reconstructed with 3.0 mm axial slices. Acquisition parameters included a gantry rotation time of 0.35 sec, collimation of 20 mm x 280 mm, tube voltage of 120 kV, and tube current of 200 mA, with a 25 cm displayed field of view.

The images were performed at a temporal resolution of 330 ms and spatial resolution 0.24 mm. The estimated radiation dose was approximately 1 mSv (dose length product 90 mGycm). The 3.0 mm images were loaded into the GE Advantage workstation and calcific plaques in coronary arteries were quantified using the SmartScore 4.0 package (GE Healthcare, Chicago, IL, USA). Agatston scores were calculated according to the method described by Agatston et al. (451).

6.1.9 Statistical methods

Participants that met the eligibility criteria were consecutively selected from the electronic clinical records. Patients were grouped as responders or non-responders of the questionnaire. Statistical analyses were performed with the use of STATA software, version 14.1 (StataCorp LLC., College Station, TX, USA).

Demographic and clinical characteristics were initially compared between responders and non-responders of the questionnaire. Time between CT scan and survey response was calculated in months. The prevalence of traditional cardiovascular risk factors was calculated as the presence of the risk factors divided by the total number of the cohort and expressed as a percentage. The Australian absolute CVD risk score (9) was also estimated.

Continuous variables were tested for normal distribution with the Kolmogorov–Smirnov test. Continuous variables with normal distribution were expressed as means and standard deviations; non-normal variables were reported as medians and interquartile ranges. Categorical variables
were expressed as percentages. Normally distributed continuous variables were compared with Student's t-test; Wilcoxon rank-sum test was used to compare non-normally distributed variables. The frequencies of categorical variables were compared using Pearson's chi-square or Fisher's exact test. Logistic regression analysis was performed to determine whether the time between CT scan and survey was a predictor of more favourable responses.

6.1.10 Ethical considerations

Data from Chapter 3 study were obtained as part of the clinical audit approved by the Tasmanian Health and Medical HREC (Ethics 1 and 2). The surveys were previously sent to patients as part of the audit performed by WB at the Calvary Lenah Valley Hospital. An exemption from ethics review with reference number RA/4/20/4872 was awarded by the UWA Human Research Ethics Office (Ethics 14), constituting ethical clearance.
6.2 Results

6.2.1 Patients characteristics

The characteristics of patients according to response to the questionnaire are shown in Table 22. There were no significant differences in the demographic, clinical or biochemical characteristics between responders and non-responders to the survey. The CACS was not different between the groups either.

The demographic, clinical and biochemical characteristics of the patients who responded to the survey, in relation to CACS result, are shown in Table 23. Ninety-one out of 144 patients contacted, completed and returned the survey (participation rate: 63.2%). The overall mean age was 58 ± 9 years and 59% were men.

Approximately half the cohort had a history of hypercholesterolaemia, a third part had a history of hypertension, a quarter had a family history of premature CAD, 6% had a history of type 2 DM, and 3% were current smokers at the time of the cardiac CT scan. Approximately one third of patients were on antihypertensive medication, 19% on antiplatelet and the same proportion on statins. The median time of statin treatment was 13.5 months (IQR 61.3) and the most frequently used was atorvastatin (27.5%). The overall median time between the CT scan and survey was 15 months (IQR 7.0).
Table 22. Demographic, clinical and biochemical characteristics of patients according to response to the questionnaire

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>Responders</th>
<th>Non-responders</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>144</td>
<td>91</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>88 (61.1)</td>
<td>54 (59.3)</td>
<td>34 (64.1)</td>
<td>0.568</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.3 ± 9.4</td>
<td>58.6 ± 8.7</td>
<td>57.7 ± 10.4</td>
<td>0.582</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>9 (6.3)</td>
<td>6 (6.6)</td>
<td>3 (5.6)</td>
<td>0.823</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>52 (36.1)</td>
<td>35 (38.5)</td>
<td>17 (32.0)</td>
<td>0.442</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>7 (4.9)</td>
<td>3 (3.3)</td>
<td>4 (7.5)</td>
<td>0.253</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>79 (55.6)</td>
<td>51 (56.0)</td>
<td>28 (52.8)</td>
<td>0.744</td>
</tr>
<tr>
<td>Family history premature CAD, n (%)</td>
<td>39 (27.1)</td>
<td>20 (22.0)</td>
<td>19 (35.8)</td>
<td>0.071</td>
</tr>
<tr>
<td>Pre-statin TC, mmol/L</td>
<td>6.3 ± 1.3</td>
<td>6.3 ± 1.4</td>
<td>6.2 ± 1.1</td>
<td>0.416</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>4.0 ± 1.2</td>
<td>4.0 ± 1.3</td>
<td>3.9 ± 0.9</td>
<td>0.711</td>
</tr>
<tr>
<td>Pre-statin triglyceride, mmol/L</td>
<td>1.3 (1.0)</td>
<td>1.3 (1.0)</td>
<td>1.4 (0.9)</td>
<td>0.756</td>
</tr>
<tr>
<td>Pre-statin HDL-C, mmol/L</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>0.647</td>
</tr>
<tr>
<td>Pre-statin non-HDL-C, mmol/L</td>
<td>4.8 ± 0.1</td>
<td>4.8 ± 1.5</td>
<td>4.7 ± 1.1</td>
<td>0.521</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>28 (19.4)</td>
<td>18 (19.8)</td>
<td>10 (18.8)</td>
<td>0.984</td>
</tr>
<tr>
<td>On antihypertensive, n (%)</td>
<td>46 (31.9)</td>
<td>31 (34.1)</td>
<td>15 (28.3)</td>
<td>0.474</td>
</tr>
<tr>
<td>On antiplatelet, n (%)</td>
<td>30 (20.8)</td>
<td>17 (18.7)</td>
<td>13 (24.5)</td>
<td>0.405</td>
</tr>
<tr>
<td>Coronary artery calcium score, AU</td>
<td>8.0 (71.0)</td>
<td>10.0 (62.0)</td>
<td>4.0 (81.0)</td>
<td>0.607</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range). CAD indicates coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and AU, Agatston units. p value for responders versus non-responders.
Table 23. Demographic, clinical and biochemical characteristics of patients according to the presence and absence of coronary artery calcification

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>CACS &gt;0</th>
<th>CACS = 0</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>91</td>
<td>63</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>54 (59.3)</td>
<td>41 (65.0)</td>
<td>13 (46.4)</td>
<td>0.095</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.6 ± 8.7</td>
<td>60.3 ± 7.7</td>
<td>54.6 ± 9.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n (%)</td>
<td>6 (6.6)</td>
<td>6 (9.5)</td>
<td>0 (0.0)</td>
<td>0.091</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>35 (38.5)</td>
<td>29 (40.0)</td>
<td>6 (21.4)</td>
<td>0.026</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>3 (3.3)</td>
<td>2 (3.1)</td>
<td>1 (3.5)</td>
<td>0.922</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>51 (56.0)</td>
<td>35 (55.5)</td>
<td>16 (57.1)</td>
<td>0.745</td>
</tr>
<tr>
<td>Family history premature CAD, n (%)</td>
<td>20 (22.0)</td>
<td>12 (19.0)</td>
<td>8 (28.5)</td>
<td>0.311</td>
</tr>
<tr>
<td>Pre-statin TC, mmol/L</td>
<td>6.3 ± 1.4</td>
<td>6.2 ± 1.5</td>
<td>6.6 ± 1.1</td>
<td>0.336</td>
</tr>
<tr>
<td>Pre-statin LDL-C, mmol/L</td>
<td>4.0 ± 1.3</td>
<td>3.9 ± 1.3</td>
<td>4.2 ± 1.0</td>
<td>0.403</td>
</tr>
<tr>
<td>Pre-statin triglyceride, mmol/L</td>
<td>1.3 (1.0)</td>
<td>1.4 (1.7)</td>
<td>1.2 (1.0)</td>
<td>0.609</td>
</tr>
<tr>
<td>Pre-statin HDL-C, mmol/L</td>
<td>1.5 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>0.118</td>
</tr>
<tr>
<td>Pre-statin non-HDL-C, mmol/L</td>
<td>4.8 ± 1.5</td>
<td>4.8 ± 1.6</td>
<td>4.9 ± 1.1</td>
<td>0.635</td>
</tr>
<tr>
<td>On statins, n (%)</td>
<td>18 (19.8)</td>
<td>16 (25.4)</td>
<td>2 (7.1)</td>
<td>0.044</td>
</tr>
<tr>
<td>On antihypertensive, n (%)</td>
<td>31 (34.1)</td>
<td>27 (42.8)</td>
<td>4 (14.2)</td>
<td>0.008</td>
</tr>
<tr>
<td>On antiplatelet, n (%)</td>
<td>17 (18.7)</td>
<td>17 (26.9)</td>
<td>0 (0.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Time since CT scan, months</td>
<td>15.0 (7.0)</td>
<td>15 (10.0)</td>
<td>16 (6.0)</td>
<td>0.720</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation or median (interquartile range). CACS indicates coronary artery calcium score; CAD, coronary artery disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and CT, computed tomography. p value for CACS >0 versus CACS = 0.
6.2.2 Patient characteristics according to cardiac CT scan findings

Fifty (55%) patients included in the present study had an indication for cardiac CT scan for CACS according to the CSANZ CAC scoring position statement (448,452). The overall median CACS was 10.0 AU (IQR 62.0). The cardiac CT scan was positive (CACS >0) in 63 patients (69%); among those patients, the median CACS was 33.0 AU (IQR 151.0).

Patients with positive test were significantly older and had a higher prevalence of hypertension than those with negative test (CACS = 0). Between patients with positive and negative tests, there were no significant differences in the proportion of males and current smokers, as well as in the prevalence of type 2 DM, hypercholesterolaemia and a family history of premature CAD.

There were no significant differences in the median time between the CT scan and the survey as well as in the pre-statin plasma concentrations of TC, LDL-C, TG, HDL-C, and non-HDL-C between patients with positive and negative test. The proportion of patients on statin, antihypertensive and antiplatelet medications was significantly higher in patients with positive test than in those with negative test ($p = 0.044$, $p = 0.008$ and $p = 0.002$, respectively).

6.2.3 Responses to questionnaire

Figure 15 depicts the responses in relation to the understanding of the test (A) and the health perception (B). Overall, there was a high understanding and a favourable perception of the CACS measurement. Specifically, over 96% of the patients reported that they knew the rationale of why the test was done, were informed of the outcome and understood the results. With respect to health perception, 85% of patients considered that the test was very important for their health; 66% perceived the results as positive for their health; 45% considered that it made a difference in their treatment for cholesterol.
Figure 15. Responses to questionnaire from all patients
Figure 16 depicts the proportion of patients in relation to the understanding of the test according to absence (A) and presence (B) of CAC. It can be seen that there were no significant differences in the proportion in relation to the responses between those with negative and positive test.

The proportion of patients in relation to the health perception according to the absence (A) and presence (B) of CAC is depicted in Figure 17. It can be seen that between patients with negative and positive test, there were not significant differences in the proportion in relation to the importance of the test and the influence the test results had in the way they view their health. Nevertheless, patients with a CACS of zero more frequently considered that the result did not influence their perception of cholesterol treatment compared with those with a CACS >0 (32.1% and 14.3%, respectively; \( p = 0.048 \)). Among the 19 patients (21%) who considered that the result did not influence their perception of cholesterol treatment, two were on statin therapy. The time elapsed between the CT scan and survey was not a predictor of more favourable survey responses.
Figure 16. Test understanding responses according to the absence and presence of coronary artery calcium
Figure 17. Health perception responses according to the absence and presence of coronary artery calcium
6.3 Discussion

New and main findings
The principal finding was that patients without symptomatic CAD have a high understanding and a favourable health perception of the nature of the cardiac CT scan results. Importantly, patients without CAC more frequently considered that the test results did not influence the way they see their treatment for cholesterol. The present investigation is one of the first studies that evaluate patients' understanding and health perception after a CACS measurement. The patients' socioeconomic status and access to health care were uniformly high, therefore this was most likely not a confounder in the present study.

Previous studies
Screening images during consultation have previously been demonstrated to enhance patient understanding and provide reassurance (490). Several current clinical guidelines have recommended that CACS measurement should be used in risk assessment, particularly in intermedia-risk or selected borderline-risk adults (256,354,355). According to the CSANZ CAC scoring position statement (448,452), 55% of surveyed patients in the present study, had an indication for cardiac CT scan for CACS.

Preceding studies have established that the use of CAC measurement can have a favourable effect (491,539,540) on health-related behaviour (539,540) by improving adherence to cholesterol-lowering therapy (491) and smoking cessation (492,493). These effects were stronger when CAC was identified (326,494).

In the present study, patients without CAC more frequently considered that the test results did not influence their perception of cholesterol treatment. The previous finding leads to questioning whether those patients with detected CAC adhere to therapy more frequently. This question has been addressed in studies with pre-test and follow-up questionnaires which have detected diagnosis dependent psychological effects following cardiac CT scanning. A
study conducted in New Zealand by Devcich et al. (495), in which 45 non-acute cardiac patients referred for diagnostic CTCA completed questionnaires prior to testing and following the receipt of test results. Illness perceptions and intentions to take cardiac medications, as well as diet and exercise intentions were measured (exercise and dietary behaviours were measured at follow-up six weeks later). Compared to positive testing patients, those with normal test results reported significant changes toward more positive illness perceptions following testing, with improvements in emotional effect of illness, illness concern, consequences, and personal control of illness. The illness perception of treatment control was seen as more important among positive testing patients, although both groups reported increases in illness coherence. Health behaviour intentions (cardiac medication and exercise intentions) and physical activity at follow-up increased for positive testing patients only.

In another study by Ladapo et al., (496) 351 adult patients without diagnosed CAD who underwent initial evaluation for ischaemic heart disease with stress testing with imaging or CTCA were surveyed. Initial test results were positive in 11% of patients. 28% of participants did not feel their initial test was very important for their health, 18% did not have an accurate understanding of their results, and 38% did not have strong preferences for completing recommended follow-up. Subsequent test or procedures were performed in 12% of patients. In adjusted analyses, patients who had a precise understanding of their initial test result were less likely to undergo follow-up test or procedures if the initial test was positive. However, a systematic review by Mamudu et al. (25) showed that CAC investigations enhanced medication adherence and impact other important domains as beneficial behavioural changes to improve CAD.

Previous evidence involving studies with follow-up, suggest that screening for cardiovascular risk factors leads to behavioural or lifestyle modifications (541). Furthermore, and more important, some previous studies have shown that behavioural or lifestyle changes could potentially impact the progression of CAC (542–545). In a study by Pérez de Isla et al. (326) including
exclusively FH patients, there were changes in patients management and care after knowing cardiac CT scan results. For example, there was a significant increase in intensity of therapy, decrease of plasma LDL-C concentrations and reduction of smoking. The change was more intense in patients with CAD demonstrated by cardiac CT scan.

**Limitations and strengths**

There are several limitations to this investigation. Limitations include the absence of sample size calculation and that it was conducted in a single private centre. The retrospective nature of the questionnaire with 37% of non-responders could bias the results despite there were no demographic and clinical differences between responders and non-responders to the questionnaire. The survey was in most of the cases self-reported and not applied immediately after the CT scan, therefore, mistakes or imprecisions could have occurred. Additionally, the time between the scan and survey could have an impact on patients' perception and understanding or even on their interest to respond to the questionnaire.

A brief, simplistic and superficial questionnaire including only multiple-choice questions was employed. Hence, all responses require a much deeper understanding in order to determine whether patients understood the questions and the specific reasons why they gave their answers. An additional limitation was the inability to assess the effect of the test results in treatment adherence and lifestyle changes because pre-test and follow-up surveys were not performed. Finally, the psychological status and health literacy of patients was unknown, therefore, those who answered negatively could have had literacy issues or depressive illness and anxiety, which could influence their responses.

The main strengths of the present study are the clinical setting in which it was conducted, the inclusion of asymptomatic patients with no history of CAD and the systematic and standardised approach employed by an experienced Cardiologist for communicating the risk following the estimation of CACS on cardiac CT scanning. Other strengths are the similarity in the demographic,
clinical and biochemical characteristics between responders and non-responders to the questionnaire and the relatively high participation rate in spite of a long time between the cardiac CT scan and the survey.

**Scope for further work and practical implications**

Further work is required to address aspects of patient-centred research to improve shared decision-making in cardiac CT scanning and how physicians communicate the results. Future studies should also include a focused group of patients selected according to current guidelines recommendations, from more diverse populations, public and private centres, including a larger sample size and long-term follow-up to assess the impact of the test.

Upcoming studies should include trials of behaviour and adherence modification and the discussion of the difference in the way people see their treatment for cholesterol in relation with the presence and absence of CAC. The health literacy and psychological status of patients should be stablished. Deeper questions must be formulated in the questionnaires and the understanding of the questions by responders must be guaranteed.

The findings in the present study suggest that the direct and non-invasive observation of subclinical coronary atherosclerosis is well accepted by patients. There is also a high level of understanding regarding the rationale and the results and they consider that is very important for their cardiovascular risk. Knowing the level of understanding and perception is important in clinical practice because physicians and patients can use cardiac CT scanning for a more personalised and precise shared decision-making. Moreover, a positive test could encourage patients to become more involved in their medical care with a higher adherence to therapy, a healthier lifestyle, and therefore, a higher risk reduction.
6.4 Conclusion

Asymptomatic patients attending a private coronary prevention clinic who had a cardiac CT scan for CACS, had a high level of understanding of the rationale and nature of the investigation, as well as a favourable health perception of the test results. The systematic and standardised approach employed by an experienced Cardiologist to communicate the risk following the estimation of CACS on cardiac CT scanning could explain the high level of satisfaction. Patients with CACS of zero more frequently considered that the result did not influence their perception of cholesterol treatment.

Given these points, a cardiac CT scan for CACS appears to be well accepted and understood by patients and the test could be valuable in risk stratification, motivational care, reinforcing healthy behaviours, and intensifying treatments of cardiovascular risk factors including hypercholesterolaemia. Consequently, precision medicine tool, such as cardiac CT scanning for CACS, could be included routinely as part of coronary risk assessment.
Chapter Seven: Concluding Discussion and Perspectives
**Principal results and interpretations**

This thesis identified several gaps in knowledge which were addressed in the foregoing chapters and four principal conclusions were derived.

First, in middle-aged asymptomatic patients, attending a private coronary prevention clinic, the number of traditional cardiovascular risk factors was a significant predictor of the presence of coronary atherosclerosis as assessed by the estimation of CACS employing cardiac CT scanning. By contrast, the Australian absolute CVD risk score was not a significant predictor of the presence of coronary atherosclerosis. It appears, therefore, that a risk factor counting method may be a better predictor of the presence of subclinical coronary atherosclerosis than the absolute risk score. This allows a more personalised coronary risk assessment leading to clearer risk communication and easier implementation of modifiable risk factors treatment.

Second, among middle-aged asymptomatic subjects, the CACS was significantly higher in those with a phenotypic diagnosis of FH compared with those without FH. This association was independent of family history of premature CAD and other cardiovascular risk factors. The higher CACS in FH patients was associated with high plasma LDL-C concentrations. Given this, cholesterol is probably a more powerful predictor of subclinical coronary atherosclerosis, than other familial and non-familial cardiovascular risk factors. This suggests that coronary risk in patients with FH is not determined by a family history of CAD per se, but by cumulative burden of plasma LDL-C present from an early age.

Third, among asymptomatic patients with a phenotypic diagnosis of FH, CACS was significantly higher in those with a pathogenic mutation affecting the LDL receptor pathway than in those without a mutation. This association was independent of statin therapy, treated plasma lipid and lipoprotein concentrations and other cardiovascular risk factors. In spite of cholesterol-lowering therapy, the presence of a genetic diagnosis of FH predicts those at greater risk of CAD. It appears, therefore, that the presence of coronary atherosclerosis is determined primarily by the level of LDL-C which is in turn
dependent on the presence of a pathogenic mutation affecting the LDL receptor pathway. Given this, a genetic test should be offered to patients with phenotypic FH to stratify their risk of CAD. Genetic testing may also be employed to select therapies and direct the intensity of LDL-C lowering and to enable more precise cascade testing of family members for FH.

Fourth, asymptomatic patients attending a private coronary prevention clinic who had a cardiac CT scan for CACS, had a high level of understanding of the rationale and nature of the investigation, as well as a favourable health perception of the test results. Patients with CACS of zero more frequently considered that the result did not influence their perception of cholesterol treatment. Given these points, a cardiac CT scan for CACS is well accepted and understood by patients and the test could be valuable in risk stratification, motivational care, reinforcing healthy behaviours, and intensifying treatments of cardiovascular risk factors including hypercholesterolaemia.

Limitations
This thesis has a number of limitations mainly related to the design of the studies. First, this is a series of observational studies that were cross-sectional and case-control that included highly selected participants. No interventions and longitudinal studies analysing the impact of CAC assessment on coronary risk were performed, therefore, studies included in this thesis did not prove causality. Second, while statistical significance was employed in the studies, the sample sizes were of comparatively low statistical power and might admit additional tests of the null hypothesis. Third, only CACS was employed in analyses, so that non-calcific coronary atherosclerosis was not accounted for. Fourth, the nature of the family history of CAD was not assessed and no genetic risk scores were considered.

Future studies
Further research is suggested to address the above limitations. Future studies ought to be multicentre, population-based including more diverse participants and larger sample sizes. The evaluation of different clinical
approaches for coronary risk prevention and other imaging methods for assessing the distribution of coronary atherosclerosis, such as CTCA, is required. Other measurements derived from the CACS estimation such as the number of lesions and vessels affected, lesion size, volume, and plaque density should also be implemented.

Future longitudinal studies are required in younger patients with FH, particularly in those with a pathogenic mutation, to assess whether the estimation of CACS might improve risk stratification; this could also help to determine the age when a cardiac CT scan should be carried out in this high-risk population. Other non-invasive extra coronary imaging methods not requiring ionising radiation such as ultrasound, might be employed in younger adults with FH to assess the subclinical atherosclerotic burden. Finally, further work is required to address aspects of patient-centred research to improve shared decision-making related to coronary risk assessment and cardiac CT scanning. Upcoming studies should include trials of behaviour and treatment adherence modification following the non-invasive estimation of CACS.

Concluding perspectives

There is compelling evidence supporting the superior role of CACS in risk assessment, particularly in individuals with intermediate cardiovascular risk (425,426,510,511). Although there are guidelines and position statements recommending the estimation of CACS in selected patients (354,355,469,546), the use of CAC scoring in coronary prevention does not have a strong level of recommendation. This could be attributed to concerns about ionising radiation, uncertainties regarding downstream testing and the reluctance to alter traditional methods of coronary risk assessment. Also important is the absence of randomised controlled trials demonstrating the value of employing CACS in modifying CAD risk. However, the CAUGHT-CAD trial (516) is currently being carried out in Australia and it aims to show definitive evidence of the efficacy and cost-effectiveness of CAC testing. This study may provide useful evidence to inform the guidelines about the place of CACS in decision-making regarding
prevention of patients with a family history of premature CAD. Furthermore, cardiac CT scanning for CACS alone has no Medicare rebate in Australia leading to additional costs to the patient.

With the growth of precision medicine, it is likely that the use of non-invasive cardiovascular imaging will be incorporated routinely into coronary prevention programs. As patients and clinicians are willing to base the decision-making on more accurate and personalised risk assessment, the value of CACS is highly likely to grow in the future as more studies demonstrate their benefits in managing patients at risk of CAD.
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Appendices
Appendix 1. Chapter three - histograms of coronary artery calcium score using four data transformations

Histograms by transformation
Appendix 2. Chapter three - area under ROC curve for multivariable logistic model of risk factor counting predicting the presence of CACS >0
Appendix 3. Chapter three - predictors of coronary artery calcification
(defined as CACS >200) in univariate logistic regression

<table>
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<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>0.99</td>
<td>0.94 - 1.04</td>
<td>0.745</td>
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<tr>
<td>Male sex</td>
<td>1.10</td>
<td>0.41 - 3.00</td>
<td>0.844</td>
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<tr>
<td>Hypertension</td>
<td>1.72</td>
<td>0.65 - 4.54</td>
<td>0.277</td>
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<tr>
<td>Glucose</td>
<td>0.61</td>
<td>0.25 - 1.47</td>
<td>0.277</td>
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<tr>
<td>Smoking</td>
<td>2.82</td>
<td>0.51 - 15.71</td>
<td>0.236</td>
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<tr>
<td>Hypercholesterolaemia</td>
<td>0.41</td>
<td>0.15 - 1.12</td>
<td>0.083</td>
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<tr>
<td>Family history premature CVD</td>
<td>1.69</td>
<td>0.61 - 4.67</td>
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<td>Statin therapy</td>
<td>0.75</td>
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<td>Australian absolute CVD risk score</td>
<td>0.94</td>
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<tr>
<td>Number of cardiovascular risk factors</td>
<td>1.26</td>
<td>0.72 - 2.19</td>
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CACS indicates coronary artery calcium score; and CVD, cardiovascular disease.
*p value for univariate logistic regression analyses.
Appendix 4. Chapter three - area under ROC curve for multivariable logistic regression model 1 (CACS >0)

Area under ROC curve = 0.8003
Appendix 5. Chapter three - area under ROC curve for multivariable logistic regression model 2 (CACS >100)

Area under ROC curve = 0.8079
Appendix 6. Chapter five - histograms by coronary artery calcium score transformation
Appendix 7. Chapter six - patients' understanding and perception survey

Dr Warrick Bishop
MBBS FRACP
CARDIOLOGIST
Provider: No 080955/AF

Calvary Cardiac Centre
49 Augusta Road
LENAH VALLEY 7008
Telephone 03 62 280300
Fax 03 62 789221

Patients Understanding and Perceptions Regarding Risk Assessment with Cardiac CT Scanning

Date: __________________________

Understanding:

1. Do you know why the heart scan was done?
   Yes☐ No☐

2. Were you informed of the scan results?
   Yes☐ No☐

3. Did you understand the results?
   Yes☐ No☐

Comments: __________________________________________

Perceptions:

1. How important for your health do you think the test was?
   Nil ☐ Slight ☐ Somewhat ☐ Very☐

2. Did the test results influence the way you viewed your health and risk of heart disease?
   No ☐ Slight ☐ Somewhat ☐ Very☐

3. Did the test results make a difference to the way you viewed your treatment for cholesterol?
   No ☐ Slight ☐ Somewhat ☐ Very☐

Comments: __________________________________________
Ethics Approvals Documentation
Ethics 1. Chapter three - ethics approval

14 June 2017

Dr Warrick Bishop
CI- Calvary Cardiac Centre

Sent via email

Dear Dr Bishop

REF NO: H0016382
TITLE: Audit of patients undergoing CT coronary angiography and coronary calcium scoring

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The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation on 07 June 2017 to be conducted at the following site(s):

Calvary Cardiac Centre

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the Health and Medical HREC. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the National Statement on the Ethical Conduct in Human Research (NHMRC 2007 updated 2014).

Therefore, the Chief Investigator’s responsibility is to ensure that:

(1) The individual researcher’s protocol complies with the HREC approved protocol.

(2) Modifications to the protocol do not proceed until approval is obtained in writing.
from the HREC. Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.

(3) Section 5.5.3 of the National Statement states:

Researchers have a significant responsibility in monitoring approved research as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies and take prompt steps to deal with any unexpected risks.

The appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested. http://www.utas.edu.au/research-admin/research-integrity-and-ethics-unit-rieu/human-ethics/human-research-ethics-review-process/health-and-medical-hrec/managing-your-approved-project

(4) All research participants must be provided with the current Patient Information Sheet and Consent Form, unless otherwise approved by the Committee.

(5) The Committee is notified if any investigators are added to, or cease involvement with, the project.

(6) This study has approval for four years contingent upon annual review. A Progress Report is to be provided on the anniversary date of your approval. Your first report is due 7 June 2018. You will be sent a courtesy reminder closer to this due date.

(7) A Final Report and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact me on (03) 6226 6254.

Yours sincerely

Jude Vienna-Hallam
Ethics Administration Officer
221

Ethics 2. Chapter three - ethics approval amendment

04 July 2017

Dr W Bishop
C/- Menzies Institute for Medical Research

Sent via email

Dear Dr Bishop

REF NO:  H0016382
TITLE:  Audit of patients undergoing CT coronary angiography and coronary calcium scoring

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<td>Amendment – additional personnel Cristian Vargas Garcia</td>
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The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above amendment documentation on 28 June 2017.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the National Statement on Ethical Conduct in Human Research (NHMRC 2007).

Should you have any queries please do not hesitate to contact me on (03) 6226 6254.

Yours sincerely

Jude Vienna-Hallam
Ethics Administration Officer
Ethics 3. Chapter three - UWA ethics approval recognition

Our Ref: RA4/1/0238

25 July 2017

Dr Warrick Bishop

Dear Doctor Bishop

HUMAN RESEARCH ETHICS OFFICE – NOTIFICATION OF ETHICS APPROVAL FROM ANOTHER ETHICS COMMITTEE

Project: Audit of Patients Undergoing CT Coronary Angiography & Coronary Calcium Scoring - Recognition University of Tasmania HREC Approval H00163482

Thank you for your correspondence notifying this office of your project’s review and approval by a non-UWA Research Ethics Committee. It is noted that you have ethics approval from Tasmania Health & Medical Human Research Ethics Committee, approval number H00163482.

The students and researchers identified as working on this project are:

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<th>Name</th>
<th>Institution</th>
<th>Details</th>
<th>Role</th>
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<tr>
<td>Dr Warrick Bishop</td>
<td>Calvary Hospital</td>
<td></td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>Winthrop Professor Gerald Watts</td>
<td>Medical School</td>
<td></td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Ma Jing Pang</td>
<td>Medical School</td>
<td></td>
<td>Co-Investigator</td>
</tr>
</tbody>
</table>

Student(s): Cristian Vargas Garcia

Although The University of Western Australia reserves the right to subject any research involving its staff and students to its own ethics review process, in this case, the UWA Human Ethics Office recognizes the existing approval of the non-UWA ethics committee.

1. Approving HREC to receive annual reports, amendments and notification of adverse events

You are reminded that the approving ethics committee remains the monitoring committee for this project. You must correspond with them for matters regarding amendments, adverse events, annual and final reporting.

If you have any queries, please contact the HEO at humanethics@uwa.edu.au.

Please ensure that you quote the file reference – RA4/1/0238 – and the associated project title in all future correspondence.

Yours sincerely,

Mark Davies
Manager, Human Ethics
Good morning

A new research project has arisen related to the **CAUGHT-CAD heart study** and I write to see if you would be interested in participating. The study team is headed by Prof Gerald Watts and consists of Dr Cristian Vargas Garcia & Dr Jing Pang.

The aim of the new study is to evaluate the role of cardiac CT scanning in assessing the risk of heart disease in people who have an inherited condition called Familial Hypercholesterolaemia (FH), which causes very high cholesterol in the family and can lead to very early heart disease.

Given that you have a family history of early onset heart disease, have undergone a CT scan of the heart and do not have the condition FH, you are well placed to have your CAUGHT-CAD heart study result contribute to this study as the control group (meaning that you do not have FH and we can use your information as a comparison).

There is **no significant imposition on your time and no effect on your participation in the CAUGHT-CAD study**. If you agree to participate in this new study, I will provide the study team with non-identifiable data from the CAUGHT-CAD data. This means the data the study team receive will not be able to identify you in any way.

This new study has ethics approval from Royal Perth Hospital Human Research Ethics Committee. I have attached a copy of the ‘Participant Information Sheet’ for you to read before making any decision. Further a consent form is attached for you to read, sign, scan and return to me if you agree to your information being used for the new study.

If after reading the information you still have questions, please contact me via return email with your preferred contact number. I will call to explain the study and answer any questions you may have.

Looking forward to hearing from you in relation to your participation in this new study.

Kind regards

**Jackie Ryan**  
MNurs(NursPract) MNurs, GDipHealthSc, CertCC, RN  
Clinical Research Coordinator  
CAUGHT-CAD Study  
Level 5, MRF Building, Rear of 50 Murray Street  
Perth WA 6000
Ethics 5. Chapter four - participant information sheet and consent form

Royal Perth Hospital

Participant Information Sheet

Cardiac Computerised Tomography and Inherited Hypercholesterolaemia

Investigators:
Professor Gerald F Watts
Jacqueline Ryan NP, Dr Jing Pang & Dr Cristian Vargas Garcia
Royal Perth Hospital

You are invited to take part in this research project, because you previously participated in the CAUGHT-CAD Heart Study. This information sheet explains what will be involved should you decide to participate. Please read the information carefully and ask any questions you might have. You may also wish to discuss the project with a relative or friend or your GP.

What is the purpose of this study?

People who have the inherited condition Familial Hypercholesterolaemia (FH) are at increased risk of developing heart disease, but the risk for those with FH can vary among individuals.

The aim of this study is to evaluate the role of CT scanning of the heart to improve the accuracy of assessing the true risk of heart disease in individuals with FH. The results may be used to inform individuals of their true risk of heart disease and to guide management of cholesterol lowering treatment. This could ultimately lead to greater reduction and progression of heart disease in FH.

The results of this research will be used by the study doctor Cristian Vargas Garcia to obtain a Masters of Clinical Research degree.

What will participation in this study involve?

You are being invited to participate in this study because you do not have FH and the CT scan and clinical information you provided as part of your participation in the CAUGHT-CAD study is the ideal "control" data for this new project.

If you agree to participate, you will not have to do anything. The CAUGHT-CAD study coordinator will provide us with your CAUGHT-CAD clinical data, CT scan, and blood test results. The CAUGHT-CAD coordinator will make the data "non-identifiable" before providing it to us for use in this new study. That means you will not be able to be identified in any way from the data provided, with no personal information (such as your name) being provided.

To address this study's aims we will compare the data from CAUGHT-CAD participants ("controls") with data from a group of patients with FH ("cases"). Each group will have a minimum of 138 people.

What are the possible benefits of taking part?

There will be no direct benefit to you from your participation in this study. However, possible benefits may include the improvement of care for individuals with FH and other inherited conditions.

What are the possible risks and disadvantages of taking part?

There are no foreseeable risks or burdens for you. This study does not involve any active participation and will only use non-identifiable data already collected as part of CAUGHT-CAD.
What will happen to information about me?

The CAUGHT-CAD study coordinator will supply the researchers conducting this project with your CAUGHT-CAD data in electronic non-identifiable format. This means it will contain no information that can identify you, such as your name or address.

The information will be stored on the University of Western Australia’s networked drives and will only be used for the purpose of the present research study.

It is anticipated that the results of this research study will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified.

What happens when the research study ends?

This study is part of a Master’s of Clinical Research and it is expected that the results will generate several presentations at scientific meetings and published works, as well as a Master’s thesis.

Do I have to participate in this study?

Participation in any research study is voluntary. If you do not want to take part, you do not have to. You are also free to withdraw if you first agree to participate, but later change your mind. Note, however, that once the data has been supplied to the team conducting this study, it cannot be withdrawn because it has been made non-identifiable (and we cannot then identify your specific data and remove it).

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your participation in the CAUGHT-CAD Heart Study.

Contacts for further information

If you want any further information concerning this project, you may contact the clinical investigators:

<table>
<thead>
<tr>
<th>Name</th>
<th>Gerald Watts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Coordinating Principal Investigator</td>
</tr>
<tr>
<td>Telephone</td>
<td>0415698140</td>
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<tr>
<td>Email</td>
<td><a href="mailto:gerald.watts@uwa.edu.au">gerald.watts@uwa.edu.au</a></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Jackie Ryan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position</td>
<td>Clinical Research Coordinator (CAUGHT-CAD study)</td>
</tr>
<tr>
<td>Telephone</td>
<td>08 92240388</td>
</tr>
<tr>
<td>Email</td>
<td><a href="mailto:caught-cad-am@uwa.edu.au">caught-cad-am@uwa.edu.au</a></td>
</tr>
</tbody>
</table>

This project has been granted ethical approval by the Royal Perth Hospital (RPH) Human Research Ethics Committee (HREC). If you have any concerns about the conduct of the project or your rights as a research participant, please call (08) 9224 2292 or email: EMHS.REG@health.wa.gov.au and quote the ethics approval number (RGS0000000748).
Consent Form
Cardiac Computerised Tomography and Inherited Hypercholesterolaemia

Investigators:
Professor Gerald F Watts
Jacqueline Ryan NP, Dr Jing Pang & Dr Cristian Vargas Garcia
Royal Perth Hospital

Declaration by Participant
I have read the Participant Information Sheet or someone has read it to me in a language that I understand.
I understand the purposes of the research described in the project.
I have had an opportunity to ask questions and I am satisfied with the answers I have received.
I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the project without affecting my participation in the CAUGHT-CAD Heart Study.
I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) ____________________________
Signature ____________________________ Date ____________________________

Declaration by CAUGHT-CAD Research Coordinator

Date returned by email (scanned) or mail:

Where requested, I have given a verbal explanation of this study by phone and I believe that the participant has understood that explanation.

Name of CAUGHT-CAD Research Coordinator (please print) ____________________________
Signature ____________________________ Date ____________________________

1A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.
Ethics 6. Chapter four - ethics approval

Royal Perth Hospital Human Research Ethics Committee

20 December 2017

Professor Gerald Watts
50 murray street
Perth WA 6001

Dear Prof Watts

PRN: RGS0000000748
Project Title: Cardiac Computerised Tomography and Inherited Hypercholesterolaemia
Protocol Number: Version 2, 29 November 2017

Thank you for submitting the above research project for ethical review. This project was considered by the Royal Perth Hospital Human Research Ethics Committee at its meeting held on 13 December 2017.

Decision: Conditional Approval

The Committee recommended approval of the project on receipt of satisfactory responses to the questions below.

The Chairperson is authorised to review the responses and approve on behalf of the Committee.

Research merit and integrity (aims, outcomes and conduct)

1. The Committee supported this case control study that will compare coronary artery calcium (CAC) scores in people with asymptomatic familial hypercholesterolemia (FH) with rates of CAC in non-FH controls to determine the incidence of subclinical disease in people with asymptomatic FH (NS 1.1)

Justice (recruitment, unfair burden, no exploitation, access to benefits)

No Issues

Beneficence (benefits & risks of harm and consent)

No Issues

Respect (privacy, confidentiality and consent)

2. The Committee noted that access to CT imaging and other data for the FH group will rely on existing consent obtained as part of their enrolment in the FHWA cohort (NS 2.2.14 - 2.2.17)

3. For the consent of the non-FH control group (former participants in the "CAUGHT-CAD" study) the Committee requested revision of the supplied Participant Information Sheet & Consent Form (PICF) to simplify the formal and sometimes stilted language and explain that there are no active requirements to participation (NS 2.2.3)

Please provide the requested information as soon as possible. Your response should include a letter addressing the issues mentioned above, along with any revised forms and documents uploaded into the RGS project workspace.

If no response is received within four months from the date of this letter, the project will be considered withdrawn and you will be required to resubmit the project with full documentation.
If you have any queries about the HREC’s consideration of your project, please contact the HREC Administrative Officer on (08) 9224 2292. A copy of the HREC’s Terms of Reference, Standard Operating Procedures, membership and standard forms can be obtained on request from the Research Ethics & Governance Office, or from the website: http://ww2.health.wa.gov.au/About-us/East-Metropolitan-Health-Service/About/Human-Research-Ethics-and-Governance

Yours sincerely

[Signature]

DR RMIN GHARBI
Chairman | Royal Perth Hospital Human Research Ethics Committee
Prof Gerald Watts  
Consultant Physician  
Royal Perth Hospital  

Dear Prof Watts  

Study Title: Cardiac Computerised Tomography and Inherited Hypercholesterolaemia  
PRN: RG500000001748  

Thank you for submitting the above research project for governance review. I am pleased to advise you that East Metropolitan Health Service Executive has granted authorisation for this research project to be conducted at the following participating site(s):  

Royal Perth Hospital  

The documents approved for use are those listed on the Royal Perth Hospital Human Research Ethics Committee (HREC) approval letters dated 121 December 2017.  

Site authorisation of this project is valid from 8 March 2018 subject to continued ethical approval from the Royal Perth Hospital Human Research Ethics Committee and compliance with the ‘Conditions of Site Authorisation for a Research Project’ (Appendix A).  

Should you have any queries about East Metropolitan Health Service Executive’s consideration of your project, please contact the Research Governance Office at EMHS.REG@health.wa.gov.au or on 08 9224 2260. The Research Governance Office’s Standard Operating Procedures are available from the Research Governance Office or from http://ww2.health.wa.gov.au/About-us/East-Metropolitan-Health-Service/About/Human-Research-Ethics-and-Governance.  

I wish you every success in your research.  

Yours sincerely  

Dr Aref Anwar  
EXECUTIVE DIRECTOR  

Research Ethics & Governance  
Level 3 Colonial House, Royal Perth Hospital, GPO Box X2213 Perth WA 6847  
Telephone: (08) 9224 2260 / (08) 9224 2282  
Email: EMHS.REG@health.wa.gov.au
CONDITIONS OF SITE AUTHORISATION TO CONDUCT A RESEARCH PROJECT
ROYAL PERTH HOSPITAL, EAST METROPOLITAN HEALTH SERVICE

The following general conditions apply to the research project authorised to be conducted at the site(s) nominated in the accompanying letter. The acceptance of the site authorisation will be deemed to be an acceptance of these conditions by all investigators involved in the project at the nominated site(s).

1. The responsibility for the conduct of the project at a site lies with the nominated Principal Investigator (PI) at that site. All correspondence should be signed by the PI.

2. The PI will inform the RPH REG Office about any changes to the project. The PI is responsible for submitting any amendments to the approved documents listed on the approval letter, or any new documentation to be used in the project. Any new or amended documentation should be submitted in a timely manner and cannot be implemented at this site until they have received Lead HREC approval for their use.

3. The PI will notify the RPH REG Office of their inability to continue as PI at the site(s) and will provide the name and contact information of their replacement.

4. The PI will notify the RPH REG Office of any departures of named site investigators. The PI will also notify the RGO if any new site investigators join the project.

5. The PI is responsible for reporting site adverse events, using the standard forms available from the website. Reporting requirements are as per the WA Health Research Governance and Single Ethical Review Standard Operating Procedures. Additional reports, other than those outlined, that are submitted will be returned without acknowledgement.

6. The annual report that is submitted to the HREC should also be submitted to the RPH REG Office. This should include the site specific information which should be completed by the site PI.

7. RPH has the authority to audit the conduct of any project without notice. Exercise of this authority will only be considered if there are grounds to believe that some irregularity has occurred, if a complaint is received from a third party or the site decides to undertake an audit for Quality Improvement purposes.

8. The site can conduct random monitoring of any project. The PI will be notified if their project has been selected. The PI will be given a copy of the monitor’s report along with the HREC and REG Office.

9. Complaints relating to the conduct of a project should be directed to the RPH REG Office and will be promptly investigated according to the site Standard Operating Procedures.

10. The PI is reminded that records of consent or authorisation for participation in a project form part of the Acute Hospital Patient Record and should be stored with that record in accordance with the WA Health Patient Information Retention and Disposal Schedule (Version 2) 2000. A copy of the ‘Participant Information Sheet’ should also be included in the medical records as part of informed consent documentation.

11. Once the project has been closed at RPH, the PI is required to submit to the RPH REG Office a copy of the final report that is submitted to the Lead HREC. This should include the site specific information which should be completed by the site PI. If the report is not received within 30 days the project will be closed and archived. An outstanding final report could impact on the PI’s ability to apply for approval for future projects.

12. If a project is suspended or terminated the PI must ensure that the RPH REG Office is informed of this and the circumstances necessitating the suspension or termination of the project. Such notification should include information as to what procedures are in place to safeguard participants.

13. If a project fails to meet these conditions the RPH REG Office will contact the investigator(s) to request they rectify the identified issues. If, after being contacted by the RGO, the issues are not addressed the site authorisation will be withdrawn.
Our Ref: RA/4/20/4848

18 September 2018

Winenthrop Professor Gerald Watts
Medical School
MBDP: M570

Dear Professor Watts

HUMAN RESEARCH ETHICS OFFICE – NOTIFICATION OF ETHICS APPROVAL FROM ANOTHER ETHICS COMMITTEE

Project: Cardiac Computerised Tomography and Inherited Hypercholesterolaemia - Recognition Royal Perth Hospital HREC Approval RGS0000000748

Thank you for your correspondence notifying this office of your project's review and approval by a non-UWA Research Ethics Committee.

It is noted that you have ethics approval from Royal Perth Hospital, approval number RGS0000000748.

The students and researchers identified as working on this project are:

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution Details</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winenthrop Professor Gerald Watts</td>
<td>Medical School</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>Mrs Jacqueline Ryan</td>
<td>Medical School</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Ms Jing Pang</td>
<td>Medical School</td>
<td>Co-Investigator</td>
</tr>
</tbody>
</table>

Student(s): Cristian Vargas Garcia

Although The University of Western Australia reserves the right to subject any research involving its staff and students to its own ethics review process, in this case, the UWA Human Ethics Office recognizes the existing approval of the non-UWA ethics committee.

1. Approving HREC to receive annual reports, amendments and notification of adverse events

You are reminded that the approving ethics committee remains the monitoring committee for this project. You must correspond with them for matters regarding amendments, adverse events, annual and final reporting.

If you have any queries, please contact the HEO at humanethics@uwa.edu.au.

Please ensure that you quote the file reference – RA/4/20/4848 – and the associated project title in all future correspondence.

Yours sincerely

Mark Davies
Manager, Human Ethics
Ethics 9. Chapter five - ethics approval

Ref: EC 2012/063
(This number must be quoted on all correspondence)

Dr Timothy Bates
Internal Medicine
(on behalf of FHWA Committee)
Royal Perth Hospital

Dear Tim

EC 2012/063 CT Coronary Angiography in Familial Hypercholesterolemia

Thank you for submitting the above project for approval by the RPH Ethics Committee.

Under the revised National Statement (NS) (March 2007) it would appear that this project meets the conditions set out in paragraphs 5.1.22 and 5.1.23 and 2.1.7 for low to negligible risk research and has been reviewed by the Ethics Committee’s low risk sub-committee.

I am pleased to advise that the sub-committee has approved your study.

The Committee is obliged by the provisions of the revised “National Statement” of the NH&MRC (2007) to monitor progress of all studies until completion. Therefore, this approval is granted on the understanding that you will advise of any protocol amendments and submit an annual report to the Committee.

Yours sincerely

Prof Frank van Bockxmeer
Chairman, Royal Perth Hospital Ethics Committee

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The RPH Human Research Ethics Committee (HREC) is constituted and operates in accordance with NH&MRC Guidelines.

Ethics Office Level 5 Colonial House, Royal Perth Hospital, GPO Box X2213 Perth WA 6001
Tel (08) 9224 2292 | Fax (08) 9224 3688 | Email rph@health.wa.gov.au
21 September 2018

Professor Gerald Watts
School of Medicine
Royal Perth Hospital

Dear Professor Watts

Project Title: *CT coronary angiography in Familial Hypercholesterolemia*
REG Number: 2012-063
HREC: Royal Perth Hospital Human Research Ethics Committee (EC00270)
Site: Royal Perth Hospital

The following amendment/s (and associated documents) have been approved by the RPH HREC and the EMHS site/s:

<table>
<thead>
<tr>
<th>Amendment/Documents</th>
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<tr>
<td>Change to Project Investigators:</td>
</tr>
<tr>
<td>Addition of Cristian Garcia as Associate Investigator</td>
</tr>
</tbody>
</table>

If this project is also being conducted at non-EMHS sites, please submit a copy of this approval letter to the Research Governance Office of those sites, as evidence of approval by the HREC.

Yours sincerely,

MARK WOODMAN
Delegate of the Chair
Royal Perth Hospital HREC

MELISSA JARVIS
Research Governance Officer
East Metropolitan Health Service

cc: Jing Pang

Research Ethics & Governance
Level 2 Kirkman House, Royal Perth Hospital, GPO Box X2213 Perth WA 6847
Telephone: (08) 9224 2260 / (08) 9224 2292
Email: EMHS.REG@health.wa.gov.au
Ethics 11. Chapter five - UWA ethics approval recognition

Our Ref: RAA/20/4866
24 September 2018

Winthrop Professor Gerald Watts
Medical School
MB0P: M570

Dear Professor Watts

HUMAN RESEARCH ETHICS OFFICE – NOTIFICATION OF ETHICS APPROVAL FROM ANOTHER ETHICS COMMITTEE

Project: CT coronary angiography In Familial Hypercholesterolemia - Recognition Royal Perth Hospital HREC Approval 2012-063

Thank you for your correspondence notifying this office of your project’s review and approval by a non-UWA Research Ethics Committee. It is noted that you have ethics approval from Royal Perth Hospital, approval number 2012-063.

The students and researchers identified as working on this project are:

<table>
<thead>
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<tr>
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<td>Medical School</td>
<td>Chief Investigator</td>
</tr>
<tr>
<td>Ms Jing Peng</td>
<td>Medical School</td>
<td>Co-Investigator</td>
</tr>
<tr>
<td>Mr Cristian Vargas Garcia</td>
<td>Student Body</td>
<td>Co-Investigator</td>
</tr>
</tbody>
</table>

Although The University of Western Australia reserves the right to subject any research involving its staff and students to its own ethics review process, in this case, the UWA Human Ethics Office recognizes the existing approval of the non-UWA ethics committee.

1. Approving HREC to receive annual reports, amendments and notification of adverse events

You are reminded that the approving ethics committee remains the monitoring committee for this project. You must correspond with them for matters regarding amendments, adverse events, annual and final reporting.

If you have any queries, please contact the HEO at humanethics@uwa.edu.au.

Please ensure that you quote the file reference – RA/4/20/4866 – and the associated project title in all future correspondence.

Yours sincerely

Mark Davies
Manager, Human Ethics

The University of Western Australia
Cerreya WA 6022 Australia
T +61 8 6488 3793/4703
F +61 8 6488 2770
E humanethics@uwa.edu.au
CRICOS Provider Code 00126B

234
Ethics 12. Chapter four and five - consent form biobank for inherited disorders of lipid metabolism

Royal Perth Hospital
Lipid Disorders Clinic
Telephone (08) 9224 8092  Fax (08) 9224 1319

Consent Form
BioBank for Inherited Disorders of Lipid Metabolism

Surname: ___________________  Given Names: ___________________
Date of Birth: _______________  Sex: ___________________  UMRN No. ___________________
Address: ___________________

☐ Index case  ☐ Family member, if yes, what is the relationship to the index case? ___________________

Participant Consent
I, _______________________________ have read the Information Sheet entitled ‘BioBank for Inherited Disorders of Lipid Metabolism’.

Dr _______________________________ has explained to me what this involves and I understand the consequences of donating a blood sample (“biospecimen”) for genetic testing and for clinical research. I have had the opportunity to ask questions and am satisfied with the answers given.

I hereby consent to donating biospecimens including blood and any material derived from it such as serum, plasma, cell lines and DNA for genetic testing, storage, and for my associated medical information to be used for research purposes on the understanding that:

1) the biospecimens will only be used to test for inherited disorders of lipid metabolism, associated cardiovascular complications and responses to treatments.

2) prior approval will be obtained from the Royal Perth Hospital Human Research Ethics Committee for any research involving my biospecimens and associated clinical information.

3) research data arising from my de-identified biospecimens and clinical information may be published and will be made available to me on request.

4) my de-identified biospecimens may be shared with external researchers in the field, for the study of inherited disorders of lipid metabolism and their treatment. Yes ☐ / No ☐

5) I agree that I may be approached in the future concerning further involvement in research studies or providing additional information. Yes ☐ / No ☐.

6) my biospecimens will be removed from storage and destroyed on my written request to the Director of the Lipid Disorders Clinic or the Director of Clinical Services at Royal Perth Hospital without prejudice to me or my family’s care at Royal Perth Hospital.

_____________________________  ______________________  ___________________
Signature                        Date                        Print name

_____________________________  ______________________  ___________________
Parent/Guardian Signature       Date                        Print name

_____________________________  ______________________  ___________________
Health Professional name and designation  Signature of Health Professional  Date

BioBank for Inherited Disorders of Lipid Metabolism, Consent Version 3, 26 September 2017
Ethics 13. Chapter four and five - national familial hypercholesterolaemia registry information and consent form for participants

National Familial Hypercholesterolaemia Registry

FH Registry Information and Consent Form for Participants

This Information and Consent Form is for Participants aged 18 years and over. Additional forms for unaffected family members and children under 18 years of age are available.

INFORMATION FOR PARTICIPANTS

We invite you to register with the Australian Familial Hypercholesterolemia Registry.

- To join the Registry you will need to sign an information and consent form. If you are younger than 18 years of age and can understand this information, you will need to complete the form for children under 18 years of age and we also require your parent or guardian to co-sign it.
- Whatever your age, please discuss registration with your family and/or your doctor, and do not hesitate to contact us if you have any questions.
- If you are the parent or guardian of a child who is not old enough to understand this form, please sign the form specifically for parents/guardians of children under 18 years of age.
- The section you need to sign to confirm that you agree to participate is at the end of the form.
- Before you sign the form it is important that you understand what is involved and what happens to the information you provide.
- This information and consent form contains answers to some of the questions you might have.
- If you have any questions please contact the relevant person in your state before signing the form. You will find a list of contact details at the end of this document.
- This project has been approved by the local Human Research Ethics Committee.

“What is a patient Registry and why would I want to participate in one?”

A patient Registry is a place where medical information, family history and other related information from individuals is collected and stored for medical research and/or for improving medical care of a condition.

The purpose of this Registry is to collect and store medical information and other information from individuals with familial hypercholesterolemia (FH) and their family members. The data will be used to:

- improve the care of patients through the co-ordination of diagnosis and therapy;
- ensure new intervention strategies and clinical best practice are available in an equitable and consistent manner across the community;
• improve the functions and capacity of the health network to provide quality care to those with FH;
• provide opportunities for the recruitment of patients into clinical trials; and
• facilitate participation in studies for the benefit of the community and the advancement of medical science.

The FH Registry will be supervised nationally by the FH Australasia Network (Australian Atherosclerosis Society). Additionally an FH Registry National Advisory Board, which is a committee of experts from each state in Australia and New Zealand, will provide guidance and advice on the management of the Registry. For more information, please refer to the Terms of Reference of the FH Registry Charter provided by your treating doctor and/or please refer to the Australian Familial Hypercholesterolemia Registry website.

The Registry is supported financially by the FH Australasia Network and there is no cost to you to participate.

"Whose data are being collected in this Registry?"
Registry information will be collected on participants who:
• are diagnosed with FH,
• are biologically related to someone who is diagnosed with FH,
• carry a mutation in a gene that is associated with significant increased risk of developing FH.

"You" will refer to the individual diagnosed with FH and whose data will be entered in the Registry.

"What information will be recorded by the Registry?"
The Registry will record demographic information including name, address, date of birth, email and contact telephone numbers as well as treating doctor’s name, address, telephone number and email. Clinical information such as family history of FH, history of cardiovascular disease and cardiovascular disease risk factors, treatment and lipid concentrations will be provided by your treating doctor and recorded. If genetic testing has been done, the affiliated laboratory services will be asked to provide details of the results. This information will be entered by your clinic co-ordinator or treating doctor when you are registered onto the Registry.

"How long will data be kept?"
Data will be kept for the duration of the operation of the Registry unless you choose to withdraw from the Registry. Personal demographic data will be kept separately from the clinical and laboratory data. Only people involved in managing the Registry, with specific authority, will be able to access this demographic data (i.e. personal identifying data such as name and address).

If the Registry needs to stop operation the data will be retained for 60 years and then all records will be destroyed.
“How will my privacy be protected?”

Your personal identifying details (name, address etc.) and other information will be stored as an individual electronic record. This individual record will be assigned a Unique Identification Number. All information will be stored in a secure and confidential manner in order to prevent a person from being identified by anyone other than those directly involved with their clinical care. Personal identifying information will never be given to a clinical trial co-ordinator or other third party without first obtaining that person’s (or their guardian’s) written agreement.

This file will be subject to the regulations on data protection,1,2 at both state and national levels, and we will only transfer non-identifiable data to researchers under national laws1,2. Any information we collect from this Registry that can identify you will be treated as confidential. All confidential information shall be encrypted and stored securely, in accordance with each state’s and national privacy laws.

There is minimal risk in taking part in the Registry. The Registry includes questions that can be sensitive and some participants may feel uncomfortable answering. You do not have to share any information you do not want to. Another unlikely risk is potential breaches in the computer system. In the event that there is a breach in the Registry’s computer system all participants will be notified.

If we publish any research or other documents based on data from the Registry, this research will never identify you by name.

Third parties wishing to have access to data in the Registry (such as researchers or companies planning clinical trials or conducting research on new treatments) will only have access to clinical and genetic information along with your Unique Identification Number. We will never transfer any personal information which could identify you. Researchers using information with the personal identifying code cannot identify you personally from the information they can access. Only the person in charge of the Registry (the National Coordinator) or a person explicitly appointed to that position will be able to access to your personal information and identify you.

Before a third party is granted access to the Registry data they must have the approval of a Human Research Ethics Committee. Your data will not be made available to employers, government departments, insurance companies or educational institutions.

The Registry can only disclose your personal information identifying you with your specific permission and will remain confidential except in the case of a legal requirement to provide information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research.

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1 The Federal Privacy Act 1988 is Australia’s national law for the protection of personal information when handled by Federal and ACT Government Agencies and many private sector organisations. Within the Act, eleven Information Privacy Principles have been developed to govern things such as the collection, storage, use and disclosure of personal information. The Principles also provide individuals with certain rights to access their personal information and correct any errors.

2 National Health & Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research (2007); The primary purpose "... is the protection of the welfare and the rights of participants in research..." and the secondary purpose "... is to facilitate research that is or will be of benefit to the researcher’s community or to humankind...".
situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility.

“Who will have access to my information on the Registry?”
Your data will be stored securely and no unauthorized people in the Registry will be able to gain access to any information about you. Staff in charge of the Registry might need to gain access to information in your medical records to seek information to include in the Registry. Only people specifically authorised by the Registry will be able to do this.

“Will relationship to family members be linked to my record?”
It is very useful for the Registry to have a record of your family history. With your consent, the Registry proposes to link your record to the records of your blood relatives, using only your Unique Identification Number. The link will show your Unique Identification Number and your relationship to all registered affected and unaffected family members. Only those people with access specifically to your records will be able to see your details. They will not see any details about your relative other than their Unique Identifier and their relationship to you. The same restrictions will apply to their records and the Registry link to you.

One purpose of a Registry is that participants could volunteer for clinical trials.

“What is a clinical trial?”
A clinical trial is research in which a treatment or diagnostic test is investigated in a selection of relevant people in a scientific and ethically appropriate manner. Clinical trials are conducted in accordance with ethical guidelines and, in certain circumstances, in accordance with the Therapeutic Goods Act.

“How will my participation in the Registry relate to clinical trials?”
When a clinical trial is being planned, it is important that individuals who are suitable for that trial are able to be identified and contacted. The best way of ensuring this can happen is to collect data on all individuals with FH and their family members in a single Registry.

You are completely free to make your own decision about your participation in any trial we inform you about. If you decide not to take part in a particular trial, your data will still be kept in the Registry and we will continue to inform you, through your doctor, about other trials unless you tell us not to.

If, after careful consideration and discussion with your doctor, you decide to take part in the trial you will need to review and sign a separate consent form. Your doctor will then contact us at the Registry, and we will in turn, send the required information to the researchers running the trial.

The information in the Registry will also allow us to provide you with new information relevant to standards of care for FH, and to collect a small amount of statistical

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The Therapeutic Goods Act 1969 establishes a uniform, national system of controls to ensure the quality, efficacy and timely availability of therapies in Australia. The Act governs how clinical trials are conducted in Australia.
information that may be used to improve Australian and international knowledge of FH.

“What about other research?”
Information from participants of this Registry may be used for research to better understand FH and to improve treatments for FH.

Your information (without personal identifying information such as name, address, date of birth) may also be shared with other global databases to advance the treatment and outcomes of people with FH worldwide. This research may include diagnostic, epidemiological, health service planning, social planning and genetic research, with the overall aim of informing best practice for the care of FH.

“I want to be involved in a clinical trial. If I register, is this guaranteed?”
Although one of the main goals of the Registry is to make it easier for affected individuals to participate in research, there is no guarantee that those participants will be eligible for a trial.

“What do I have to do to participate?”
If you agree to take part in this project, you should read this patient information and sign the consent form at the end.

Your treating doctor or the clinic coordinator will enrol you in the Registry and add some personal data and some information about your condition. It is very important that the Registry is able to collect your clinical information and laboratory test results. The clinical information will be provided to the Registry by your treating doctor and the laboratory test results will be provided by the testing laboratory. This data will be entered into the Registry by the clinic coordinator or your doctor where you have enrolled.

“I don’t want to be involved in a clinical trial. Should I still register?”
We hope that you will still be willing to register, even if you don’t want to take part in a clinical trial. Your information may still be useful to researchers who are trying to learn more about individuals with your condition.

“What are my options if I do not want to be in the Registry?”
You do not have to join this Registry. Participation is voluntary. You do not need to participate in this Registry to remain a member of the FH community. Your decision not to participate in this Registry will not affect your healthcare.

By signing this form you do not give away any legal rights or benefits to which you are otherwise entitled. If you do join, you can change your mind and withdraw from the Registry at any time and request to remove any of your information that has not been assigned yet to any specific study. You will not be able to remove any information that already has been assigned to a specific study. If you decide not to sign this form, there will not be any effect on your regular health care, medical treatment or insurance benefits.
“Who do I contact with questions?”
If you have any questions about the registration process or about participation in the Registry, please contact your clinical coordinator for the Registry. To inquire about your rights as a participant in the Registry, you may also contact the ethics committee that approved this Registry (contact details are provided at the end of this form).

For additional information regarding the terms and conditions of the Registry or the privacy policy please go to Terms and Conditions and Privacy Policy sections of the Registry website.

“What if I want to withdraw from the Registry?”
Should you change your mind and wish to withdraw your data from the Registry, you will be free to do so without having to provide any explanation. Contact the clinical coordinator where you enrolled and all of your data will be removed from the database.
INFORMED CONSENT

1. I understand that my participation in the Registry is voluntary and that I can change my mind and withdraw at any time.

2. I understand that all reasonable attempts will be made to protect my privacy and my family’s privacy. I understand that my personal information will be protected and saved in the Registry using a code. However, there is a very small risk that my personal information could be revealed.

3. I understand that by agreeing to participate, I may be contacted by the Registry or an agent of the Registry to update or correct my health information regularly.

4. I understand that I may not personally benefit from participating in the Registry or from the use of my de-identified medical information in any research study.

5. I understand that any information that has already been given to a specific study cannot be removed by the Registry.

6. I understand that I can withdraw from the Registry at any time and if I do so my information will be removed from the Registry database. I also understand that any information that has already been given to a specific study cannot be removed by the Registry even if I have withdrawn from the Registry.

7. I consent to my de-identified medical information being used for clinical trials and other medical studies related to FH provided those studies have been approved by an institutional ethic committee.

8. I consent to my de-identified information being used for research studies about diseases that are not associated with FH provided those studies have been approved by an institutional ethic committee.

9. I consent to my de-identified information being shared with other databases whose primary focus is the treatment and outcomes of people with FH.

10. To improve the quality of the family history data on the Registry, we propose to link your record to any other affected and/or unaffected family member or relative on the Registry. The link will only show your Unique Identification Number and your relationship to the relative. I consent to my record being linked to the records of other relatives on the Registry.

I consent to all of the above  ☐ Yes / ☐ No

I would like to be contacted about clinical trials or other studies that I can participate  ☐ Yes / ☐ No

I would like information on familial hypercholesterolemia be sent to me via my email or home address.

☐ Yes / ☐ No

1Please note that if we inform your doctor about the existence of a trial, this does not imply that we endorse it. In order to participate in any trial, you will need to discuss it with your family and your doctor and will be required to fill out a separate informed consent form that relates to that specific trial.
The nature of the Registry has been fully explained to me. I have understood the patient information and informed consent form and have received a copy to take away with me. I have had the opportunity to ask questions, and all my questions have been answered to my satisfaction. Upon reflection, I agree to participate in this Registry.

Name of participant

________________________________________

Signature of participant  Date

________________________________________

Name of Person Obtaining Consent  Position

________________________________________

Signature of Person Obtaining Consent

________________________________________

Date

________________________________________
PARTICIPANT REGISTRATION DETAILS

First name: ____________________________

Family name: __________________________

Date of Birth (dd/mm/yyyy) __________________________

Address: ____________________________

                                                  
                                                  
                                                  
                                                  
Postcode __________________________

Telephone: __________________________

Mobile phone: __________________________

Email: __________________________

If you would like to register directly with the Registry please provide the name of your doctor below giving us permission to contact your doctor directly if we require further information to complete your registration.

You have my permission to contact my doctor for my personal details:

Doctors Name: __________________________

Clinic / Medical Practice Address: __________________________

                                                  
                                                  
Clinic / Medical Practice Telephone: __________________________

Specialist Name: __________________________


## CONTACT DETAILS

If you would like any additional information or need to tell us about any change in your data, or if you wish to withdraw your data from the Registry, please contact the person from the hospital you attend listed below:

<table>
<thead>
<tr>
<th>State</th>
<th>Hospital</th>
<th>Contact Person</th>
<th>Phone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA</td>
<td>Royal Perth Hospital</td>
<td>W/Prof Gerald Watts</td>
<td>(08) 9224 0245</td>
<td><a href="mailto:gerald.watts@uwa.edu.au">gerald.watts@uwa.edu.au</a></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clinical Coordinator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WA</td>
<td>Royal Perth Hospital</td>
<td>Clin/Prof John Burnett</td>
<td>(08) 9224 3121</td>
<td><a href="mailto:john.burnett@health.wa.gov.au">john.burnett@health.wa.gov.au</a></td>
</tr>
<tr>
<td>WA</td>
<td>Royal Perth Hospital</td>
<td>A/Prof Timothy Bates</td>
<td>(08) 9224 2244</td>
<td><a href="mailto:timothy.bates@health.wa.gov.au">timothy.bates@health.wa.gov.au</a></td>
</tr>
<tr>
<td>WA</td>
<td>Royal Perth Hospital</td>
<td>A/Prof Damon Bell</td>
<td>(08) 9224 2453</td>
<td><a href="mailto:damon.bell@health.wa.gov.au">damon.bell@health.wa.gov.au</a></td>
</tr>
</tbody>
</table>

If you have any complaints about any aspect of the Registry, the way it is being conducted or any questions about being a participant in general, please contact the ethics committee that approved the Registry:

<table>
<thead>
<tr>
<th>State</th>
<th>Human Research Ethics Committee</th>
<th>Address</th>
<th>Phone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA</td>
<td>Royal Perth Hospital</td>
<td>Office 212, Level 2, Southern Research Facility</td>
<td>(08) 6151 1180</td>
<td><a href="mailto:smhs.reg@health.wa.gov.au">smhs.reg@health.wa.gov.au</a></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Perkins), Fiona Stanley Hospital, 102-118 Murdoch Drive, Murdoch WA 6150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Locked Bag 100, Palmyra DC WA 6961</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Our Ref: RA/4/2044872

26 September 2018

Winthrop Professor Gerald Watts
Medical School
MBDP-M70

Dear Professor Watts

HUMAN RESEARCH ETHICS OFFICE – EXEMPTION FROM ETHICS REVIEW

Patients Perception & Understanding of Cardiovascular Risk Assessment Employing the Estimation of Coronary Artery Calcium Using Cardiac Computed Tomography Scanning

Based on the information you have provided to the Human Ethics office in relation to the above project, the described activity has been assessed as exempt from ethics review at the University of Western Australia.

However, should there be any significant changes to the protocol, you must contact the HREO to determine whether your exempt status remains valid or whether you will be required to submit an application for ethics approval.

If you have any queries please contact the Human Ethics office at humanethics@uwa.edu.au.

Please ensure that you quote the file reference – RA/4/2044872 – and the associated project title in all future correspondence.

Yours sincerely

Mark Davies
Manager, Human Ethics

Name       Faculty / School Role
Winthrop Professor Gerald Watts  Medical School  Chief Investigator
Dr Warrick Bishop               Calvary Hospital Co-Investigator
Ms Jing Pang                    Medical School  Co-Investigator

Student(s): Cristian Vargas Garcia

The University of Western Australia

The University of Western Australia
Crawley WA 6029 Australia

T +61 8 6488 2703 / 4103
F +61 8 6488 8779

E humanethics@uwa.edu.au
CRICOS Provider Code: 00126G